applied biosystems

TaqMan Gene Expression Assay solutions

Proven performance for fast, reliable results

ThermoFisher SCIENTIFIC

The leader in gene expression analysis

We are the leader in gene expression analysis, providing world-class sample preparation with Applied Biosystems™ technologies, real-time PCR using Applied Biosystems™ TaqMan™ or Applied Biosystems™ SYBR™ Green chemistry, and industry-leading real-time PCR instruments and data analysis software.

Applied Biosystems™ TaqMan™ assay technology is the gold standard in performance, quality, and content for gene expression analysis. Developed using long-standing bioinformatic expertise in primer and probe design, and stringent testing across applications and integrated platforms, TaqMan Assays provide you with the most reliable and robust real-time PCR solutions.

With over one and a half million predesigned and preoptimized assays across a growing list of model species, a wide range of formats to scale to your needs, and a robust manufacturing quality system, we have a complete suite of solutions that will enable you to get fast, reliable, and accurate gene expression results.

Contents

TaqMan Gene Expression Assays

Proven 5´ nuclease–based real-time PCR chemistry

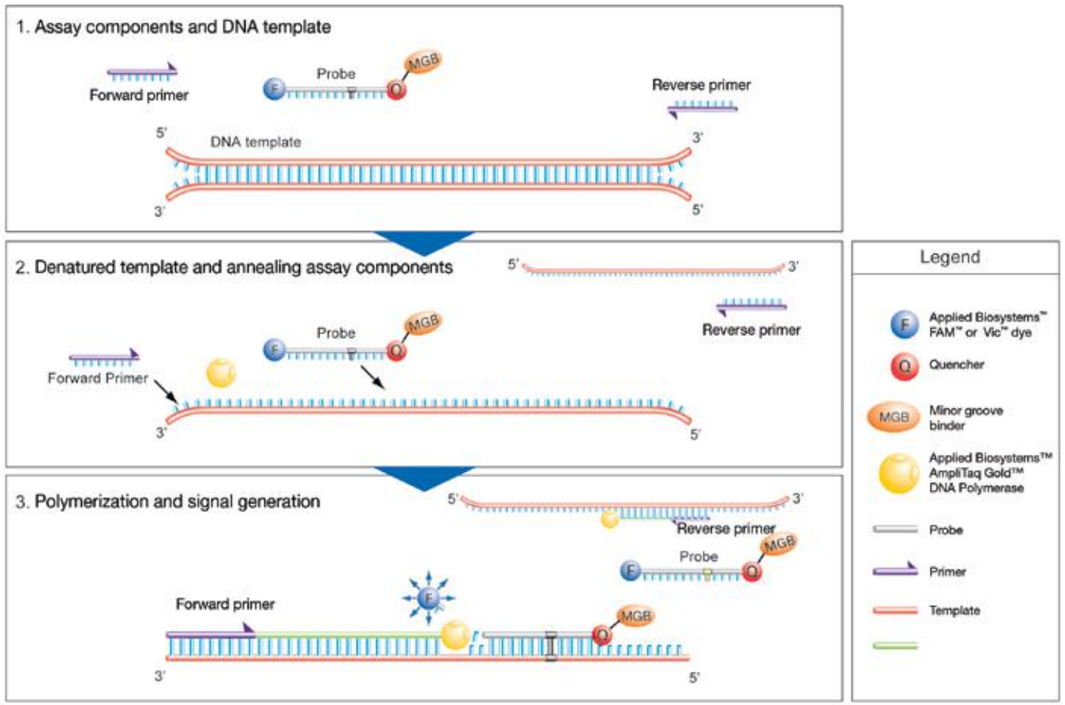
Get results you can trust

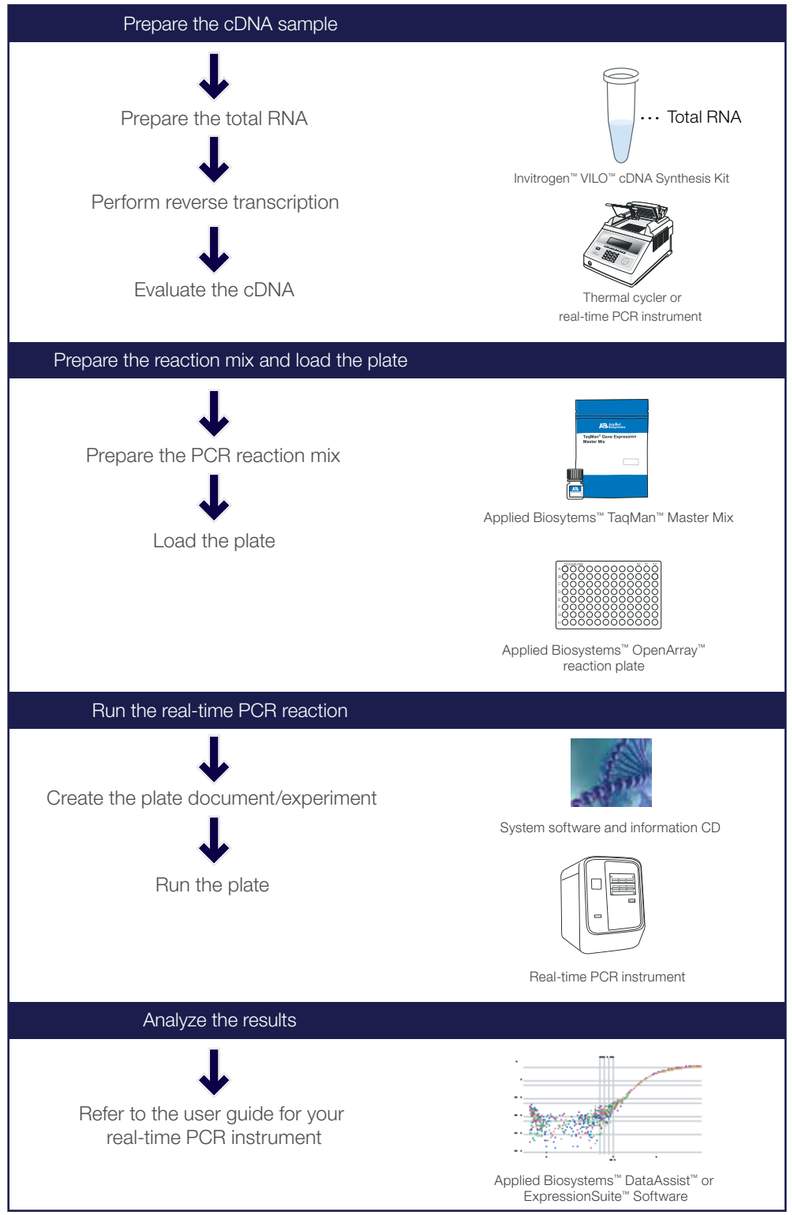
TaqMan Gene Expression Assays are referenced in tens of thousands of publications and are considered the gold standard for gene expression quantification by scientists around the world.

TaqMan Gene Expression Assays are based on 5´ nuclease chemistry, and each assay contains the primer and probe set for your target of interest. Here's how an assay works (Figures 1 - 3):

1. At the start of the real-time PCR reaction, the temperature is raised to denature the double-stranded cDNA. During this step, the signal from the fluorescent dye on the 5´ end of the Applied Biosystems™ TaqMan™ probe is quenched by the MGB–nonfluorescent quencher on the 3´ end of the probe.
2. In the next step, the reaction temperature is lowered to allow the primers and probe to anneal to their specific target sequences.
3. Taq polymerase synthesizes a complementary DNA strand using the unlabeled primers and template. When the polymerase reaches the TaqMan probe, its endogenous 5´ nuclease activity cleaves the probe, separating the dye from the quencher.

With each cycle of PCR, more dye molecules are released, resulting in an increase in fluorescence intensity proportional to the amount of amplicon synthesized.





The largest selection of predesigned assays

Spend time on results, not assay design and optimization

With TaqMan predesigned assays, spend your time generating results, not designing and optimizing assays.

- Detect virtually any gene product—more than 1.5 million predesigned assays, and custom design for everything else
- Assays for nearly every human, mouse, and rat gene in the RefSeq database
- Available for 25 species, and some pathogens
- Assays for multiple locations per transcript and across nearly every exon junction in human
- Strain-neutral assays for mouse and rat

To learn more and order, go to **thermofisher.com/taqmangex**

- Not finding what you're looking for in our predesigned assay collection? The Applied Biosystems™ Custom TaqMan™ Assay Design Tool lets you design and order a TaqMan Assay to detect any gene from any organism. Design and order your assays at **thermofisher.com/cadt** Custom TaqMan Assays are typically delivered in 5–12 business days.
- Also, try Applied Biosystems™ TaqMan™ Endogenous Controls—a collection of TaqMan Assays targeting commonly used control gene products for sample input normalization in real-time PCR.

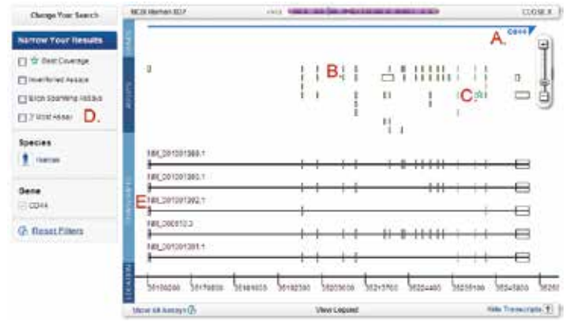
Predesigned TaqMan Gene Expression Assays
(as of November 2015)

Species	Number of assays	Gene coverage (%)*
Human *(H. sapiens)*	205,707	99.8%
Mouse *(M. musculus)*	176,510	99.5%
Chinese hamster *(C. griseus)*	154,743	88.2%
Rat *(R. norvegicus)*	146,589	89.2%
Cow *(B. taurus)*	103,562	99.6%
Rice *(O. sativa)*	99,822	95.6%
Arabidopsis *(A. thaliana)*	97,879	93.8%
Nematode *(C. elegans)*	92,687	95.1%
Rhesus monkey *(M. mulatta)*	69,310	55.8%
Zebrafish *(D. rerio)*	63,712	77.3%
Frog *(X. tropicalis)*	56,764	87.3%
Dog *(C. familiaris)*	55,558	64.3%
Chicken *(G. gallus)*	48,432	85.1%
Fruit fly *(D. melanogaster)*	41,607	94.0%
Sweet corn *(Z. Mays)*	38,493	59.5%
Cynomolgus monkey *(M. fascicularis)*	37,652	80.5%
Pig *(S. scrofa)*	16,247	90.3%
Fission yeast *(S. pombe)*	6,538	94.3%
Rabbit *(O. cuniculus)*	5,927	80.9%
Baker's yeast *(S. cerevisiae)*	5,524	93.4%
Horse *(E. caballus)*	3,891	72.8%
Soybean *(G. max)*	3,456	13.5%
Guinea pig *(C. porcellus)*	2,037	64.3%
Grape *(V. vinifera)*	965	25.3%
Wheat (T. aestivum)	760	43.6%
Summary	**1,534,372**	**81.1%, 25 species**

*Percent coverage refers to genes in the RefSeq database.

There are multiple assays for my gene product. How do I choose the right one?

Genomic alignment maps on our website make it easy to see exactly what gene products are detected and how they align to the genomic locus. The top of the map shows the target gene. Below it, all TaqMan Gene Expression Assays for target gene products are shown relative to the genomic locus map. The known transcripts from the locus are shown below, with their RefSeq accession numbers.



A. Gene symbol

B. Alignment of TaqMan amplicons to the gene. Hover over an assay to see its name and assay number as well as the transcripts it detects. Click on an assay to open an assay details pane for more information and to add the assay to your shopping cart.

C. Assays providing the best coverage are marked with a star symbol.

D. Narrow your results by specifying the type of assay you need.

E. All RefSeq transcripts that map to the gene locus, showing exon usage

The TaqMan Assays qPCR guarantee

We stand behind every predesigned TaqMan Assay. We are committed to helping you achieve your research goals and believe our predesigned TaqMan primer and probe sets establish the benchmark for high-quality and easy-to-use real-time PCR products.

We want you to be happy with your purchase and confident in the genomic tools we provide. Therefore, we guarantee every TaqMan Assay in terms of:

- **Quality**—high-quality manufacturing for reproducible results from lot to lot
- **Performance**—superior sensitivity, specificity, and accuracy
- **Content**—the largest collection of primer and probe sets using the world's best and most extensively validated assay design pipeline
- **Results**—enables you to obtain data you can trust

If you are not satisfied with the performance of a predesigned TaqMan Assay, we'll replace it at no cost or credit your account. For more information, and to see the full terms and conditions of the guarantee, go to **thermofisher.com/taqmanguarantee**

Proven performance

Reliable reagents for confidence in your results

TaqMan MGB probes bind more tightly—shorter, more specific probes

TaqMan probes include an MGB moiety at the 3′ end that increases the T_m of the probe and stabilizes probe–target hybrids. This means that TaqMan probes can be significantly shorter than traditional probes, providing better sequence discrimination and flexibility to accommodate more targets.

Nonfluorescent quencher (NFQ) maximizes sensitivity

TaqMan probes incorporate an NFQ to absorb (quench) signal from the fluorescent FAM or VIC dye label at the other end of the probe. The properties of the NFQ combined with the short length of MGB probes result in lower background signal than with non-MGB/NFQ probes. Lower background noise results in increased sensitivity and precision in your data.

TaqMan probe outperforms non-MGB probe in real-time PCR

	C_t		Standard deviation	
Input	TaqMan Assay	Non-MGB assay	TaqMan Assay	Non-MGB assay
10 ng	9.72	13.35	0.02	0.15
1 ng	13.36	16.82	0.04	0.18
0.1 ng	16.76	20.23	0.07	0.13
10^{-2} ng	20.19	23.72	0.04	0.13
10^{-3} ng	23.64	27.31	0.03	0.10
10^{-4} ng	27.01	30.66	0.04	0.12
10^{-5} ng	30.24	32.82	0.13	0.19

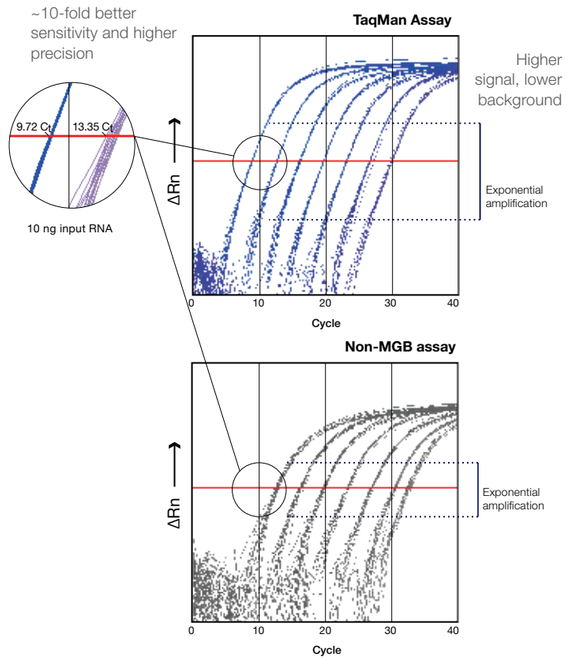


Figure 2. TaqMan probes provide better sensitivity and precision. Comparison of two 5′ nuclease PCR assays for 18S rRNA. Ten-fold dilutions of Universal Human Reference RNA (10–10^{-5} ng) were prepared and analyzed in 11 replicate real-time PCR reactions using either the TaqMan Gene Expression Assay (FAM dye–labeled, with NFQ) or the non-MGB assay (FAM dye–labeled, with BHQ). Real-time PCR was run according to the respective manufacturers' recommended conditions. Across a 6-log range of input template, the TaqMan Assay displayed earlier C_t values and better reproducibility across all data points. In addition, the TaqMan Assay had higher signal and lower background, resulting in better sensitivity and higher precision.

- **Specificity:** Advanced primer/probe sequence selection criteria plus MGB probe enhancement deliver the specificity and reproducibility you need for confidence in your results. Your results are generated from amplification of the intended target, not from nonspecific dye binding or amplification of closely related genes or pseudogenes.
- **Sensitivity:** The NFQ on TaqMan probes minimizes background, and intelligent PCR primer and probe design maximizes amplification efficiency. Get better sensitivity and accuracy—reliably detect targets present at 10 or fewer copies.
- **Reproducibility:** Accurately reproduce results from well to well, day to day, and lab to lab—even across manufacturing lots.
- **Wide dynamic range:** Detect from a handful to millions of target molecules with the same reaction setup. Capture the full spectrum of expression variability in virtually any experimental scenario.
- **High amplification efficiency:** All TaqMan Gene Expression Assays have a PCR efficiency of 100% (±10%). Use the comparative C_t ($\Delta\Delta C_t$) method of quantification confidently.
- **Ease of use:** All assays use a single, universal thermal cycling profile. Run any assay combination on a single plate. Avoid instrument-programming errors.
- **Comprehensive assay information:** Genomic mapping data are provided prior to purchase.

Detect as few as 10 target molecules with high sensitivity and large dynamic range

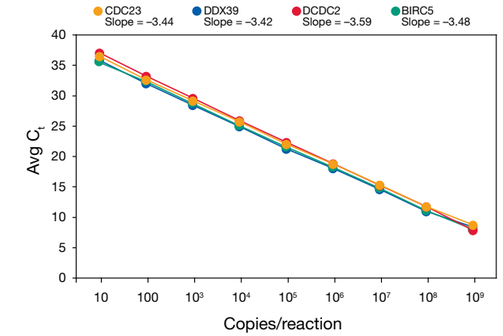


Figure 3. Sensitivity and wide dynamic range. Sequential 10-fold dilutions of synthetic sense RNA corresponding to 4 gene products—CDC23, DDX39, DCDC2, and BIRC5—were added to a background of yeast RNA to evaluate the sensitivity and dynamic range of TaqMan Gene Expression Assays. Samples containing 50 to 5 x 109 target molecules were reverse transcribed, and 20% of each RT reaction was used in quadruplicate PCR reactions using TaqMan Gene Expression Master Mix. Reactions containing as few as 10 copies were detected (C_t ~35).

Reproducible quantification with virtually 100% amplification efficiency

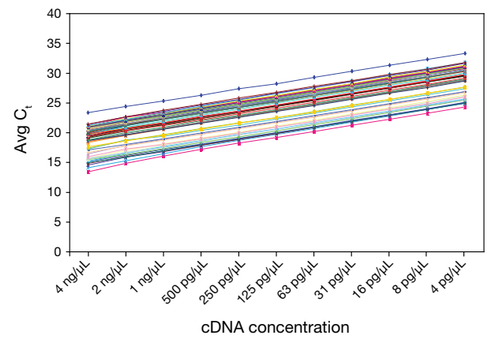


Figure 4. Reliable performance and wide dynamic range. TaqMan Gene Expression Assays were used to analyze expression of 60 targets across a 2-fold dilution series of universal reference cDNA, from 4 ng/µL to 4 pg/µL. The average slope of the lines is 1.02. TaqMan Assays exhibit virtually 100% amplification efficiency at each cycle of PCR: each target molecule is copied, doubling the fluorescence signal.

Specificity for your mRNA target

TaqMan Assay design helps ensure target mRNA specificity: readily distinguish even highly homologous sequences

Specificity is built into the TaqMan Assay design pipeline. As a result, assays detect only their intended targets. Even TaqMan Gene Expression Assays for members of highly homologous gene families typically amplify their targets with C_t values at least 10 cycles earlier than the closest homolog, or with at least 1,000-fold discrimination if equal numbers of the two targets are present.

TaqMan Gene Expression Assays are designed to detect only their intended targets, easily discriminating among highly homologous sequences.

HOX gene family members *HOXA10*, *HOXC10*, and *HOXD10* share ~80% sequence homology

Gene	RefSeq ID	TaqMan Assay ID	Homology
HOXA10	NM_018951.3	Hs00172012_m1	—
HOXC10	NM_017409.3	Hs00213579_m1	81%
HOXD10	NM_002148.3	Hs00157974_m1	79%

Clear gene expression results for HOX gene family members

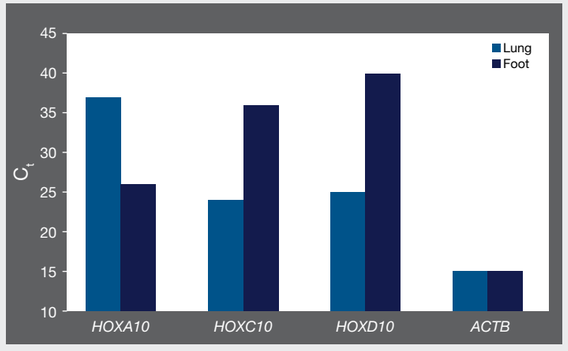


Figure 5. TaqMan Gene Expression Assays detect only their intended targets, even among the highly homologous HOX gene family members. In vertebrates, as in *Drosophila*, location-appropriate expression of members of the HOX gene family is essential for normal embryogenesis. Tissue-specific expression of 3 closely related HOX genes, comparable to published data, was easily detected using TaqMan Gene Expression Assays.

Advanced bioinformatics

TaqMan Gene Expression Assays are designed using our sophisticated design pipeline that has been stringently validated by functionally testing more than 18,000 assays (a statistically significant subset). Since then, our customers have consistently confirmed through their own validation experiments that TaqMan Gene Expression Assays enable reliable, reproducible results.

This process is used to design all TaqMan Gene Expression Assays, including inventoried assays, made-to-order assays, and Applied Biosystems™ Custom Plus assays. We offer ~73,000 inventoried assays and over 1.5 million made-to-order assays, which are manufactured when an order is placed. Applied Biosystems™ Custom Plus TaqMan™ RNA Assays are ideal for newly identified genes and specific splice variants, and offer the same performance as predesigned TaqMan Assays.

TaqMan Assay design and manufacture

Target selection
mRNA sequences (NCBI)

Preprocessing
–Map to genome
–Mask SNPs, repeats, and discrepancies
–Identify exon–exon junction

Assay design
Thermodynamic and chemistry parameters
–Balance T_m for universal thermal cycling
–Avoid secondary structure, optimize GC content
–Optimize amplicon size
–Eliminate primer-dimer formation

***In silico* QC**
–Score assays for target specificity
–Score assays for genome specificity

Assay selection
High-quality TaqMan Gene Expression Assays

Perform stringent assay formulation QC

Confirm oligo identity by mass spectrometry

Online ordering

Flexible formats

A variety of formats for different research needs

Configurations to fit your research goals

Are you analyzing hundreds (or thousands) of samples, and expression from a handful of genes? Or does your research involve a few samples that need to be analyzed for a long list of mRNA targets? No matter what experiment you are performing, there is a TaqMan Gene Expression Assay format and real-time PCR instrument for your research needs.

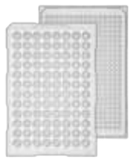
TaqMan Gene Expression Assay formats

Single tubes

- Low entry price
- Flexible
- Run on any real-time PCR instrument

384-well microfluidic cards

- Low cost per reaction
- Optimal for medium to large projects
- Run on Applied Biosystems™ QuantStudio™ 7 & 12K Flex, ViiA™ 7, and 7900HT Real-Time PCR Systems



96- or 384-well plates

- Optimal for small to medium projects
- Balances flexibility with streamlined reaction setup
- Run on any 96- or 384-well real-time PCR instrument

OpenArray plates

- Lowest cost for large projects
- Ultimate throughput
- Run on QuantStudio 12K Flex Real-Time PCR System

TaqMan Gene Expression Assays (single tubes)

Predesigned assays come in four different sizes so that you can order only the number of assays appropriate for your research. In addition, for made-to-order assays in small, medium, and large sizes, you can choose FAM or VIC dye labeling, and non–primer-limited or primer-limited formulation. (Extra small assays are only available with FAM dye labels.)

For more information, go to **thermofisher.com/allgenes**

Size	No. of reactions*	Concentration	Reporter dye	Cat. No.
Extra small (inventoried)†	75	20X	FAM	4453320
Extra small (made-to-order)‡	75	20X	FAM	4448892
Small (inventoried)†	250	20X	FAM	4331182
Small (made-to-order)‡	360	20X	FAM or VIC	4351372, 4448489 (VIC) 4448484 (VIC-PL**)
Medium (made-to-order)‡	750	20X	FAM or VIC	4351370, 4448490 (VIC) 4448485 (VIC-PL**)
Large (made-to-order)‡	2,900	60X	FAM or VIC	4351368, 4448491 (VIC) 4448486 (VIC-PL**)

*Reaction number is based on 20 µL reaction size.
**Primer-limited.
† Inventoried assays are typically delivered in 1–4 business days.
‡ Made-to-order assays are typically delivered in 5–12 business days.

Applied Biosystems™ TaqMan™ Arrays: 96-well plates or 384-well microfluidic cards

- Configure a Custom TaqMan Array containing inventoried predesigned assays, or select from our gene signature assay collections
- TaqMan Gene Expression Assays are loaded into one of two TaqMan Array formats: 96-well plates (Fast or standard) or 384-well microfluidic cards

(To include made-to-order or custom assays on your plate or card, order using our Applied Biosystems™ TaqMan™ Custom Plating Service, or contact your sales representative for other options.)

Custom TaqMan Array 96-well plates

- Choose any inventoried TaqMan Gene Expression Assay
- 6-plate minimum order
- Choose standard (20 µL rxn) or Fast (10 µL rxn) format

Typically delivered in 4–14 business days

To learn more and order, go to **thermofisher.com/arrayplates**

Assays + controls	Assay replicates	Samples per plate	Name	Cat. No. (standard)	Cat. No. (Fast)
95 + 1*	1	1	Format 96	4391524	4413255
92 + 4**	1	1	Format 96 +	4391525	4413256
47 + 1*	2	1–2	Format 48	4391526	4413257
44 + 4**	2	1–2	Format 48 +	4391527	4413258
31 + 1*	3	1–3	Format 32	4391528	4413259
28 + 4**	3	1–3	Format 32 +	4391529	4413260
15 + 1	6	1–6	Format 16	4413264	4413261
12 + 4	6	1–6	Format 16 +	4413265	4413262
7 + 1	12	1–12	Format 8	4413266	4413263

*Available with one manufacturing control assay for 18S ribosomal RNA. These formats are required for plates with assays for rhesus, canine, or a mixture of species.
**Includes the manufacturing control assay for 18S ribosomal RNA, plus assays for 3 additional candidate endogenous control genes: *GAPDH*, *HPRT1*, and *GUSB*, appropriate for human, mouse, or rat sample analysis.

Custom TaqMan Array 384-well microfluidic cards

- Choose any inventoried TaqMan Gene Expression Assays
- 10-card minimum order
- Run on the QuantStudio 7 & 12K Flex, ViiA 7, and 7900HT Fast Real-Time PCR Systems
- No robotics required: cards have 8 sample-loading ports, each connected to 48 wells containing dried-down TaqMan Assays
- 1 µL reactions (2 µL including channel filling and overage)
- Typically delivered in 3–4 weeks

To learn more and order, go to **thermofisher.com/arraycards**

Assays + controls*	Assay replicates	Samples per card	Name	Cat. No.
11 + 1	4	8	Format 12	4342247
15 + 1	3	8	Format 16	4346798
23 + 1	2 (or 4)	8 (or 4)	Format 24	4342249
31 + 1	3	4	Format 32	4346799
47 + 1	1 (or 2)	8 (or 4)	Format 48	4342253
63 + 1	3	2	Format 64	4346800
95 + 1	1 (or 2)	4 (or 2)	Format 96a	4342259
95 + 1	2 (or 4)	2 (or 1)	Format 96b	4342261
191 + 1	2	1	Format 192	4346802
380 + 4	1	1	Format 384	4342265

*These arrays are available with one manufacturing control assay for 18S ribosomal RNA.

Applied Biosystems™ TaqMan™ Array Gene Signature Plates and Cards

- Predesigned, preloaded TaqMan Assays for gene products specific to pathways, biomarkers, or disease target classes to facilitate drug discovery and disease research
- Endogenous control panels are also available to identify the best housekeeping gene products for your research
- Gene signature plates are typically delivered in 5–10 business days, and gene signature cards in 1–4 business days

Here is a sampling of what's available:

– Apoptosis

– Endogenous controls

– Cancer

– Immune system and inflammation

– Cell cycle proliferation and regulation

– Neurology

– Development and stem cells

– Signal transduction

– ECM matrix and adhesion

– Toxicology and drug metabolism

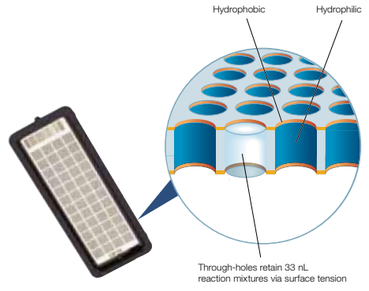
To see the complete collection of 96-well gene signature plates, go to **thermofisher.com/signatureplates**
To see the collection of 384-well gene signature microfluidic cards, go to **thermofisher.com/signaturecards**

OpenArray Real-Time PCR Plates

- TaqMan Assays loaded and dried down into the 3,072 through-holes on OpenArray Real-Time PCR Plates
- Process up to 576 samples to obtain over 43,000 data points, with a single operator in an 8-hour day, without the use of robotics
- For use with the QuantStudio 12K Flex Real-Time System with an Applied Biosystems™ OpenArray™ block configuration and supporting reagent kits only
- OpenArray plates with inventoried assays are typically delivered in 4–5 weeks, and within 5–6 weeks for custom assays

To learn more about OpenArray technology on the QuantStudio 12K Flex system,go to **thermofisher.com/openarray**

Assays + controls	Assay replicates	Samples per plate	Name	Cat. No.
18	3	Up to 48	Format 18	4471124
56	1	Up to 48	Format 56	4471125
112	1	Up to 24	Format 112	4471126
168	1	Up to 16	Format 168	4471127
224	1	Up to 12	Format 224	4471128



TaqMan Custom Plating Service: 96- or 384-well plates

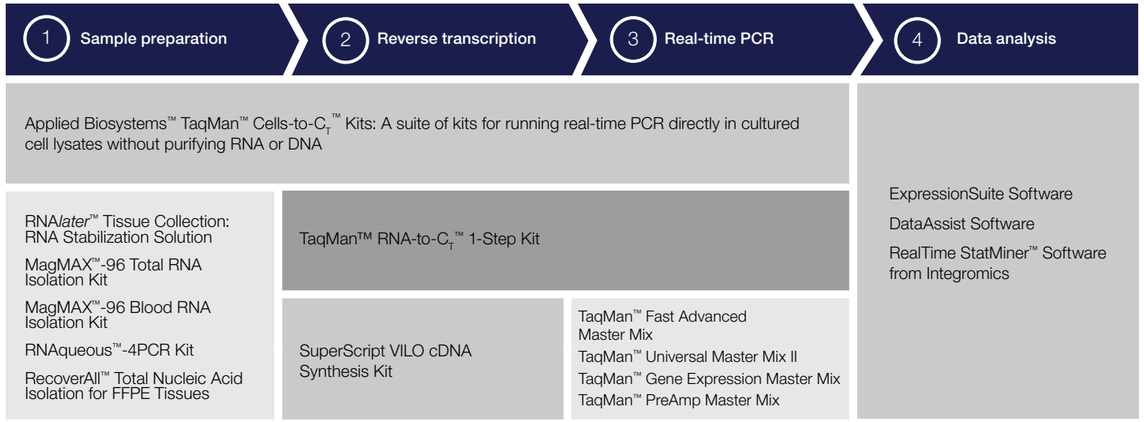
Configure 96- or 384-well plates with any TaqMan Gene Expression Assays, including custom assays designed to your target sequences and made-to-order assays.

- Set up custom configurations of any TaqMan Assays, including inventoried, made-to-order, custom, or Custom Plus gene expression assays or custom TaqMan probes and primers
- Choose 96- or 384-well plate, and Fast or standard format
- Receive in dried-down or liquid formulation
- Typically delivered in 2–5 weeks

Complementary reagents

Everything you need for reliable results

We provide everything you need for real-time PCR analysis, starting with isolating RNA from virtually any sample type, to reverse transcription into cDNA, optional preamplification to stretch small samples for analysis of many gene products, and of course, real-time PCR data analysis.



TaqMan chemistry vs. SYBR Green chemistry for real-time PCR

We offer two types of chemistries to detect PCR products using real-time PCR instruments:

- TaqMan Assay chemistry (also known as “fluorogenic 5´ nuclease chemistry”)
- SYBR Green I dye chemistry

	TaqMan Assay–based detection	SYBR Green–based detection
Overview	Uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR cycles	Uses SYBR Green I dye, or similar: dye binds to double-stranded DNA, to detect PCR product as it accumulates during PCR cycles
Specificity	High	Low
Sensitivity—low copies	High	Variable*
Reproducibility	High	Variable*
Multiplexing	Yes	No
Predesigned assays	Yes	No
User design and optimization	No	Yes
Cost	High	Low*
Gene expression quantitation	High	Low
DNA quantitation	Yes	Yes (pathogen detection)
ChIP	Yes	Yes
SNP genotyping	Yes	No
MicroRNA	Yes	No
Copy number	Yes	No
Somatic mutation detection	Yes	No
Pathway analysis	Yes	No

*Depends on template quality and primer design/optimization.

Support at every step of your workflow

Consistent reliability from manufacturing to follow-up

Quality manufacturing and stringent quality control
TaqMan Assays are manufactured in-house under rigorous quality processes at our ISO 13485–certified manufacturing facilities, and are never outsourced.

Comprehensive worldwide support
Whether you need help finding a TaqMan Assay for your target, deciding which format best suits your needs, placing your order through our online ordering system, or setting up your reactions, our global sales and technical support teams are here to help.

Technical support
If you have questions about how to use TaqMan Assays or how to analyze results, call or email our technical support specialists. These scientists are skilled in experimental planning and design, are expert troubleshooters, and are familiar with a wide variety of applications that use TaqMan Assays.

Rapid delivery
We continually strive to minimize delivery time on TaqMan Assay products. To that end, we have implemented streamlined order processing systems that interface with our new manufacturing facilities to help reduce delivery times.

Everything you need to meet the MIQE guidelines for peer-reviewed publications
The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines, published by Bustin et al. in *Clinical Chemistry* (April 2009), are meant to ensure that real-time PCR experiments are meaningful, accurate, and reproducible. We support this initiative and commend the MIQE scientists for their leadership.

We provide the following for easier adherence to these guidelines:

- **TaqMan Assay annotation**—Information requested under the real-time PCR target, oligonucleotide, and protocol sections of the guidelines is provided in your assay shipment and on our website. All biologically relevant information is available, including assay location, transcripts detected, and amplicon size. Protocols with recommended reagents and reaction conditions are also available on our website.
- **Publications**—There are >9,900 peer-reviewed publications that cite TaqMan Assays, so including the TaqMan Assay ID in lieu of sequences is sufficient and widely accepted.
- **Instrument software**—Applied Biosystems™ instrument software reports C_t values for quantification. The C_t can be used to generate standard curves, determine slope, and derive R2 values. To help adhere to the MIQE guidelines, the term quantification cycle (C_q) may be used directly in place of C_t.
- **Data analysis**—We offer data analysis software, including ExpressionSuite and DataAssist Software; simple-to-use tools for calculating relative gene expression using statistical analysis and visualization; and RealTime StatMiner Software (Integromics) for additional statistical analysis workflows.

applied
biosystems

Find out more at **thermofisher/allgenes**

ThermoFisher
SCIENTIFIC

TaqMan® Gene Expression Assays—single-tube assays

Pub. No. 4401212 **Rev.** D

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Gene Expression Assays User Guide—single-tube assays* (Pub. No. 4333458). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of TaqMan® Gene Expression Assays—single-tube assays. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan® Gene Expression Assays User Guide—single-tube assays* (Pub. No. 4333458).

Procedural guidelines

Guidelines for preparing cDNA templates

- For optimal reverse transcription, input RNA should be:
  - Free of inhibitors of reverse transcription (RT) and PCR
  - Dissolved in PCR-compatible buffer
  - Free of RNase activity

    Note: We recommend using RNase Inhibitor (Cat. No. N8080119) or RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).
  - Nondegraded total RNA
- For the input RNA amount, follow the recommendations provided by the cDNA kit.
- Small amounts of cDNA can be pre-amplified. Use TaqMan® PreAmp Master Mix (Cat. No. 4391128) or TaqMan® PreAmp Master Mix Kit (Cat. No. 4384267).
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.

Procedural guidelines for performing real-time PCR

- Protect the assays from light and store as indicated until ready for use. Excessive exposure to light can negatively affect the fluorescent probes of the assays.
- Run technical replicates in triplicate to identify outliers.

Perform PCR amplification

Before you begin (60X assays)

Dilute 60X assays to 20X working stocks with TE, pH 8.0, then divide the solutions into smaller aliquots to minimize freeze-thaw cycles. The size of the aliquots depends upon the number of PCR reactions you typically run. An example dilution is shown in the following table.

1. Gently vortex the tube of 60X assay, then centrifuge briefly to spin down the contents and eliminate air bubbles.
2. In a 1.5-mL microcentrifuge tube, dilute sufficient amounts of 60X assay for the required number of reactions.

Component	Volume
TaqMan® Gene Expression Assays (60X) or Custom TaqMan® Gene Expression Assays (60X)	40 µL
TE, pH 8.0 (1X)	80 µL
Total aliquot volume	**120 µL**

3. Store aliquots at –20°C until use.

Prepare the PCR Reaction Mix

Thaw the cDNA samples on ice. Resuspend the cDNA samples by inverting the tube, then gently vortexing.

1. Mix the Master Mix thoroughly but gently.
2. Combine the PCR Reaction Mix and assays in an appropriately-sized microcentrifuge tube according to the following table.

Component	Volume for 1 reaction	
	Standard 96-well or 48-well Plates	**384-well Plate or 96-well Fast Plate**
Master Mix (2X)[1,2]	10 µL	5 µL
TaqMan® Gene Expression Assay (20X) or Custom TaqMan® Gene Expression Assay (20X)	1 µL	0.5 µL
Nuclease-free water[3]	7 µL	3.5 µL
Total PCR Reaction Mix volume	**18 µL**	**9 µL**

[1] Recommended: TaqMan® Fast Advanced Master Mix

[2] (*Optional*) If you add AmpErase™ UNG (uracil-N-glycosylase), the final concentration must be 0.01U/ µL. Reduce the volume of water in the PCR reaction mix to compensate for additional volume from the UNG.

[3] Adjust the volume of nuclease-free water for a larger volume of cDNA.

3. Vortex the PCR Reaction Mix, then centrifuge briefly.
4. Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.

5. Add cDNA template (1 pg–100 ng in nuclease-free water), or nuclease-free water for NTC, to each well.
   - 1 µL for a 384-well plate or 96-well Fast Plate
   - 2 µL for a 96-well and 48-well Standard Plate

   Note: Be sure to adjust the volume of nuclease-free water in the PCR reaction mix for a larger volume of cDNA.

   IMPORTANT! For optimal results when using TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG, prepare the plate on ice. Run the plate within 2 hours of preparation, or store the plate at 2–8°C for up to 24 hours.

6. Seal the plate with a MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.

7. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

Set up and run the real-time PCR instrument

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

Note: The instrument must be configured with the block appropriate for the plate type.

1. Select the cycling mode appropriate for the Master Mix.

   IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on a Standard or a Fast plate format.

2. Set up the thermal protocol for your instrument.

   See "Thermal protocols" on page 2 for the thermal protocols for other Master Mixes.

   Table 1 TaqMan® Fast Advanced Master Mix (StepOne™, StepOnePlus™, ViiA™ 7, and QuantStudio™ systems with fast cycling mode)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds[2]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

[1] Optional, for optimal UNG activity.
[2] Enzyme activation can be up to 2 minutes. The time should not cause different results. See Enzyme activation time.

   Table 2 TaqMan® Fast Advanced Master Mix (7500 and 7500 Fast systems with fast cycling mode)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds[2]	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

[1] Optional, for optimal UNG activity.
[2] Enzyme activation can be up to 2 minutes. The time should not cause different results. See Enzyme activation time

3. Set the reaction volume appropriate for the reaction plate.
   - **96-well Standard (0.2-mL) Plate**: 20 µL
   - **96-well Fast (0.1-mL) Plate and 384–well Plate**: 10 µL

4. Load the plate into the real-time PCR instrument.

5. Start the run.

Analyze the results

For detailed information about data analysis, see the appropriate documentation for your instrument. Use the absolute or relative quantification ($\Delta\Delta C_t$) methods to analyze results.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
  - Adjust the baseline and threshold values.
  - Remove outliers from the analysis.
- In the well table or results table, view the C_t values for each well and for each replicate group.

For more information about real-time PCR, see *Introduction to Gene Expression Getting Started Guide* (Pub. No. 4454239) or go to **thermofisher.com/qpcreducation**.

Thermal protocols

The thermal protocols in "Set up and run the real-time PCR instrument" on page 2 are optimized for the TaqMan® Fast Advanced Master Mix.

The following tables provide thermal protocols for other Master Mixes that are compatible with TaqMan® Gene Expression Assays.

IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on a Standard or a Fast plate format.

Table 3 TaqMan® Gene Expression Master Mix or TaqMan® Universal Master Mix II, with UNG (any compatible instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	1 minute	

[1] For optimal UNG activity.

Table 4 TaqMan® Universal Master Mix II, no UNG (any compatible instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	1 minute	

Table 5 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (StepOne™, StepOnePlus™, ViiA™ 7, or QuantStudio™ system)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

Table 6 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (7500 or 7500 Fast system)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

Enzyme activation time

Using TaqMan® Fast Advanced Master Mix, the enzyme activation step can range from 20 seconds to 2 minutes. A 20–second enzyme activation step is sufficient when the template is cDNA. A longer enzyme activation time should not cause different results. The enzyme activation time for the default fast thermal cycling conditions on the instruments is 20 seconds. If a longer enzyme activation time is required, the thermal cycling conditions need to be changed before the run is started. A longer enzyme activation time can help to denature double-stranded genomic DNA when genomic DNA is used.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer: Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, CA 94566

Revision history: Pub. No. 4401212

Revision	Date	Description
D	15 May 2018	• Added procedural guidelines. • Added new instruments, Master Mixes, and other products applicable for the workflows. • Updated for general style, formatting, and branding.
C	November 2010	Baseline for this revision history.

New Fusion Transcript Assay for the BCR-ABL Translocation

Applied Biosystems® TaqMan® Gene Expression Assays for Fusion Transcripts

HEMATOPATHOLOGY UNIT, HOSPITAL CLINIC, BARCELONA

Dolors Colomer, PhD, senior consultant responsible for the molecular biology area at the Hematopathology Unit and Spain Representative for the Molecular Monitoring EUTOS Program (middle), with technicians Sandra Cabezas (right) and Sandra Martinez (left)

APPLICATION

Fusion transcript expression

TECHNOLOGIES

TaqMan® Gene Expression Assays for Fusion Transcripts

7900HT Fast Real-Time PCR System

Detecting Fusion Transcripts Caused by Chromosome Translocations

Chromosome translocations are abnormalities caused by rearrangement of chromosome sections between nonhomologous chromosomes. These rearrangements may result in chimeric genes that express fusion transcripts. Some of these transcripts can be translated into fusion proteins that affect normal regulatory pathways and stimulate abnormal cell growth. A well-known example is the *bcr-abl* chimeric mRNA (Philadelphia translocation), which is the result of a translocation of the abl gene on chromosome 9 to the *bcr* gene breakpoint cluster on chromosome 22.

In this study, new quantitative real-time PCR assays—Applied Biosystems® TaqMan® Gene Expression Assays for Fusion Transcripts, for detection of different bcr-abl transcripts (targeting p210 and p190 isoforms)—were compared to primers and probes for the same targets recommended and standardized by the Europe Against Cancer (EAC) Program and currently in widespread use [1–3]. The data presented here indicate that TaqMan® Gene Expression Assays for Fusion Transcripts have greater sensitivity and use an easier, ready-to-go workflow. The standardized assay format and protocol with an optimized master mix results in less variability in assay setup and allows laboratories to generate more reproducible data.

The Philadelphia Translocation (BCR-ABL Fusion Proteins)

BCR-ABL fusion proteins are associated with the formation of the Philadelphia translocation (Ph) and are one of the most common genetic abnormalities studied in blood cancer research. At the molecular level, the Ph chromosome, or t(9;22) (q34;q11) translocation, results from the fusion of the *bcr* gene (chromosome 22), which forms the 5' end of the fusion transcript, to the abl gene (chromosome 9), which forms the 3' end. In the vast majority of cases, the breakpoints in the *bcr* gene are found within three well-defined regions: the major breakpoint (M-bcr), minor breakpoint (m-bcr), and micro breakpoint (μ-bcr). Depending on which breakpoints are used, three main chimeric proteins of different sizes are generated (Table 1, Figure 1). These BCR-ABL chimeric proteins (p190, p210, p230) show increased, deregulated tyrosine kinase activity, which appears to deregulate normal cytokine-dependent signal transduction leading to inhibition of apoptosis, independent of growth factors.

Real-Time PCR Detects Translocations and Quantifies Expression

Current methods for identifying translocations include FISH and karyotyping, neither of which can be used to quantify the expression level of the fused gene as real-time PCR does. Real-time quantitative PCR can provide an appropriate monitoring strategy for analyzing BCR-ABL expression levels in the samples under study [5].

Table 1. *bcr-abl* Fusion Transcripts and Resulting Fusion Proteins.

Breakpoint Designation	Chrm 22 (*bcr* Gene) Break Location	Chrm 9 (abl Gene) Break Location	Variant Transcript Designation	Chimeric Protein Size (Name)
M-*bcr* (exons 12-16)	Intron 13	Intron 1	b2-a2 (e13-a2)	210 kDa (p210)
	Intron 14	Intron 1	b3-a2 (e14-a2)	" "
	Intron 13	Intron 2	b2-a3 (rare) (e13-a3)	" "
	Intron 14	Intron 2	b3-a3 (rare) (e14-a3)	" "
m-*bcr*	Intron 1	Intron 1	e1-a2	190 kDa (p190)
µ-*bcr* (rare)	Intron 19	Intron 1	e19-a2	230 kDa (p230)

Improved *bcr-abl* PCR Assays

Real-time PCR is the gold standard for quantitative measurement of nucleic acid. In collaboration with Applied Biosystems, EAC researchers developed primers and probes to detect specific *bcr*-abl fusion transcripts [1,2]. Recently Applied Biosystems has improved on these primer and probe designs, creating new TaqMan® Gene Expression Assays for all of the *bcr*-abl fusion transcripts (Table 3). Selected transcripts were annotated, and bases located at the fusion transcript breakpoint, known SNPs, and repetitive sequences were masked. TaqMan® minor groove binder (MGB) assays were then designed using the Applied Biosystems® bioinformatics design pipeline. The assays were designed such that the primers and probes bound on either side of the fusion transcript breakpoint (Figure 2), and each assay design was checked by in silico quality control.

TaqMan® Gene Expression Assays vs. EAC Assays

As proof of principle, the TaqMan® Gene Expression Assay designs were first tested using plasmids containing the translocation variant and human samples containing the translocation event. Amplification only occurred in samples containing the fusion transcript, confirming assay specificity (data not shown). Subsequently, researchers in the Hematopathology Unit of the Hospital Clinic in Barcelona used human samples to compare the Applied Biosystems® TaqMan® Gene Expression Assays for *bcr-abl* fusion transcripts and the EAC primer and probe designs [1,2]. (Note: The *bcr-abl* TaqMan® Gene Expression Assays include assays for several fusion transcripts for which there were no corresponding EAC designs.) The experimental procedure is provided in the sidebar, *Technical Details for Fusion Assays*. Results follow.

Detection of *bcr-abl* Fusion Transcript

Although it has been recommended to use a fixed threshold value using EAC designs (threshold 0.1), better results have been obtained using TaqMan® Gene Expression Assays with Automatic Analysis provided by the 7900HT Fast Real-Time PCR System. Standard curves generated by amplification of dilutions of each of the fusion transcripts (cloned into Ipsogen and pCR2.1 plasmid vectors) using both TaqMan® Gene Expression Assays and the ABL endogenous control TaqMan® Assay were very reproducible. PCR efficiencies were close to 100% with R2>0.99 (Figure 3).

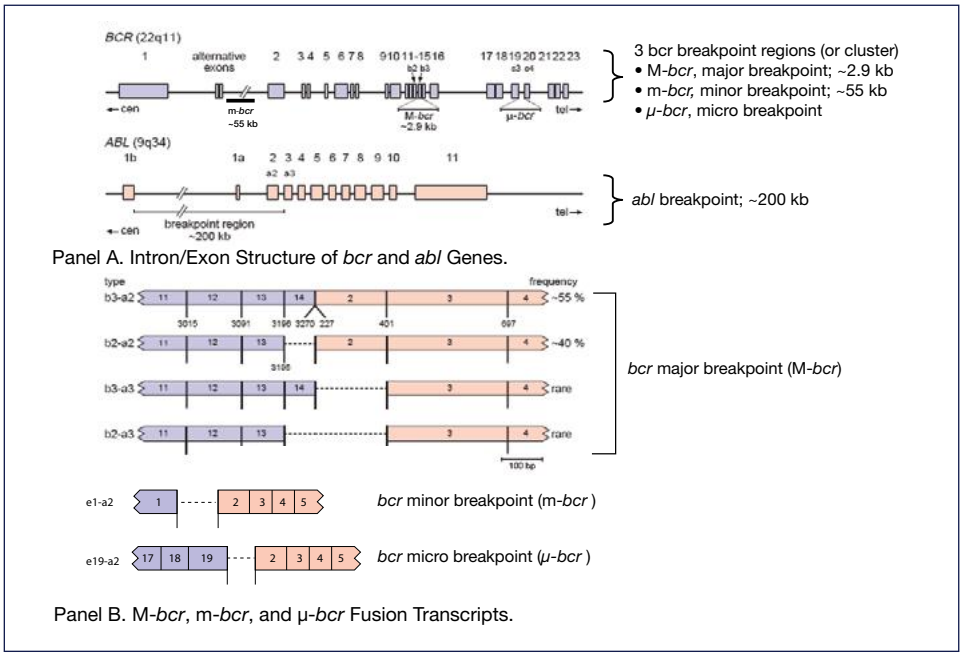


Figure 1. *bcr-abl* Chromosomal Breakpoints and Fusion Gene Transcripts. (A) Schematic diagram of the exon/intron structure of the *bcr* and *abl* genes involved in t(9;22) (q34;q11). The centromere (cen) and telomere (tel) orientation, exon numbering, and relevant breakpoint regions are indicated, including the micro breakpoint cluster region (µ-*bcr*). **(B)** Schematic diagram of *bcr-abl* major (M), minor (m), and micro (µ) transcripts. For M-*bcr*, the b3-a2 and b2-a2 transcripts are found most frequently, but sporadic cases with b3-a3 and b2-a3 transcripts have been reported. (Parts of this figure are used with permission from *Leukemia*.)

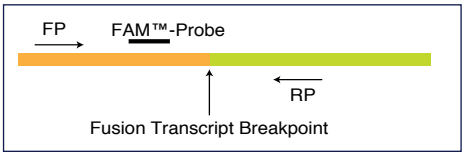


Figure 2. Design for TaqMan® Gene Expression Assays for Fusion Transcripts. Design of primer and probe binding locations [2]. Approximately 10 bp surrounding the breakpoint were masked to avoid designing the probe across this region, since the precise sequence around the breakpoint can be ambiguous. Thus, probes did not span transcript breakpoints. FP = forward primer; RP = reverse primer.

M-bcr and *m-bcr* Analysis

M-*bcr* and m-*bcr* fusion transcript quantification results were generated using the Ipsogen plasmid vector and the pCR2.1 plasmid vector (this second vector has only been used for M-bcr). A statistical study demonstrated that quantitative results obtained for M-*bcr* and m-*bcr* fusion transcripts with TaqMan® Gene Expression Assays for Fusion Transcripts were identical to those obtained with EAC assays for all 20 samples analyzed, independent of the plasmid vector used (Ipsogen or pCR2.1 plasmid vector) (Figure 4 and Figure 5).

To check the sensitivity of TaqMan® Gene Expression Assays compared with the EAC assays, samples amplified with TaqMan® Gene Expression Assays were reanalyzed using a fixed threshold value of 0.1. For M-*bcr*, the Ct values obtained with TaqMan® Gene Expression Assays were 0.49 to 1.97 lower (mean: 0.85) than Ct values obtained using EAC assays (Figure 6, Panel A). For m-*bcr*, the Ct values obtained with TaqMan® Gene Expression Assays were 1.76 to 2.53 (mean: 2.22) lower than Ct values obtained using the EAC assays (Figure 6, Panel B). This analysis demonstrates the greater sensitivity of TaqMan® Gene Expression Assays versus the EAC assays. Besides, quantitative results obtained with the fixed threshold value for both M-*bcr* and m-*bcr* translocations were almost identical to those obtained with the 7900HT Fast Real-Time PCR System's Automatic Analysis. The greater sensitivity achieved with TaqMan® Gene Expression Assays has also made possible the detection of the m-*bcr* fusion transcript in a sample that previously could not be amplified using the EAC probe and primers (data not shown).

Analysis of *abl* Expression

The expression levels of the abl endogenous control obtained with TaqMan® Assay Hs99999002_mH were higher than those obtained using the EAC designs in all 20 samples (on average 1.7 times higher). Samples were analyzed independently with the Ipsogen and the pCR2.1 plasmid vector (Figure 7).

Technical Details for Fusion Assays

Samples. Peripheral blood samples were a subset of routine samples collected by the Hematopathology Unit of the hospital, and were kept anonymous. Informed consent was obtained in accordance with the Institutional Ethics Committee of the Hospital Clinic (Barcelona, Spain) and the Helsinki declaration.

RNA and cDNA Preparation. Dra Colomer and colleagues used 10 human samples each for analysis of M-*bcr* and m-*bcr* expression. Leukocytes from BCR-ABL–positive peripheral blood samples were isolated by 2% dextran sedimentation. Total RNA was extracted from the leukocytes using TRIzol® Reagent (Invitrogen) following the manufacturer's instructions. Total RNA (1 µg; quantified by Nanodrop technology) was reverse transcribed into cDNA (50 µL reactions) using random primers and M-MLV reverse transcriptase (Invitrogen), following the protocol published by the EAC Consortium [2] (Table 2).

Fusion Transcript Assays. M-*brc* (b2-a2, b3-a2; TaqMan® Gene Expression Assay Hs030024541_ft) and m-*bcr* (e1-a2; TaqMan® Gene Expression Assay Hs03024844_ft) fusion transcript quantification was performed in two different reactions using both TaqMan® Gene Expression Assays and the EAC fusion transcript primers and probes. The Abelson (ABL) TaqMan® Gene Expression Assay (Hs99999002_mH) and EAC *abl* primers and probes were used together to amplify the endogenous control.

Quantitative real-time PCR (qPCR) for *bcr-abl* and *abl* control transcripts was performed in duplicate on the Applied Biosystems® 7900HT Fast Real-Time PCR System using standard run conditions. Reactions (25 µL) included TaqMan® Gene Expression Master Mix (Applied Biosystems) and cDNA (2 µL). A known positive and negative control were amplified for each assay.

Table 2. EAC Reverse Transcription Protocol.

Incubate 1 µg total RNA in 10 µL H_2O at 70°C for 10 min
Cool on ice and add other reagents to a final volume of 20 µL
Reverse transcriptase (either M-MLV or SuperScript® I or II): 100 U
RT buffer (according to the RTase used)
dNTP: 1 mM
DTT: 10 mM
Random hexamers: 25 µM
RNase inhibitor: 20 U
Incubate subsequently at:
Room temperature for 10 min
42ºC for 45 min
99ºC for 3 min
Place the sample at 4ºC
Dilute the final cDNA with 30 µL of H_2O

Data Analysis. For analysis of both the M-*bcr* breakpoint and *abl* endogenous control gene data, standard curves were created using the Ipsogen plasmid vector (Ipsogen) and/or the pCR2.1 T0P0® + *bcr-abl* plasmid vector [3]. For analysis of the m-*bcr* breakpoint data, standard curves were created using the Ipsogen plasmid vector. Detection of the rare M-*bcr* b3-a3 transcript was tested with a TaqMan® Gene Expression Assay (Hs 03043652_ft) on M-*bcr* b3-a3 positive samples using the protocol described above. No plasmid was available for this last fusion transcript.

Analysis of Rare Fusion Transcript Forms

A TaqMan® Gene Expression Assay (Hs03024652_ft) was able to detect the rare M-*bcr* b3-a3 transcript in human M-*bcr* b3-a3 positive samples. Figure 8 shows a time course of b3-a3 M-*bcr* transcript expression in the positive samples taken at time points out to one year, when the rare transcript was not longer detected. EAC designs were not available for detection of this transcript.

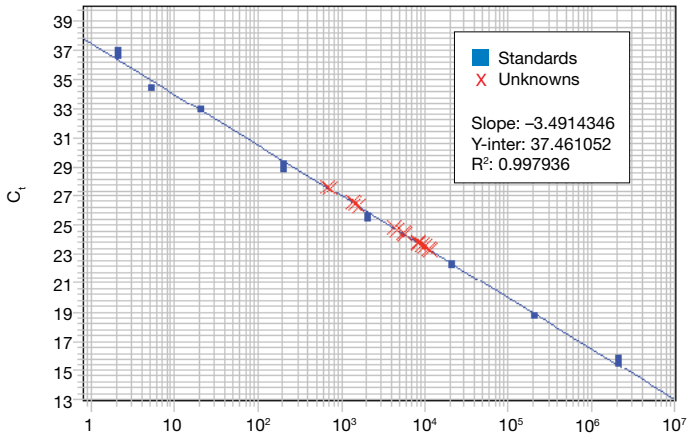
Ready-to-Use TaqMan® Assays Provide Many Advantages for Detecting Fusion Transcripts

Applied Biosystems has recently released a novel set of 165 TaqMan® Gene Expression Assays for quantitation of human fusion

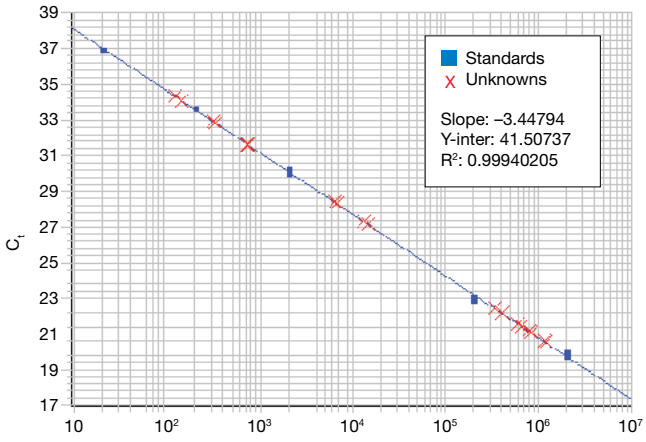
transcripts. These assays were developed with Applied Biosystems' validated bioinformatics pipeline used to design the 1.1 million TaqMan® Gene Expression Assays currently available. As with other TaqMan® Gene Expression Assays, the fusion transcript assays undergo a synthesis quality control test using mass spectrometry to verify primer and probe sequence and concentration.

In this study, Dra Colomer and colleagues use TaqMan® Gene Expression Assays for M-*bcr* (Hs030024541_ft) and m-*bcr* (Hs03024844_ft) to detect and quantify bcr-abl fusion transcripts. The TaqMan® Assays were able to detect M-*bcr* and m-*bcr* transcripts in the same samples as EAC probe and primers designed to these targets. However, the TaqMan® Gene Expression Assays provided more sensitivity, yielding transcript amplification in samples not amplifiable with the EAC designs. In addition, the single-tube 20X format of TaqMan® Gene Expression Assays and associated Applied Biosystems® reagents made the assays easier to process, and saved valuable time. "When used with the EAC program's standardized protocol, these TaqMan® Gene Expression Assays would help eliminate much of the variability seen across different laboratories due to individual primer and probe preparation protocols. Having standardized reverse transcription quantitative PCR (RT-qPCR) assays would harmonize the current technology for detecting *bcr-abl* transcripts, saving time and providing more reproducibility in results," notes Dra Colomer.

Slightly better results have been achieved using the automatic threshold software on the 7900HT Fast Real-Time PCR System to analyze results obtained with TaqMan® Gene Expression Assays. However, as it is recommended to use a fixed threshold value with the EAC primer and probe designs (threshold = 0.1), the data for all samples was reanalyzed using this fixed threshold. Under these conditions, TaqMan® Gene Expression Assays were shown to be more sensitive, allowing the amplification of a sample that was negative using EAC primer and probe designs.

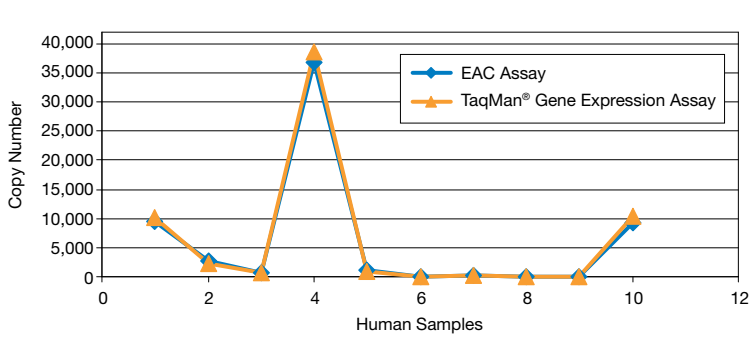


Panel A. M-*bcr* (Hs 030024541_ft) Standard Curve With pCR2.1 Plasmid Vector.

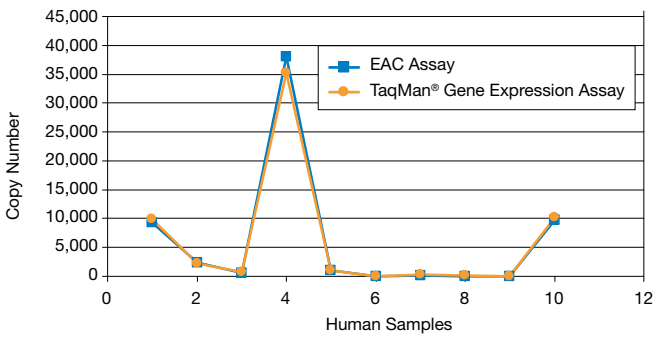


Panel B. m-*bcr* (Hs 03024844_ft) Standard Curve With Ipsogen Plasmid Vector.

Figure 3. Standard Curves Obtained Using TaqMan® Gene Expression Assays. (A) Two replicates of each of eight dilutions (2×10^6, 2×10^5, 2×10^4, 2×10^3, 2×10^2, 2×10, 5, and 2 copies/µL) of the M-*bcr* breakpoint cloned into the pCR2.1 plasmid vector were amplified, using 2 µL of each dilution. **(B)** Two replicates of each of five dilutions (2×10^6, 2×10^5, 2×10^4, 2×10^3, and 2×10 copies/µL) of the m-*bcr* breakpoint cloned into the Ipsogen plasmid vector were amplified using 2 µL of each dilution.



Panel A. M-*bcr* samples analyzed with pCR2.1 plasmid vector.



Panel B. M-*bcr* samples analyzed with Ipsogen plasmid vector.

Figure 4. M-*bcr* Copy Number Using TaqMan® Gene Expression Assays vs. EAC Assay. Copy number analysis was performed using TaqMan® Gene Expression Assay (Hs 030024541_ft) and the comparable EAC assay with either **(A)** pCR2.1 plasmid vector or **(B)** Ipsogen plasmid vector. The TaqMan® Gene Expression Assay gave the same results as the EAC assay in both cases.

The TaqMan® Assays were also able to successfully detect the rare M-*bcr* transcript b3-a3 and the µ-*bcr* transcript e19-a2 in human samples (data not shown).

It is especially important to note that these particular assays provide new tools to researchers, since there are no comparable EAC probe and primer designs for these specific translocations.

Applied Biosystems provides researchers a standardized, easy-to-use workflow to quantify the different bcr-abl fusion transcripts. This workflow makes it possible to obtain rapid and reproducible results within and between laboratories.

Acknowledgments

We acknowledge Beatriz Cabot, Applied Biosystems Senior Field Application Specialist, Molecular Biology, and Marisa Checa, Applied Biosystems Field Application Specialist, Molecular Biology, for their generous contribution in this collaboration.

References

1. p190 primers and probe: ENF401, ENR561, ENF541; p210 primers and probe: ENF501, ENR561, ENP541.

2. Gabert J, Beillard E, van der Velden VH et al. (2003) Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—A Europe Against Cancer Program. *Leukemia* 17:2318–2357.

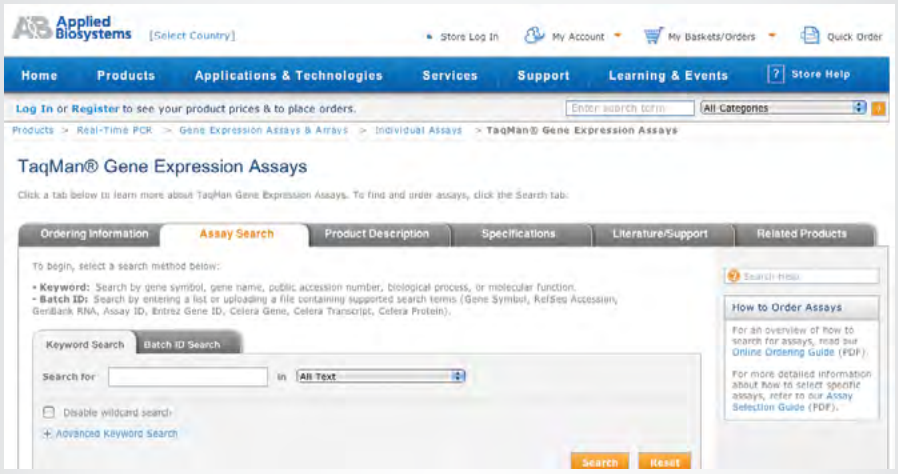
3. Beillard E, Pallisgaard N, Bi W et al. (2003) Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR)—A Europe Against Cancer Program. *Leukemia* 17:2474–2486.

4. Hughes T, Deininger M, Hochhaus A , et. al. (2006) *Blood* (ASH Annual Meeting Abstracts),108:28–371.

5. Melo JV (1996) The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. *Blood* 88:2375–2384.

Simplifying Fusion Transcript Detection and Quantitation With Better Tools TaqMan® Gene Expression Assays for Fusion Transcripts

TaqMan® Gene Expression Assay products enable researchers to conduct fusion studies quickly and easily by eliminating the time-consuming processes involved in assay development. As all of the assays are ready to use (primer and probes formulated in single-tube, 20X mix), it is easier to set up the reaction and to compare results with other researchers and labs directly and accurately (see Table 3). See Tables 3 and 4 and page 8 for ordering information.



You can search online for the assay you need at **info.appliedbiosystems.com/gexassays.**

Table 3. *bcr-abl* and Endogenous Control TaqMan® Gene Expression Assays.

bcr-abl TaqMan® Gene Expression Assays		
Assay ID	**Transcript**	**Assay Accession No.**
Hs03024541_ft	b2-a2	AJ131467.1
Hs03024541_ft	b3-a2	AJ131466.1
Hs03024844_ft	e1-a2	AF113911.1
Hs03205538_ft	e19-a2	AM491363.1
Hs03043652_ft	b3-a3	AM491360.1
Hs03043652_ft	b3-a2	AJ131466.1
Hs03024646_ft	b2-a3	AY043457.1
Hs03024646_ft	b3-a3	AM491360.1
Hs03024646_ft	b2-a2	AJ131467.1
Endogenous Control TaqMan® Gene Expression Assay		
Assay ID	Assay Accession No.	
Abelson (ABL)	Hs 99999002_mH	

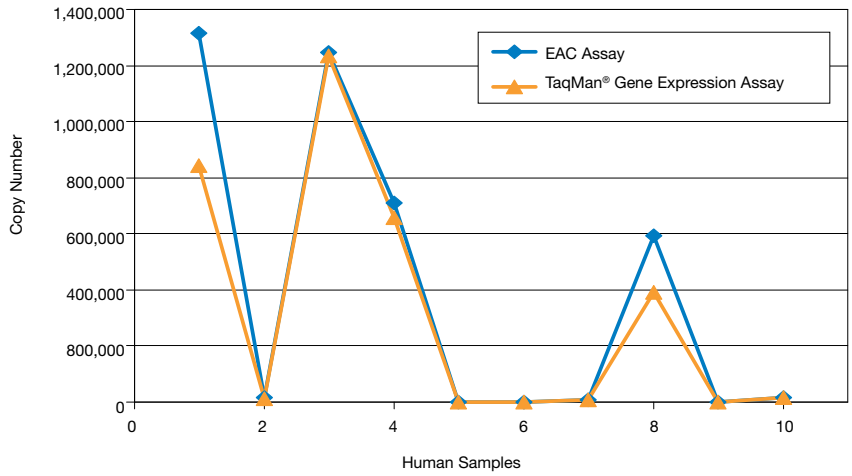
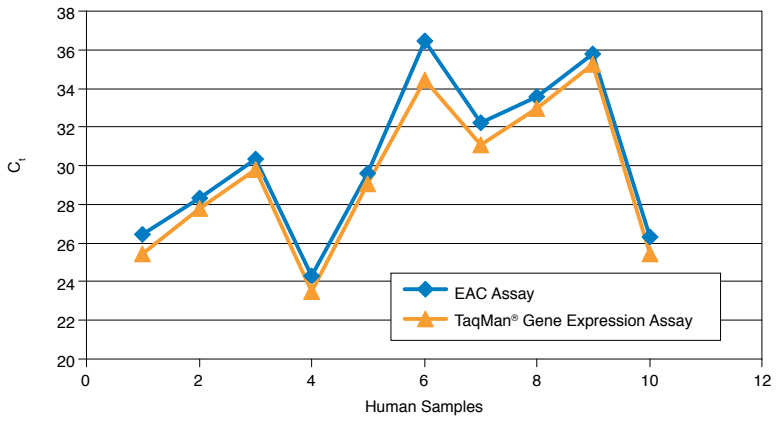
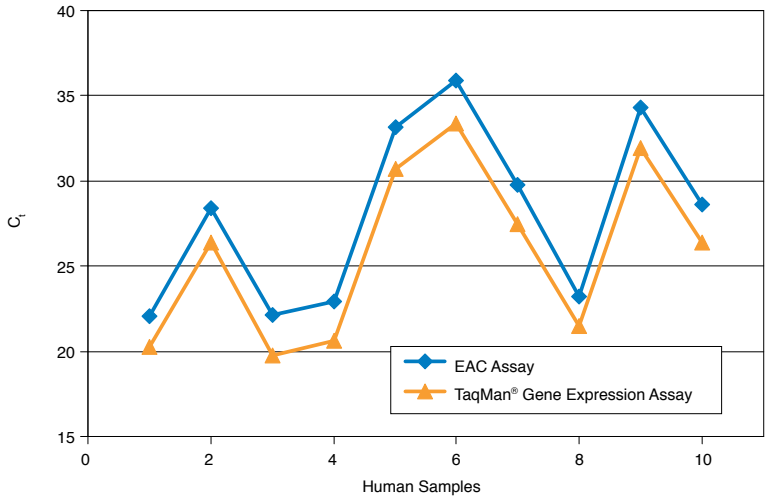


Figure 5. m-*bcr* Copy Number Using TaqMan® Gene Expression Assay vs. EAC Assay. Copy number analysis was performed using TaqMan® Gene Expression Assay Hs03024844_ft and the comparable EAC assay with the Ipsogen plasmid vector. The TaqMan® Gene Expression Assay gave the same results as the EAC assay.



Panel A. M-*bcr* Samples.



Panel B. m-*bcr* Samples.

Figure 6. C_t Values for M-*bcr* Using TaqMan® Gene Expression Assays vs. EAC Assays. Reanalysis of M-*bcr* samples (**A:** TaqMan® Gene Expression Assay Hs 030024541_ft and comparable EAC assay using a fixed 0.1 threshold value) and of m-*bcr* samples (**B:** TaqMan® Gene Expression Assay Hs03024844_ft and comparable EAC assay). The TaqMan® Gene Expression Assays were consistently more sensitive than the EAC assays.

Applied Biosystems® TaqMan® Gene Expression Master Mix

The TaqMan® Gene Expression Assay workflow can be further simplified by incorporating Applied Biosystems® TaqMan® Gene Expression Master Mix. The Gene Expression Master Mix comes concentrated with all needed reagents premixed, decreasing hands-on time for dilution, mixing, and pipetting. TaqMan® Gene Expression Master Mix delivers sensitive and specific detection across a broad range of template quantities, down to a single copy of target. For ease of use, TaqMan® Gene Expression Master Mix uses universal thermal cycling conditions and users can set up the reaction at room temperature. See page 8 for ordering information.

LeukoLOCK™ Total RNA Isolation System

The LeukoLOCK™ Total RNA Isolation System is an innovative method for cellular fractionation of whole blood, and total RNA stabilization and extraction from the leukocyte population. It has been optimized for use with human blood. Blood is a storehouse of cellular information; however, the presence of globin mRNA in RNA prepared from whole blood can interfere with downstream expression profiling applications. The LeukoLOCK™ System employs filter-based leukocyte-depletion technology to isolate leukocytes from whole blood, and Ambion® RNA*later*® to stabilize the cells on the filter. By excluding red blood cells, the RNA that is purified from captured leukocytes is inherently depleted of globin mRNA, which improves sample utility for expression profiling and other applications. See page 8 for ordering information.

High Capacity cDNA Reverse Transcription Kit

The High Capacity cDNA Reverse Transcription Kit delivers extremely high-quality, single-stranded cDNA from total RNA. It contains all components necessary for the quantitative conversion of 0.02 to 2 µg total RNA in a single 20 µL reaction to single-stranded cDNA. Downstream applications include real-time PCR, standard PCR, and microarrays. See page 8 for ordering information.

Custom Plating Service

The TaqMan® Custom Plating Service offers the convenience of pre-plated TaqMan® Gene Expression Assays, Custom Assays, and Custom Probe/Primer Sets in 96- or 384-well plates. Set up custom configurations using TaqMan® Gene Expression Assays (Inventoried, Made-to-Order, and Custom) and Custom TaqMan® Probes and Primers. You can select from a variety of reaction volumes and receive assays in dried or liquid formulation.

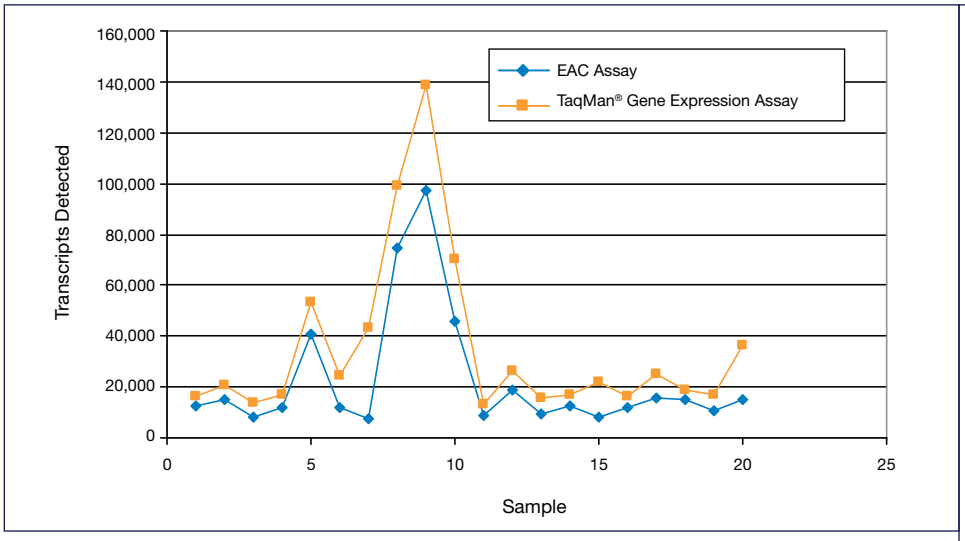


Figure 7. Better Detection of *abl* Expression Levels Using TaqMan® Gene Expression Assays. Samples (20) were amplified using probe and primer designs for the abl endogenous control transcript. Higher *abl* expression levels were detected with the TaqMan® Gene Expression Assay Hs99999002_mH than with the EAC designs (pCR2.1 plasmid vector). The same data was obtained using the Ipsogen plasmid (data not shown).

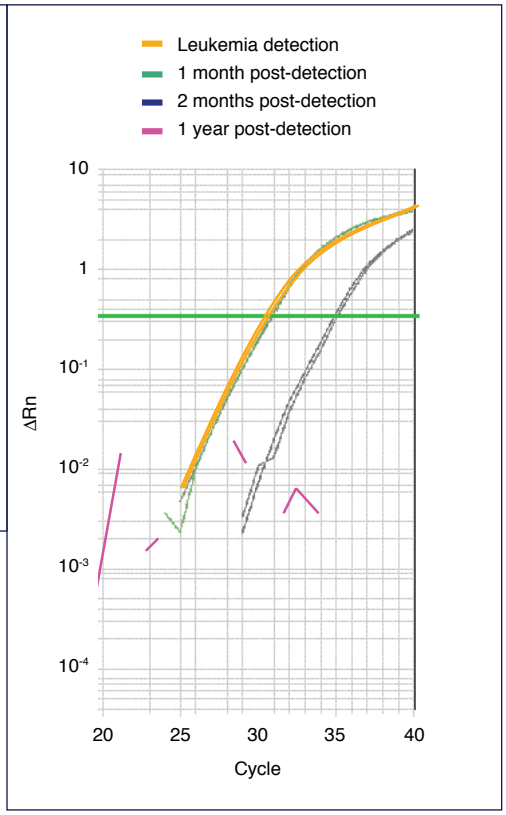


Figure 8. TaqMan® Assays Make Possible Detection of Rare M-*bcr* Transcript. Detection of the rare b3-a3 M-*bcr* translocation in a positive sample, over a time course out to 1 year. Amplifications were performed using the TaqMan® Gene Expression Assay Hs03024652_ft.

Ordering Information

Description	Size	Part Number
LeukoLOCK™ Total RNA Isolation System	20 rxn*	AM1923
High Capacity cDNA Reverse Transcription Kit	200 rxn*	4368814
TaqMan® Gene Expression Assays for Fusion Transcripts		
Inventoried	250 rxn	4331182†
Made-to-Order	360 rxn	4351372
TaqMan® Gene Expression Master Mix, 1 Mini-Pack (1 x 1 mL)	40 rxn*	4370048
TaqMan® Universal PCR Master Mix, 1-Pack (1 x 5 mL)	200 rxn*	4304437
7900HT Fast Real-Time PCR System with Standard 96-well Block Module	1 instrument	4329003

*Available in other sizes or in bundles.

†See list below for specific assays.

TaqMan® Gene Expression Assays for *bcr-abl* Fusion Transcripts

Assay ID	Transcript	Assay Accession No.
Hs03024541_ft	b2-a2	AJ131467.1
Hs03024541_ft	b3-a2	AJ131466.1
Hs03024844_ft	e1-a2	AF113911.1
Hs03205538_ft	e19-a2	AM491363.1
Hs03043652_ft	b3-a3	AM491360.1
Hs03043652_ft	b3-a2	AJ131466.1

Endogenous Control TaqMan® Gene Expression Assay

Assay ID	Assay Accession No.
Abelson (ABL)	Hs 99999002_mH

ThermoFisher SCIENTIFIC

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ThermoFisher SCIENTIFIC
The world leader in serving science

CERTIFICATE OF ANALYSIS

K0721 GeneJET Genomic DNA Purification Kit

Packaging Lot: **3170594**
Expiry Date: 31.12.2027 (DD.MM.YYYY)
Storage: at 5±3°C
Note: IMPORTANT Check Individual Components for Storage Conditions

Filling lots for components in package:

Lot	Quantity	Description
3158541	1.2 mL	Proteinase K Solution, 20mg/ml
3156100	1 mL	RNase A Solution, 10 mg/ml
3157968	30 mL	Elution Buffer
3113212	11 mL	Digestion Solution
3090483	24 mL	Lysis Solution
3152821	10 mL	Wash Solution I (concentrated)
3149595	10 mL	Wash Solution II (concentrated)
3168856	1 pack	Collection Tubes 2 ml
3129737	1 pack	GeneJET DNA Purification Colum & Collection Tubes

QUALITY CONTROL

Parameter	Method	Requirement	Result
Specific activity (RNase A)	One unit is the amount of the enzyme which produces an increase in soluble reaction products by an OD of 1.0 at A260 nm using yeast RNA as substrate in 15 minutes at 37 °C.	≥ 5000 U/mg	Conforms
Activity (Proteinase K)	The unit activity of a solution of Proteinase K is determined. One unit liberates 1 µmol of Folin-positive amino acids, measured as tyrosine, at 37°C, pH 7.5, using denatured bovine hemoglobin as the substrate.	Within range of predetermined specifications	Conforms
pH (Relevant kit components)	Measured using a pH meter.	Within range of predetermined specifications	Conforms
Density (Relevant kit components)	Measured using a densitometer.	Within range of predetermined specifications	Conforms
Refractive Index (Relevant kit components)	Measured using a refractometer.	Within range of predetermined specifications	Conforms
Conductivity (Relevant kit components)	Measured using a conductometer.	Within range of predetermined specifications	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė 

GeneJET™ Genomic DNA Purification Kit

Catalog Numbers K0721, K0722

Pub. No. MAN0012663 Rev. C00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ GeneJET™ Genomic DNA Purification Kit is designed for rapid and efficient purification of high-quality genomic DNA from various mammalian cell culture and tissue samples, whole blood, bacteria, and yeast. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation.

The standard procedure takes less than 20 minutes following cell lysis and yields purified DNA of more than 30 kb in size. Isolated DNA can be used directly in PCR, Southern blotting and enzymatic reactions.

Technology overview

Depending on the starting material, samples are digested with Proteinase K in either the supplied Digestion or Lysis Solution. RNA is removed by treating the samples with RNase A. The lysate is then mixed with ethanol and loaded on the purification column where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

Table 1 Typical genomic DNA yields from various sources

Source	Amount	DNA yield
Mammalian blood	200 µL	4–6 µg
Mouse heart	10 mg	10–15 µg
Mouse tail	0.5 cm	8–10 µg
Rat liver	10 mg	10–20 µg
Rat spleen	5 mg	20–30 µg
Rat kidney	10 mg	25–30 µg
Rabbit ear	20 mg	5–10 µg
Bacillus pumilis cells	2×10^9 cells	10–15 µg
Escherichia coli cells	2×10^9 cells	10–15 µg
HeLa cells	2×10^6 cells	15–20 µg
Jurkat cells	5×10^6 cells	25–30 µg
Saccharomyces cerevisiae cells	1×10^8 cells	3–5 µg

Contents and storage

IMPORTANT! Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

Item	Cat. No. K0721 (50 reactions)	Cat. No. K0722 (250 reactions)	Storage
Proteinase K Solution	1.2 mL	5 × 1.2 mL	• Upon receipt, store the unopened vial at 15–25°C. • After use, store the vial at –20°C.
RNase A Solution	1.2 mL	5 × 1 mL	
Digestion Buffer	11 mL	55 mL	15–25°C
Lysis Solution	24 mL	2 × 60 mL	
Wash Buffer 1 (concentrated)	10 mL	40 mL	
Wash Buffer 2 (concentrated)	10 mL	40 mL	
Elution Buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA)	30 mL	150 mL	
Collection Tubes (2 mL)	50	250	
GeneJET™ Genomic DNA Purification Columns and Collection Tubes	50	250	Store at 15–25°C for up to 6 months. For longer periods, store at 2–8°C.

Procedural guidelines

IMPORTANT! Wear gloves when handling the Lysis Solution and Wash Buffer I as these reagents contain irritants.

To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples and perform extractions from fresh material or material that has been immediately frozen and stored at -20°C or -70°C.

Before the first use of the kit

1. Add the indicated volume of ethanol (96-100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) prior to first use:

–	Cat. no. K0721 (50 reactions)		Cat. no. K0722 (250 reactions	
–	Wash Buffer I	Wash Buffer II	Wash Buffer I	Wash Buffer II
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96–100%)	30 mL	30 mL	120 mL	120 mL
Total Volume	40 mL	40 mL	160 mL	160 mL

2. After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

Before each use

Check the Digestion Solution and Lysis Solution for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.

Prepare buffers

Prepare buffers as described in the following table.

Buffer	Components
Mammalian cell lysate preparation	• PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, pH 7.4) • TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
Gram-positive bacteria lysate preparation	• Gram-positive bacteria lysis buffer (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100) • Add lysozyme to 20 mg/mL immediately before use
Yeast lysate preparation	Yeast lysis buffer (5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA)

Purify genomic DNA from mammalian tissue or rodent tails

1. Grind up to 20 mg of mammalian tissue, 10 mg of spleen tissue, 0.6 cm of rat tail clip, or 0.5 cm mouse tail clip in liquid nitrogen using a mortar and pestle.

   Note: Alternatively, cut the tissue into small pieces or disrupt it using a homogenizer.

2. Collect the tissue into a 1.5 mL microcentrifuge tube then resuspend in 180 µL of Digestion Solution.

3. Add 20 µL of Proteinase K Solution then mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

4. Incubate the sample at 56°C until the tissue is completely lysed and no particles remain. During the incubation, vortex the vial occasionally or use a shaking water bath, rocking platform, or thermomixer.

   Table 2 Recommended incubation times

Quantity	Recommended incubation time
5 mg of tissue (except spleen)	1 hour
10 mg of tissue (except spleen)	2 hours
20 mg of tissue (except spleen)	3 hours
5 mg of spleen tissue	2 hours
10 mg of spleen tissue	3 hours
Mouse tail (0.5 cm), rat tail (0.6 cm)	6 hours

   Note: Lysis time varies on the type and amount of tissue processed. In some cases, the incubation time should be prolonged to 6–8 hours or overnight (for rodent tail) until complete lysis occurs.

5. Add 20 µL of RNase A Solution, then mix by vortexing. Incubate at room temperature for 10 minutes.

6. Add 200 µL of Lysis Solution. Mix thoroughly by vortexing for 15 seconds until a homogeneous mixture is obtained.

7. Add 400 µL of 50% ethanol, then mix by pipetting or vortexing.

8. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution.

9. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube.

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

10. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through and place the purification column back into the collection tube.

11. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

12. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again for 1 minute at ≥12,000 × *g*.

13. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

14. To elute genomic DNA, add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane. Incubate at room temperature for 2 minutes then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, <5 mg of tissue) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

15. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from cultured mammalian cells

1. Collect suspension cells or adherent cells using the following methods:

    - **Suspension cells**: Collect up to 5 × 10^6 cells in a centrifuge tube. Pellet cells by centrifugation at 250 × *g* for 5 minutes. Discard the supernatant. Rinse cells once with PBS to remove remaining medium, then repeat the centrifugation step. Discard the supernatant.
    - **Adherent cells**: Remove the growth medium from a culture plate containing up to 2 × 10^6 cells. Rinse cells once with PBS to remove residual medium. Discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells to a microcentrifuge tube then pellet them by centrifugation at 250 × *g* for 5 minutes. Discard supernatant.

2. Resuspend the collected cells in 200 µL of TE buffer or PBS.

3. Add 200 µL of Lysis Solution and 20 µL of Proteinase K Solution to the cell pellet. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

4. Incubate the sample at 56°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

5. Add 20 µL of RNase A Solution, then mix by vortexing. Incubate the mixture at room temperature for 10 minutes.

6. Add 400 µL of 50% ethanol, then mix by pipetting or vortexing.

7. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

8. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

    Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

9. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

10. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

11. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

12. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

13. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes, then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

14. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from mammalian blood

1. Add 400 µL of Lysis Solution and 20 µL of Proteinase K Solution to 200 µL of whole blood. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

2. Incubate the sample at 56°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

3. Add 200 µL of ethanol (96–100%), then mix by pipetting or vortexing.

4. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

5. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

6. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

7. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

8. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

9. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

10. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes, then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

11. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from gram-negative bacteria

1. Harvest up to 2 × 10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation at 5,000 × *g* for 10 minutes. Discard the supernatant.

2. Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution, then mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

3. Incubate the sample at 56°C for 30 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

4. Add 20 µL of RNase A Solution. Mix by vortexing, then incubate the mixture at room temperature for 10 minutes.

5. Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 seconds until a homogeneous mixture is obtained.

6. Add 400 µL of 50% ethanol then mix by pipetting or vortexing.

7. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

8. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

9. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

10. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

11. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

12. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

13. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

14. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from gram-positive bacteria

Prepare Gram-positive bacteria lysis buffer with 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100. Add lysozyme to 20 mg/mL immediately before use.

1. Harvest up to 2 × 10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation at 5,000 × *g* for 10 minutes. Discard the supernatant.

2. Resuspend the pellet in 180 µL of gram-positive bacteria lysis buffer. Incubate at 37°C for 30 minutes.

3. Add 200 µL of Lysis Solution and 20 µL of Proteinase K. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

4. Incubate the sample at 56°C for 30 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

5. Add 20 µL of RNase A Solution. Mix by vortexing then incubate the mixture at room temperature for 10 minutes.

6. Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 seconds until a homogeneous mixture is obtained.

7. Add 400 µL of 50% ethanol then mix by pipetting or vortexing.

8. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

9. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

10. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

11. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

12. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

13. Discard the collection tube containing the flow-through solution, then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

14. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

15. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from yeast

1. Harvest up to 1 × 10^8 yeast cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation at ≥12,000 × *g* for 5–10 seconds. Discard the supernatant.

2. Resuspend the pellet in 500 µL of Yeast lysis buffer. Incubate at 37°C for 1 hour.

3. Centrifuge cells at 3,000 × *g* for 10 minutes. Discard the supernatant.

4. Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution then mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

5. Incubate the sample at 56°C for 45 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

6. Add 20 µL of RNase A Solution. Mix by vortexing then incubate the mixture at room temperature for 10 minutes.

7. Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 seconds until a homogeneous mixture is obtained.

8. Add 400 µL of 50% ethanol then mix by pipetting or vortexing.

9. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

10. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

    Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

11. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

12. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

13. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

14. Discard the collection tube containing the flow-through solution, then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

15. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

16. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from buccal swabs

1. To collect a sample, scrape the swab 5–6 times against the inside cheek.
2. Swirl the swab for 30–60 seconds in 200 µL of 1 × PBS.
3. Add 400 µL of Lysis Solution and 20 µL of Proteinase K Solution to 200 µL of whole blood. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
4. Incubate the sample at 56°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.
5. Add 200 µL of ethanol (96–100%), then mix by pipetting or vortexing.
6. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.
7. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.
8. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
9. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12000 × *g* for 3 minutes.
10. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12000 × *g* for 1 minute.
11. Discard the collection tube containing the flow-through solution, then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.
12. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes, then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g. ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be reduced to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.
13. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Troubleshooting

Observation	Possible cause	Recommended action
Low yield of purified DNA	Excess sample was used during lysate preparation.	Reduce the amount of starting material. Do not use more blood than indicated in lysis protocols.
	The starting material was not completely digested.	Extend the Proteinase K digestion at 56°C until complete lysis occurs and no particles remain visible in solution.
	Ethanol was not added to the lysate.	Ensure that ethanol is added to the lysate before applying the sample to the Purification Column.
	Ethanol was not mixed with the lysate.	After the addition of ethanol to the lysate, mix the sample by vortexing or pipetting.
	Ethanol was not added to Wash Buffers.	Ensure that ethanol is added to Wash Buffer WB I and Wash Buffer II before use. See “Before the first use of the kit” on page 2.

Observation	Possible cause	Recommended action
Low yield of purified DNA *(continued)*	Columns stored at room temperature (15–25°C) for longer than 6 months.	Extended room temperature storage (15–25°C) for columns beyond 6 months may lead to membrane drying and reduced DNA yield. For prolonged storage exceeding 6 months, it is recommended to store columns at 2–8°C. See "Contents and storage" on page 2.
Purified DNA is degraded	Sample was frozen and thawed repeatedly.	Avoid repeated sample freeze/thaw cycles. Use a fresh sample for DNA isolation. Perform extractions from fresh material when possible.
	Inappropriate sample storage conditions.	Store mammalian tissues at -70°C and bacteria at -20°C until use. Whole blood can be stored at 4°C for no longer than 1–2 days. For long term storage blood samples should be aliquoted in 200 µL portions and stored at -20°C.
RNA contamination	RNase A treatment was not carried out.	Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	Excess sample was used during lysate preparation.	Reduce the amount of starting material. A maximum of 2×10^9 of bacteria cells, 5×10^6 of suspension cells and 20 mg of mammalian tissue is recommended for lysate preparation.
	Tissue was not completely digested.	Extend the Proteinase K digestion at 56°C until complete lysis occurs and no particles remain.
Inhibition of downstream enzymatic reactions	Purified DNA contains residual ethanol.	If residual solution is seen in the purification column after washing the column with Wash Buffer II, empty the collection tube then re-spin the column at ≥12,000 × *g* for 1 minute.
	Purified DNA contains residual salt.	Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer I first and then proceed to washing with Wash Buffer II.

Documentation and support

Customer and technical support

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0012633 C00

Revision	Date	Description
C00	17 April 2024	• The document was updated to the current template, with associated updates to the warranty, trademarks, and logos. • The storage conditions for columns and collection tubes were updated.
B.0	7 November 2016	Updated information in manual.
A.0	17 October 2015	New document for the GeneJET™ Genomic DNA Purification Kit.

The information in this guide is subject to change without notice.

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CERTIFICATE OF ANALYSIS

K0881 GeneJET FFPE DNA Purification Kit

Packaging Lot: **3168933**
Expiry Date: 31.12.2027 (DD.MM.YYYY)
Storage: at 5±3°C
Note: IMPORTANT Check Individual Components for Storage Conditions

Filling lots for components in package:

Lot	Quantity	Description
3122950	1.2 mL	Proteinase K Solution, 20mg/ml
3082068	0.7 mL	RNase A Solution, 10mg/ml
3072450	10 mL	Elution Buffer
3166614	11 mL	Digestion Buffer
3091152	11 mL	Lysis Solution
3058148	10 mL	Wash Buffer I (concentrated)
3070145	10 mL	Wash Buffer II (concentrated)
3129737	1 pack	GeneJET DNA Purification Colum & Collection Tubes
3132300	1 pack	Collection Tubes 2 ml

QUALITY CONTROL

Parameter	Method	Requirement	Result
Specific activity (RNase A)	One unit is the amount of the enzyme which produces an increase in soluble reaction products by an OD of 1.0 at A260 nm using yeast RNA as substrate in 15 minutes at 37 °C.	≥ 5000 U/mg	Conforms
Activity (Proteinase K)	The unit activity of a solution of Proteinase K is determined. One unit liberates 1 µmol of Folin-positive amino acids, measured as tyrosine, at 37°C, pH 7.5, using denatured bovine hemoglobin as the substrate.	Within range of predetermined specifications	Conforms
pH (Relevant kit components)	Measured using a pH meter.	Within range of predetermined specifications	Conforms
Density (Relevant kit components)	Measured using a densitometer.	Within range of predetermined specifications	Conforms
Refractive Index (Relevant kit components)	Measured using a refractometer.	Within range of predetermined specifications	Conforms
Conductivity (Relevant kit components)	Measured using a conductometer.	Within range of predetermined specifications	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė 

GeneJET™ FFPE DNA Purification Kit

Catalog Numbers K0881, K0882

Pub. No. MAN0012673 Rev. B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ GeneJET™ FFPE DNA Purification Kit is designed for fast and convenient purification of DNA from various amounts of FFPE tissue. Up to 8 sections (10 µm thickness) of FFPE samples can be used for genomic DNA extraction without overnight incubation. Elimination of toxic reagents commonly used for deparaffinization allows an environmentally-friendly procedure. Each preparation recovers up to 8 µg of genomic DNA from one section that can be eluted in 20 µL to 80 µL of Elution Buffer. High quality eluted DNA can be directly used in downstream applications such as qPCR, PCR, NGS library preparation, or stored at -20 °C.

Technology overview

Sections of FFPE samples are subjected to enzymatic digestion and lysis to liberate genomic DNA. The released DNA is de-crosslinked by heat incubation. Subsequently, the resulting solution is centrifuged and the supernatant containing DNA is mixed with Binding Buffer. After addition of ethanol, the lysate is loaded onto the purification column. The bound DNA is washed to remove contaminants and then eluted with the Elution Buffer.

Contents and storage

IMPORTANT! Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

Table 1 GeneJET™ FFPE DNA Purification Kit

Item	Cat. no. K0881 (50 reactions)	Cat. no. K0882 (250 reactions)	Storage
Proteinase K Solution	1.2 mL	4 × 1.3 mL	• Upon receipt, store the unopened vial at 15–25°C. • After use, store the vial at –20°C.
RNase A Solution	0.7 mL	3 × 1 mL	
Digestion Buffer	11 mL	55 mL	15–25°C
Binding Buffer	11 mL	55 mL	
Wash Buffer 1 (concentrated)	10 mL	40 mL	
Wash Buffer 2 (concentrated)	10 mL	40 mL	
Elution Buffer	10 mL	40 mL	
Collection Tubes (2 mL)	50	250	
GeneJET™ Genomic DNA Purification Columns and Collection Tubes	50	250	Store at 15–25°C for up to 6 months. For longer periods, store at 2–8°C.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Vortex mixer, or equivalent	MLS
Thermo heating-blocks or waterbath (adjustable to 65°C and 90°C)	MLS
Centrifuge capable of ≥12,000 × *g* for 1.5 mL microcentrifuge tubes	MLS
Tubes and other consumables	
Microcentrifuge tubes (1.5 mL) with screw caps	MLS
Disposable gloves	MLS
Ethanol, 96–100% (molecular biology grade)	MLS

Procedural guidelines

IMPORTANT! Wear gloves when handling the Lysis Solution, Wash Buffer I, and Proteinase K Solution as these reagents contain irritants.

- DNA yield and quality from FFPE tissue may vary considerably depending on the tissue source, the thickness of the slice, the age of the sample, post-sampling delay before fixation, fixation time etc.
- Paraffin sections can be stored at or below 4°C for 1 year without observable effects on DNA yield and usability. Longer-term storage of FFPE sections may have negative effect on the DNA due to oxidation.
- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples. For short–term, DNA solution may be stored at 0–4°C, and for long-term at -20°C.

Before first use of the kit

1. Add the indicated volume of ethanol (96–100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) prior to first use:

–	Cat. no. K0881 (50 reactions)		Cat. no. K0882 (250 reactions)	
–	Wash Buffer I	Wash Buffer II	Wash Buffer I	Wash Buffer II
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96–100%)	30 mL	30 mL	120 mL	120 mL
Total Volume	40 mL	40 mL	160 mL	160 mL

2. After ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

Before each use

- Check the Digestion Buffer and Binding Buffer for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, then cool it back down to 25°C before use.
- Set temperature for two thermal heating-blocks or waterbaths, one at 65°C and one at 90°C.

Purify genomic DNA from FFPE samples

This protocol describes how to extract DNA from one to eight sections of FFPE tissue for sections up to 10 µm thick.

1. Add 200 µL of Digestion Buffer to a microcentrifuge tube (with screw cap to prevent evaporation) containing one or more sections (up to eight) of FFPE tissue.
2. Incubate for 3 minutes at 90°C. During the incubation, mix the sample a few times by gently shaking the tube.

   Note: Ensure tissue sections stay submerged in the solution.

3. After incubation, mix thoroughly with a vortex mixer to completely dissolve the paraffin. Cool the sample down to room temperature. If necessary, spin down briefly to clear the lid.

   Note: It is not necessary to cut off the excess paraffin. Use a microcentrifuge tube with a screw cap. Incubation time should be prolonged to 6 min if more than one section of FFPE tissue is used.

4. Add 20 µL of Proteinase K solution then mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Spin down briefly to clear the lid.

5. Incubate the sample at 65°C °C for 50 minutes in a thermoshaker or a water bath with occasional vortexing.

   Note: Lysis time varies on the type and amount of FFPE sample processed. In some cases, incubation time should be prolonged to 2 hours. Yield of DNA typically increases with extended lysis time.

6. Transfer the samples to the heat block set to 90°C then incubate for 40 minutes.

   Note: Prevent samples from being heated above 90°C for a prolonged period of time.

7. Centrifuge incubated samples at 6000 × *g* for 1 minute then transfer 200 µL of the digested lysate to a new 1.5 mL microcentrifuge tube.

   Note: Transfer the entire liquid layer to a new tube leaving behind any wax particulates. Small amounts of debris will not affect the DNA yield. When using eight sections of FFPE tissue (each 10 µm thick), the digested lysate volume is 160–180 µL.

8. Add 10 µL of RNase A solution then mix thoroughly by vortexing. Spin down briefly to clear the lid then incubate at room temperature for 10 minutes.

9. Add 200 µL of Binding Buffer then vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.

10. Add 400 µL of ethanol (96-100%) to the sample then vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.

11. Transfer the lysate to a GeneJET™ DNA Purification Column inserted into collection tube.

12. Centrifuge column at 6000 × *g* for 1 minute. Discard the collection tube with the flow through then place the column in a new collection tube.

13. Add 500 µL of Wash Buffer 1 with ethanol added then centrifuge at 8000 × *g* for 1 minute. Discard the flow-through then place the purification column back into the collection tube.

14. Add 500 µL of Wash Buffer 2 with ethanol added then centrifuge at ≥ 12000 × *g* for 3 minutes.

15. Empty the collection tube then place the purification column back into the collection tube. Re-spin the column for 1 minute at maximum speed to dry the membrane.

16. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

17. Add 60 µL of Elution Buffer directly to the center of the purification column membrane. Leave for 2 minutes at room temperature then centrifuge at 8000 × *g*vfor 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with additional 60 µL of Elution Buffer. Perform the second elution using different tube.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., one section of FFPE sample) the volume of the Elution Buffer added to the column can be reduced to 20 µL.
    - Elution volumes in the range of 20–80 µL are recommended, the default volume is 60 µL.

18. Discard the column. Use the purified DNA immediately in downstream applications or store at -20 °C.

Troubleshooting

Observation	Possible cause	Recommended action
Low yield of purified DNA	Excess sample was used during lysate preparation.	Reduce the amount of starting material. Do not use more blood than indicated in lysis protocols.

Observation	Possible cause	Recommended action
Low yield of purified DNA *(continued)*	The starting material was not completely digested.	If the suspension does not clarify during Proteinase K digestion, this could indicate that it is oxidized. Extend the Proteinase K digestion at 65°C until complete lysis occurs and no particles remain visible in solution.
	Ethanol was not added to the lysate.	Make sure that the ethanol was added to the lysate before applying the sample to the Purification Column.
	Ethanol was not added to the lysate.	After the addition of ethanol to the lysate, mix the sample by vortexing or pipetting.
	Ethanol was not added to Wash Buffers.	Make sure that ethanol was added to Wash Buffer 1 and Wash Buffer 2 before use. See “Before first use of the kit” on page 2.
	Poor sample quality.	Sample fixation, embedding and storage have a significant impact on quality and amount of the DNA in FFPE tissue samples.
	Columns stored at room temperature (15–25°C) for longer than 6 months.	Extended room temperature storage (15–25°C) for columns beyond 6 months may lead to membrane drying and reduced DNA yield. For prolonged storage exceeding 6 months, it is recommended to store columns at 2–8°C. See “Contents and storage” on page 1.
RNA contamination	RNase A treatment was not carried out.	Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	Excess sample was used during lysate preparation.	Too much starting material was used. Overloading may lead to a decrease in DNA yield.
	Tissue was not completely digested.	Insufficient disruption and / or homogenization of starting material.Extend the Proteinase K digestion at 65 °C until complete lysis occurs and no particles remain.
Inhibition of downstream enzymatic reactions	Purified DNA contains residual ethanol.	Do not let the flow-through touch the column outlet after the second wash with Wash Buffer 2. Always re-spin the column for an additional 1 minute at maximum speed (≥ 12000 × *g*) after the second wash.
	Purified DNA contains residual salt.	Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer 1 first and then proceed to wash with Wash Buffer 2.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

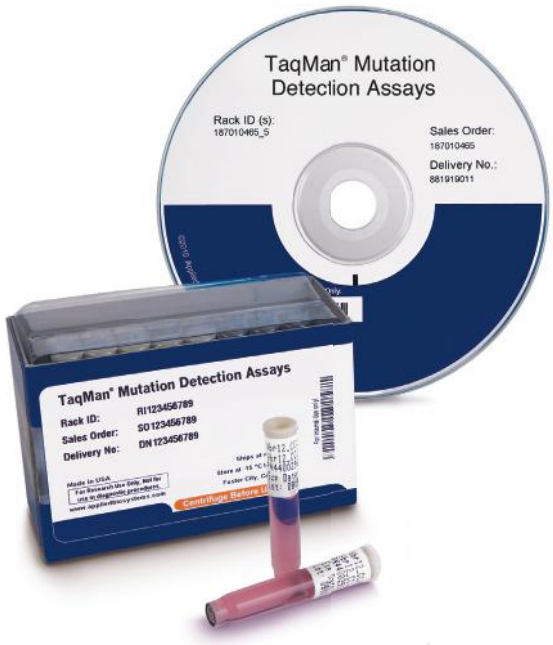
Revision history: Pub. No. MAN0012673 B

Revision	Date	Description
B	10 May 2024	• The document was updated to the current template, with associated updates to the warranty, trademarks, and logos. • The storage conditions for columns and collection tubes were updated. • The version format was changed in conformance with internal document control procedures.
1.0	28 February 2014	New document for the GeneJET™ FFPE DNA Purification Kit.

Accurate and sensitive somatic mutation detection powered by castPCR™ technology

TaqMan® Mutation Detection Assays

- High specificity—mutant allele detection is based on an allele-specific primer, while wild type background is suppressed by the proprietary MGB blocker oligonucleotide
- High sensitivity—assays can detect down to 0.1% mutation in a background of wild type DNA, as demonstrated in spiking experiments
- Wide dynamic range and excellent PCR efficiency—assays demonstrate at least 4 logs of dynamic range and an average PCR efficiency of 100% ± 10%
- Fast, simple workflow—like other TaqMan® Assays, typically requires 3 hours from sample to results, with minimum hands-on time



Cancer research samples often contain rare somatic mutations within a high background of normal wild type DNA. Many mutation detection methods compatible with tumor specimens, including gene sequencing and real-time PCR, have been reported in the literature and are commercially available. However, commercially available kits have various limitations in terms of sensitivity, specificity, cost, workflow, and turnaround time. We have developed sensitive and easy-to-use TaqMan® Mutation Detection Assays to accurately assess mutation status. TaqMan® Mutation Detection Assays were designed based on the novel competitive allele-specific TaqMan® PCR (castPCR™) technology, which combines allele-specific TaqMan® qPCR with allele-specific MGB blocker oligonucleotides that effectively suppress nonspecific amplification from the off-target allele.

Currently, the assay portfolio covers key somatic mutations identified in various cancer genes including, but not limited to, *KRAS*, *BRAF*, *HRAS*, *NRAS*, *EGFR*, *PIK3CA*, *KIT*, *PTEN*, and *TP53* genes, which have been implicated in many types of cancer. These mutations were selected from the comprehensive Sanger COSMIC database for somatic mutations. The target selection was based on frequency of occurrence and input from leading cancer researchers. We will continually add more mutation assays to cover additional cancer gene mutations. For the most updated list of available assays, refer to the TaqMan® Mutation Detection Assay index file at **lifetechnologies.com/castpcr**.

life technologies

About the assays

TaqMan® Mutation Detection Assays contain mutant allele assays, which specifically detect one or more mutant alleles, and corresponding gene reference assays, which detect mutation-free regions of the genes in which the target mutations reside (Figure 1). The validated assay set additionally includes corresponding wild type allele assays (not described here; refer to the TaqMan® Mutation Detection Assay protocol for further information).

Two experiment types

Two types of experiments are required for mutation detection analysis:

1. Detection ΔC_t cutoff determination

A mutant allele assay and corresponding gene reference assay are run on three or more wild type gDNA samples that are from the same sample type as the test samples (e.g., gDNA from FFPE tissue samples, Figure 2). ΔC_t values are calculated for each sample run with a mutant allele assay/gene reference assay pair. The average ΔC_t value for all samples is then calculated and is used to derive the detection ΔC_t cutoff value for the mutant allele assay.

2. Mutation detection

A test sample is run with one or more mutant allele assays and a corresponding gene reference assay (Figure 2). The ΔC_t value for the mutant allele assay/gene reference assay pair is calculated, and this value is compared to the previously determined detection ΔC_t cutoff value to determine the sample mutation status.

Optional use of internal positive control (IPC)

You can duplex the IPC reagents with any TaqMan® Mutation Detection Assay to distinguish true target negatives from PCR failure or inhibition (Figure 3).

Figure 1. TaqMan® Mutation Detection Assay types.

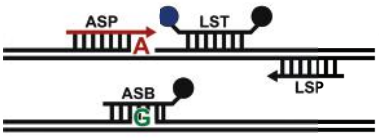
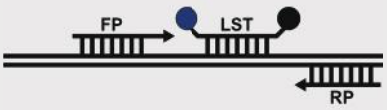
Assay type	Description	Schematic
Mutant allele assay	• Detects specific or multiple mutant alleles • An allele-specific primer detects the mutant allele • An MGB blocker oligonucleotide suppresses the wild type allele	 ASP = Allele-specific primer ASB = Allele-specific blocker (MGB) LST = Locus-specific TaqMan® probe LSP = Locus-specific primer
Gene reference assay	• Detects the gene within which the target mutations reside • A locus-specific pair of forward and reverse primers amplifies a mutation-free region of the target gene	 FP = Forward primer RP = Reverse primer LST = Locus-specific TaqMan® probe

Figure 2. Gene reference and mutant allele assays are run with a genomic DNA sample to determine the mutation status of each target mutation within the cancer gene.

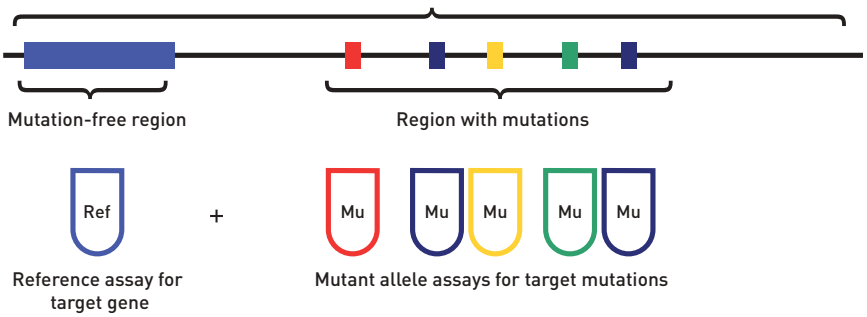
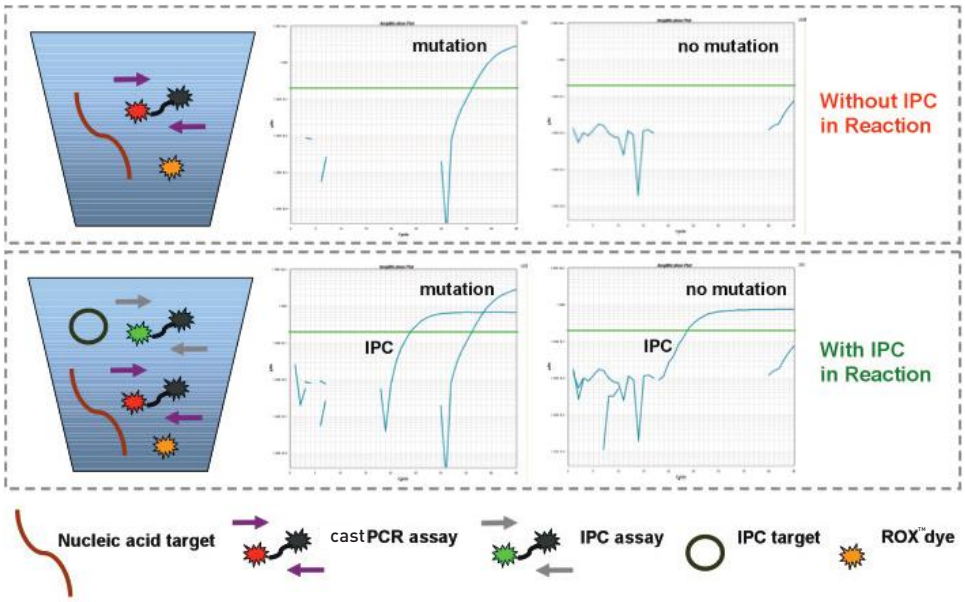


Figure 3. Internal positive controls. The TaqMan® Mutation Detection IPC Reagent Kit is a set of optional internal positive control reagents that can be duplexed with any TaqMan® Mutation Detection Assay to provide a positive PCR control result. The IPC reagents can distinguish a mutation target negative result from a PCR failure result.



Procedure

Purified gDNA, extracted from a sample with an unknown mutation status, is run with one or more mutant allele assays and the corresponding gene reference assay. For each real-time PCR reaction, the gDNA is combined with:

- **A TaqMan® Mutation Detection Assay**—contains two primers and a FAM™ dye–labeled MGB probe to detect a mutant allele or reference gene target. Mutant allele assays also contain an MGB oligonucleotide blocker.
- **TaqMan® Genotyping Master Mix**—contains AmpliTaq Gold® DNA Polymerase UP (Ultra Pure), dNTPs, and buffer
- **(Optional) TaqMan® Mutation Detection IPC Reagent Kit**—contains an internal positive control (IPC) template, two primers, and a VIC® dye–labeled TAMRA™ probe. It can be used to distinguish true target negatives from PCR failure or inhibition.

Reactions are run on a real-time PCR system, using a universal mutation detection thermal cycling protocol. After the run, the real-time PCR system's analysis software determines the C_t values for each TaqMan® Mutation Detection Assay and (optional) IPC reagent reactions. Real-time results export files can be opened in the free Mutation Detector™ Software for post-PCR data analysis. The C_t difference between each mutant allele assay and reference assay is calculated. This ΔC_t value, which represents the quantity of a specific mutant allele detected in a sample, is used to determine sample mutation status by comparison to a previously determined detection ΔC_t cutoff value. You can search for, or download a list of, currently available TaqMan® Mutation Detection Assays at **lifetechnologies.com/castpcr**.

Note: All TaqMan® Mutation Detection Assays have undergone extensive testing to ensure high sensitivity and specificity. The first set of released assays, covering 14 *KRAS*, 29 *EGFR*, and the *BRAF* V600E mutations, underwent additional testing, including determination of: the inherent amplification efficiency difference between mutant allele assays and corresponding reference assays, to enable quantitative analysis of percent mutation in a sample; and assay detection ΔC_t cutoff values using spiked cell line gDNA samples.

Assay performance

Specificity

Mutant allele detection is based on an allele-specific primer, while the wild type allele background is suppressed by the proprietary MGB blocker oligonucleotide. Assays can detect down to 0.1% mutant allele in the presence of a wild type allele background (Figure 4).

High sensitivity

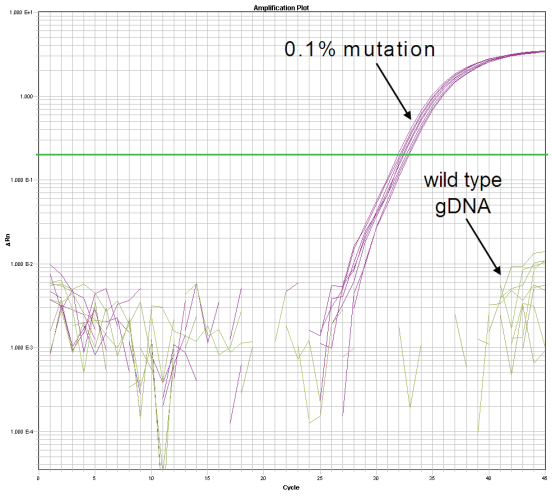
TaqMan® Mutation Detection Assays can detect as few as 1–5 mutant copies in up to one million copies of wild type background. Assay sensitivity is demonstrated using synthetic template spiking experiments (Figure 5 and 6).

Wide dynamic range and excellent PCR efficiency

Assays demonstrate up to 7 logs of dynamic range and an average PCR efficiency of 100% ± 10% (Figure 6).

Figure 4. C_t difference between 0.1% mutation samples and wild type gDNA. For each assay, 0.1% mutant allele samples were obtained by spiking 10 copies of mutant allele synthetic templates into 10,000 copies of cell line wild type gDNA. **(A)** Example of amplification plot for KRAS_522_mu assay on 0.1% mutant allele sample and wild type gDNA. **(B)** There is a significant difference in amplification C_t values between the 0.1% mutant allele sample and wild type gDNA (*P* value < 0.05 for 46 out of 48 assays in the example graph).

A



B

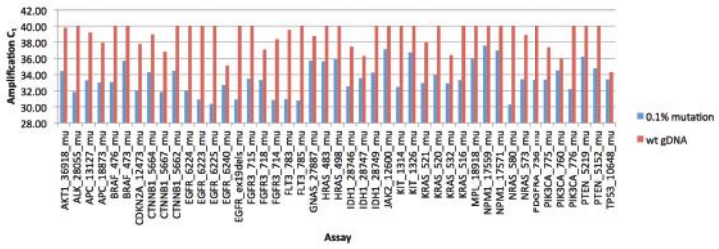


Figure 5. Assay sensitivity and selectivity. For every single assay, the sensitivity and selectivity were analyzed through synthetic template spiking experiments. 10 copies to 10^5 copies of mutant allele synthetic template were spiked into a constant background of 10^5 copies of wild type cell line genomic DNA. For a subset of the assays, 1 copy to 10^6 copies of mutant allele synthetic template were spiked into a constant background of 10^6 copies of wild type allele synthetic template. In the example shown, the BRAF_476_mu assay can detect 1 copy of mutant allele in a background of 10^6 copies of wild type allele.

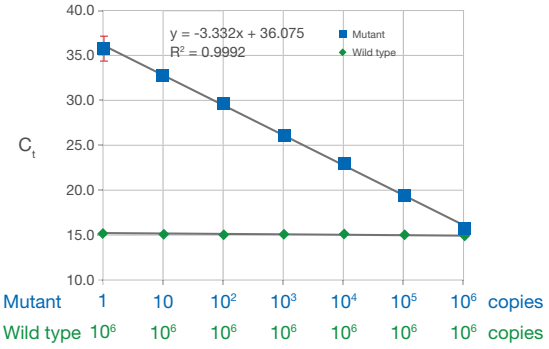
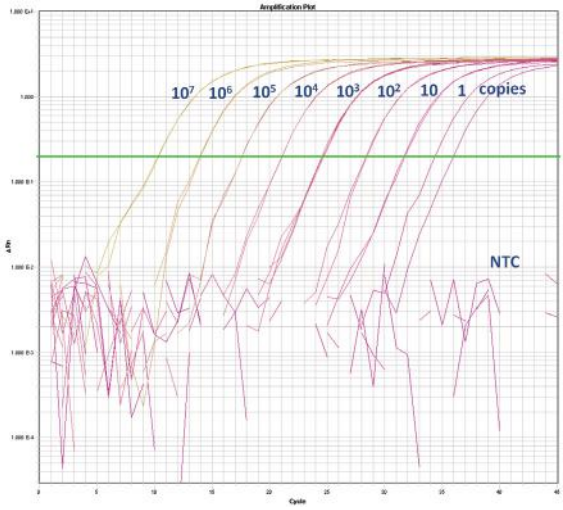


Figure 6. Assay dynamic range. Each assay was tested with 10^5 copies to 10 copies of synthetic template within a constant background of 10^5 copies of wild type genomic DNA. A subset of the assays was tested with 10^7 copies to 1 copy of synthetic template within a constant background of 10^7 copies of wild type allele synthetic template. In the example shown, the KRAS_532_mu assay has 7 logs of dynamic range, with an average PCR efficiency of 100% ± 10%.



Accuracy and reproducibility

Assays demonstrate excellent reproducibility and accurate quantification (Table 1).

Sample type compatibility

The assays can be used with gDNA samples extracted from FFPE tissues, fresh frozen tissues, and cell lines.

Data analysis software

For data analysis, Mutation Detector™ Software allows users to determine the mutation status and quantify the % mutation of their samples from TaqMan® Mutation Detection Assay data collected on the Applied Biosystems® ViiA™ 7, 7900HT, 7500, 7500 Fast, and StepOnePlus™ Real-Time PCR Systems (Table 2).

Table 1. Accuracy and reproducibility. Selected assays were tested in gDNA spiking experiments. In the example shown, G12C mutant cell line gDNA was spiked into wild type cell line gDNA at percentages ranging from 100% to 0.1%. The measured percent mutation was averaged from three experiment runs. The measured percent mutation is highly concordant with the expected percent mutation (R^2 = 0.9997). Accurate and precise quantification (CV < 20%) is obtained among the replicate runs when the target allele copy number is >30.

Copy number, target mutant allele	Expected (%)	Measured (%)	CV (%)
3,000	100.0	100.0	0.0
1,500	50.0	48.9	2.2
750	25.0	23.3	3.8
375	12.5	11.2	7.8
188	6.3	5.7	7.5
90	3.0	2.6	9.0
30	1.0	0.8	17.0
15	0.5	0.4	26.0
3	0.1	0.1	23.0

Table 2. Instrument compatibility.

Applied Biosystems® real-time PCR system	Block module	Software version
StepOnePlus™ system	Fast 96-Well Block Module	StepOne™ Software v2.X
7500 system	Standard 96-Well Block Module	SDS v1.X and v2.X
7500 Fast system	Fast 96-Well Block Module	SDS v1.X and v2.X
7900HT Fast system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	SDS v2.X
ViiA™ 7 system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	ViiA™ 7 Software v1.X
QuantStudio® 12K Flex system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	QuantStudio® Software v1.0

Ordering information

Product	Quantity	Cat. No.
TaqMan® Mutation Detection Assays	150 µL, 10X	4465804
TaqMan® Mutation Detection Reference Assays	150 µL, 10X	4465807
TaqMan® EGFR Exon 19 Deletions Assay	150 µL, 10X	4465805
TaqMan® Mutation Detection IPC Reagent Kit	1 kit	4467538

For more information and full terms of the TaqMan® Assays QPCR Guarantee, go to **lifetechnologies.com/taqmanguarantee**

life technologies

A Thermo Fisher Scientific Brand

applied biosystems

TaqMan Assays for genetic variation research

Superior performance—reliable, robust solutions

ThermoFisher SCIENTIFIC

Genetic variation: decoding the blueprint for biodiversity

Research on genetic variation in animals and plants has expanded our understanding of evolution and human diseases, accelerated the pace of drug development, and helped identify and breed agricultural traits to improve the world's food and fuel supply. Researchers are looking to uncover the association between genetic makeup and phenotypes in studies focusing on single nucleotide polymorphisms (SNPs), copy number variants (CNVs), insertion/deletions (indels), and somatic mutations. A genomics revolution, fueled by advances in biotechnology tools, has significantly increased the rate at which we are able to obtain and analyze data to better understand biodiversity.

We're at the forefront of this revolution, and our reagents, Applied Biosystems™ TaqMan™ Assays, and Applied Biosystems™ platforms for genetic variation analysis, are the preeminent real-time PCR tools for variation research.

Coupled with Applied Biosystems™ capillary electrophoresis, and Ion Torrent™ DNA sequencing systems, we offer a complete solution for genetic analysis research, from discovery to confirmation.

TaqMan Assays for analyzing genetic variation

TaqMan Assays comprise preoptimized PCR primer pairs and one or two probes (depending on product family) for allelic discrimination or quantitative real-time PCR (qPCR). Each assay contains:

- An unlabeled PCR primer pair
- An Applied Biosystems™ TaqMan™ probe with a FAM™ or VIC™ dye label on the 5′ end, and a minor groove binder (MGB) and nonfluorescent quencher (NFQ) on the 3′ end

TaqMan Assays are used to amplify and detect specific variants in target genomic DNA (gDNA). Figure 1 depicts the Applied Biosystems™ TaqMan™ SNP Genotyping Assay process. Real-time PCR using TaqMan Assays is based on the 5′ nuclease activity of *Taq* DNA polymerase.

Here's how it works:

1. TaqMan probes hybridize to the target DNA between the two unlabeled PCR primers. Signal from the fluorescent dye on the 5′ end of a TaqMan probe is quenched by the NFQ on its 3′ end through fluorescence resonance energy transfer (FRET).
2. During PCR, *Taq* polymerase extends the unlabeled primers using the template strand as a guide.
3. When the polymerase reaches the TaqMan probe, it cleaves the molecule, separating the dye from the quencher. The qPCR instrument detects fluorescence from the unquenched FAM or VIC dye.

With each cycle of PCR, more dye molecules are released, resulting in an increase in fluorescence intensity proportional to the amount of amplicon synthesized.

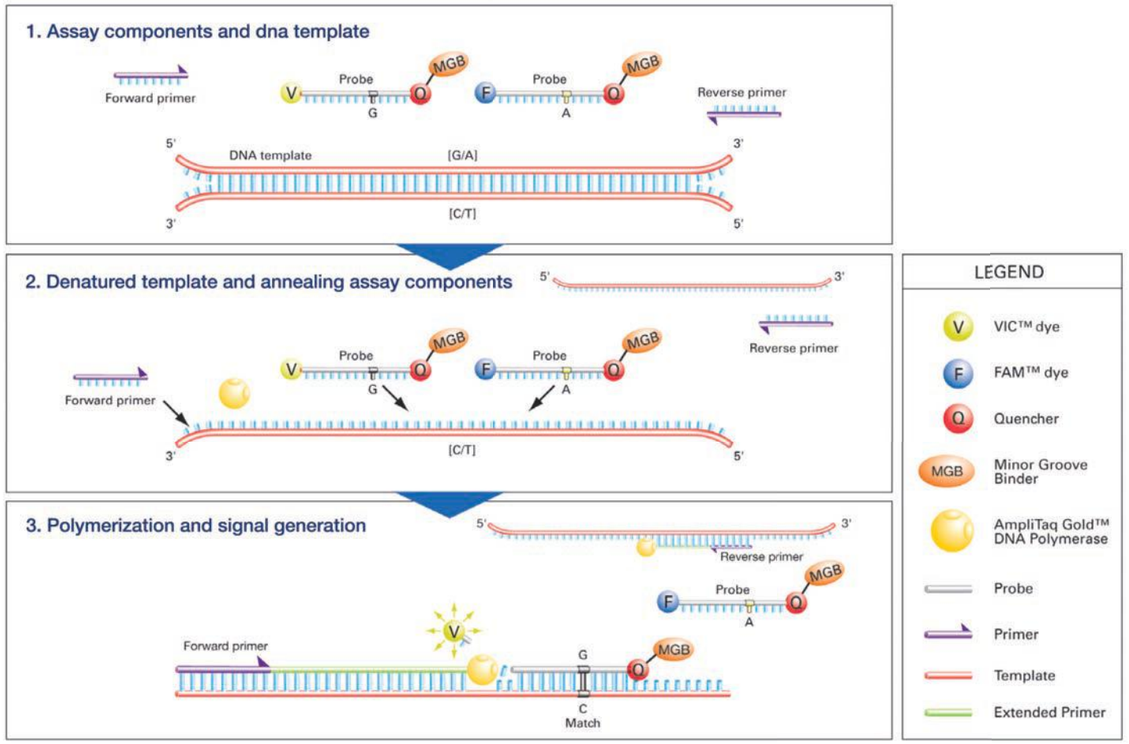


Figure 1. The TaqMan SNP Genotyping Assay. (1) The four TaqMan SNP Genotyping Assay components and the target DNA template with the SNP alleles (in brackets). **(2)** The denatured DNA target and annealing of the assay components. **(3)** Signal generation leading to specific allele detection.

TaqMan SNP Genotyping Assays

- **Better allelic discrimination**—TaqMan probes incorporate 3´ MGB technology to stabilize the probe-template complex
- **Minimize failures**—TaqMan SNP Genotyping Assays are subject to a robust design pipeline, and functional QC testing for human assays on 20 gDNA samples
- **Full-coverage assay pool**—over 7 million human SNP assays (including 160,000 validated assays tested on four ethnic populations of 45 gDNAs each) and over 10,000 mouse SNP assays
- **Simplicity**—all probes and primers are contained in a single tube: no need to optimize probe, primer, salt concentrations, or temperature; all assays use universal PCR conditions
- **Integrated run and analysis solutions**—Applied Biosystems™ instruments and associated software help you move easily from run to results

SNPs are heritable single-base pair variations that occur throughout an organism's genome. SNPs comprise the most common form of genetic variation, with some estimates of SNPs in a given human genome numbering more than 10 million. SNP genotyping plays a central role in characterizing individuals and populations, studying disease traits in humans and other organisms, and identifying genes responsible for advantageous crop traits.

TaqMan SNP Genotyping Assays provide a highly flexible technology for detection of polymorphisms within any genome. TaqMan Assays have a simple workflow and provide a quick way to generate genotyping data (Figure 2). Based on powerful TaqMan chemistry and robust probe and primer designs, and coupled to dependable Applied Biosystems instruments and software, these made-to-order assays produce high-confidence results. TaqMan Assays are ideal for genotyping applications including association studies, candidate region or gene analysis, and fine-mapping studies.

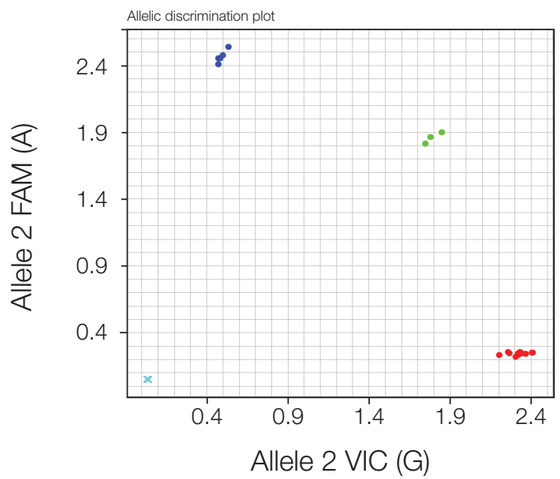


Figure 2. A three-cluster allelic discrimination plot generated with TaqMan SNP Genotyping Assay, C___1202883_20 (rs1801133) for *MTHFR* gene.

Easy online ordering

Predesigned TaqMan SNP Genotyping Assays
Find predesigned assays using our new TaqMan Assay search tool at **thermofisher.com/ordertaqman**

- Easy-to-use interface with a powerful, logical search engine
- Search by keyword (gene, SNP ID) or genomic location
- Filter by SNP type (e.g., missense mutation, intronic, UTR)
- View results on a genome alignment map for easy selection

Custom TaqMan SNP Genotyping Assays
Can't find your assay in our predesigned assay collection? Try designing a custom assay using our Applied Biosystems™ Custom TaqMan™ Assay Design Tool at **thermofisher.com/snpcadt**

- Manually enter your own custom target sequences or upload a file for batch design
- Enter custom primers and probes you have already designed to have us manufacture a ready-to-use assay for you

Simple workflow for quick results
TaqMan SNP Genotyping Assays constitute the simplest SNP genotyping technology available. We deliver your ready-to-use SNP genotyping assay in your choice of format: single-tube, 96- or 384-well plate (custom plating service), or Applied Biosystems™ TaqMan™ OpenArray™ plate (Figure 3). The rest is easy. Just combine the assay with Applied Biosystems™ TaqMan™ Genotyping Master Mix or TaqMan™ Universal PCR Master Mix and your purified DNA sample. There is no need to optimize probe, primer, salt concentrations, or temperature, because all assays use universal reagent concentrations and thermal cycling conditions. After generating an endpoint read using a thermal cycler or real-time PCR instrument, no transfers, washes, or additional reagents are required, and the plate remains sealed; just read the plate and analyze the genotypes. This helps reduce the chance of contamination, sample mix-ups, and sample loss. The simplicity of the chemistry allows you to easily automate the reaction for massively parallel genotyping studies, readily increasing the number of assays, number of samples, or both. Additionally, the analysis software allows you to auto-call genotypes, minimizing manual effort.

Simple data analysis
Applied Biosystems™ TaqMan™ Genotyper Software is a great resource for fast and accurate genotype calling. It is a free SNP genotyping data analysis tool for use with TaqMan SNP Genotyping Assays performed in 48-, 96-, or 384-well microtiter plates or OpenArray plates.

TaqMan Genotyper Software can be downloaded at **thermofisher.com/taqmangenotyper**

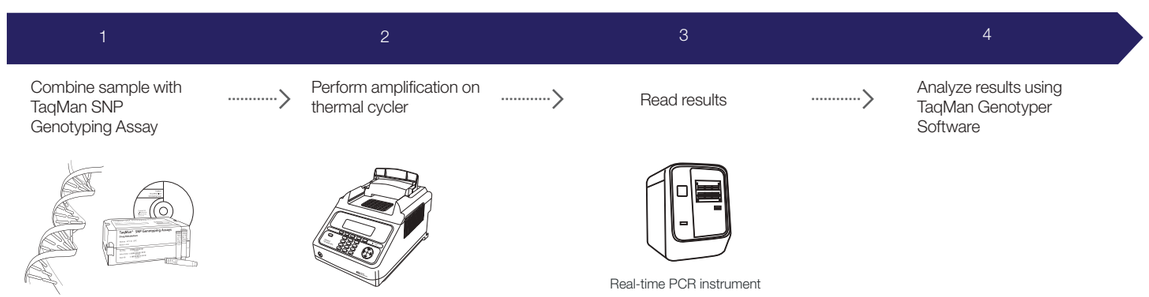


Figure 3. Workflow for TaqMan SNP Genotyping Assays.

Predesigned TaqMan SNP Genotyping Assays
Compatible Applied Biosystems™ TaqMan™ Master Mix and sample prep reagents have been developed to work in conjunction with TaqMan SNP Genotyping Assays to ensure high-quality results.

- TaqMan Genotyping Master Mix
- Applied Biosystems™ TaqMan™ Sample-to-SNP™ Kit
- Applied Biosystems™ TaqMan™ GTXpress™ Master Mix
- Applied Biosystems™ TaqMan™ Universal Master Mix II

The choice of which master mix to use depends on your sample type (tissue, blood, plant, etc.), sample preparation method (purified DNA or crude lysate), and use of fast or standard PCR cycling. For more information, go to **thermofisher.com/taqmansnp**

Ordering information

	Number of SNPs	Number of 5 µL rxns (384-well plate)	Number of 25 µL rxns (96-well plate)	Assay mix formulation	Assay type	Human assays (Cat. No.)	Nonhuman assays (Cat. No.)
Predesigned TaqMan SNP Genotyping Assays for Human and Mouse							
Small-scale	>7 million	1,500	300	40X	Made-to-order	4351379	4351384*
Medium-scale	>7 million	5,000	1,000	40X	Made-to-order	4351376	4351382*
Large-scale	>7 million	12,000	2,400	80X	Made-to-order	4351374	4351380*
Custom TaqMan SNP Genotyping Assays							
Small-scale	∞	1,500	300	40X	Made-to-order	4331349	4332077
Medium-scale	∞	5,000	1,000	40X	Made-to-order	4332072	4332075
Large-scale	∞	12,000	2,400	80X		4332073	4332076
TaqMan Drug Metabolism Genotyping Assays							
Small-scale	2,700	750	150	20X	Inventoried	4362691	N/A

***Over 10,000 mouse assays available.**

All assays are quality-control tested using a mass spectrometer to verify sequence and yield. In addition, all human (predesigned and custom) TaqMan SNP Genotyping Assays receive a genomic functional test on first synthesis. The subsequent syntheses of already-tested human assays and all nonhuman assays receive a fill volume check and mass spectrometry. All assays have a VIC dye–labeled probe, a FAM dye–labeled probe, and two target-specific primers.

Go to **thermofisher.com/taqmansnp** to order.

TaqMan Drug Metabolism Genotyping Assays

- **Excellent ADME panel coverage**—target polymorphisms in 221 genes encoding drug metabolism enzymes and associated transport proteins
- **Simple protocol**—all assays in the collection are run under the same PCR conditions, and specific allele detection is achieved with the Applied Biosystems™ TaqMan™ 5´ nuclease chemistry
- **Detects multiple polymorphisms**—detect SNPs, insertion/deletions (indels), and multinucleotide polymorphisms (MNPs)
- **Rapid receipt of order**—performance-tested assays are already in inventory, ready to ship to you.
- **Assays match databases**—assays are aligned with allele nomenclature from public allele nomenclature sites

Pharmacogenetics is the study of how a person's genetic makeup affects how he or she responds to drugs. This research offers the promise of providing information that will not only allow current drugs to be dosed and delivered more effectively but also allow the development of drugs that are specifically tailored to treat an individual.

We offer 2,700 unique Applied Biosystems™ TaqMan™ Drug Metabolism Genotyping Assays for detecting polymorphisms in 221 genes that code for various drug metabolism enzymes (DMEs) and associated transport proteins. Polymorphisms associated with these genes may influence the rate of drug metabolism within individuals, potentially affecting drug efficacy and the occurrence of side effects (Figure 4). The complex nature of these genes have had limited research conducted because few technologies and products could effectively characterize these polymorphisms. All of the assays in this collection target potentially causative polymorphisms, including those within regulatory elements, coding regions, and associated splice junctions.

TaqMan SNP Genotyping Assay technology delivers superior specificity
Each TaqMan Drug Metabolism Genotyping Assay contains two allele-specific probes and a primer pair to detect the specific SNP target. Both the probes and primers uniquely align within the genome, enabling the TaqMan genotyping technology to provide superior specificity. It is this specificity that allows these assays to detect targets residing in highly homologous gene families that may include pseudogenes.

TaqMan Drug Metabolism Genotyping Assays were developed using a high level of bioinformatics and wet-lab stringency. The assays were designed with information from several public SNP databases, including recognized public allele nomenclature sites. All assays have passed performance tests involving 180 unique DNA samples from four different populations.

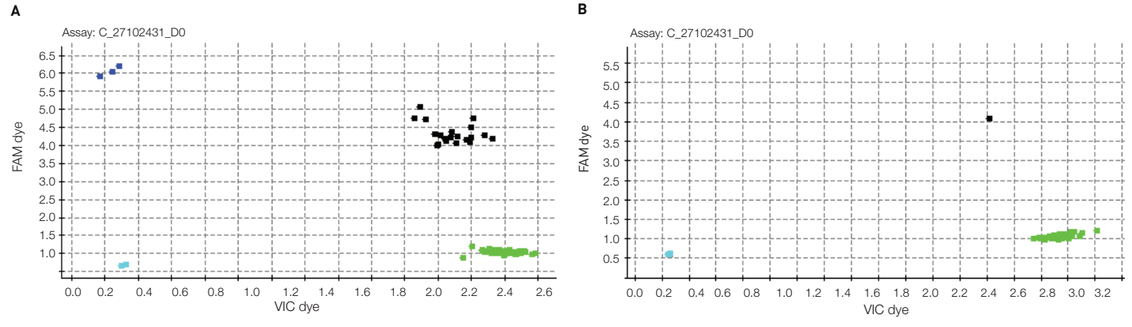


Figure 4. Allelic discrimination plots for the C__27102431_D0 assay run on (A) 45 each African-American and Caucasian, and (B) 45 each Chinese and Japanese gDNA samples. C__27102431_D0 targets the CYP2D6*4,g.1846G>A polymorphism, which encodes an mRNA splicing defect that results in a nonfunctional CYP2D6 protein. If an individual carries two nonfunctional CYP2D6 alleles, they will have the poor metabolizer (PM) phenotype and the metabolism of numerous drugs will be impacted.

Markers relevant for drug metabolism

The Applied Biosystems™ TaqMan™ DME Assay PharmaADME Core Marker Set contains a predefined group of TaqMan Drug Metabolism Genotyping and Applied Biosystems™ TaqMan™ Copy Number Assays, providing over 95% coverage of core markers in 33 ADME genes identified by the PharmaADME consortium.

This assay set greatly simplifies the study of these key putative functional genetic ADME variants and consists of:

- 164 DME assays for SNP and indel polymorphisms
- 14 copy number assays for copy number and hybrid gene variants

Assay sets are delivered in individual tubes, providing the flexibility to select a subset of assays or the entire PharmaADME Core Marker Set.

DME Assay Index

A DME Assay Index is also available with all drug metabolism assays. This file lists each assay along with context sequence, location on the NCBI assembly, the refSNP number (from dbSNP), and the common allele nomenclature from a public allele nomenclature site, when available.

Quick delivery, convenient format

For fast delivery, all assays in this collection have been manufactured and placed into inventory and are ready to ship at ambient temperature. Like other TaqMan SNP Genotyping Assays, these single-tube products consist of two allele-specific TaqMan MGB probes (labeled with either VIC or FAM dye) and two locus-specific primers. TaqMan Drug Metabolism Genotyping Assays are supplied as single tubes and in 96- and 384-well plates (custom plating service). Additionally, all products are formulated for the small-scale reaction size: a 20X single-tube assay, supporting 750 reactions at a 5 µL reaction size.

Optimized supporting reagents

Compatible TaqMan Master Mix and sample preparation reagents have been developed to work in conjunction with TaqMan Drug Metabolism Genotyping Assays to ensure high-quality results:

- TaqMan Genotyping Master Mix
- TaqMan Universal Master Mix II

Additional information about TaqMan Drug Metabolism Genotyping Assays, including links to the PharmaADME Core Marker Set and the DME Assay Index, can be found at **thermofisher.com/taqmandme**

TaqMan Copy Number Assays

- **Gold standard technology**—extraordinary accuracy and reliability; performance guaranteed for all predesigned assays**
- **Results in hours**—simplest method available to study CNV
- **Scalable solution**—automated workflow offers optimum platform for high-throughput validation of copy number changes
- **Comprehensive assay collection**—predesigned assays for human, mouse, and common vector marker/reporter genes
- **Option for custom assays**—Custom Plus and Custom TaqMan Assays for user-defined targets of interest

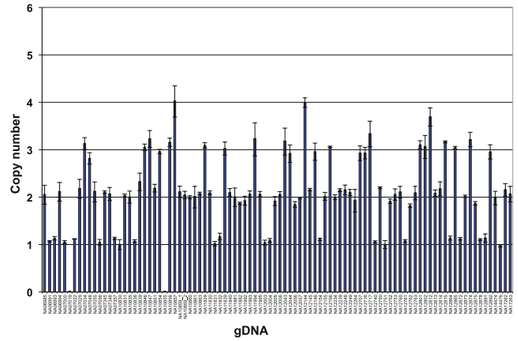
CNV, initially defined as variation in copy number of segments of DNA ≥1 kb in size, between individuals, is found in all humans as well as other animals and plants.

CNV affects a significant portion of the genome (approximately 12% of the human genome) and includes deletions, duplications, and other complex genotyping patterns. These CNVs can influence gene expression and be associated with specific phenotypes and diseases, as observed in microdeletion and microduplication syndromes.

Superior chemistry and streamlined methods offer reliable results

TaqMan Copy Number Assays combine Applied Biosystems™ TaqMan™ Assay chemistry with Applied Biosystems™ real-time PCR instruments to form a method for obtaining specific, reproducible, and easy-to-interpret copy number results (Figure 5). TaqMan Copy Number Assays are an ideal validation tool for microarray or next-generation sequencing follow-up studies and can be used to find specific targets. The workflow can be automated so that several hundred to thousands of samples can be processed in a single day.

A



B

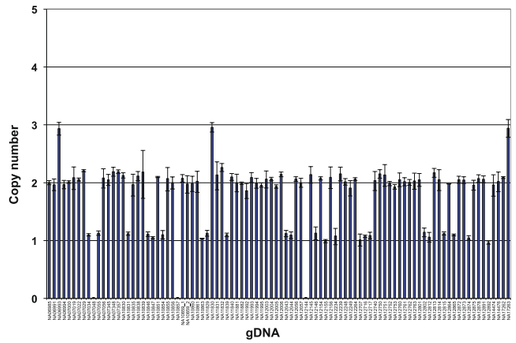


Figure 5. High specificity of TaqMan Copy Number Assays. C4A and C4B represent two isoforms of the C4 gene family. The sequences for these two genes differ in only 5 bases, but the encoded C4A and C4B proteins are functionally different. Differential detection of **(A)** C4A and **(B)** C4B is very challenging. Shown are TaqMan Copy Number Assays for C4A and C4B with the HAPMAP CEU sample set. Distinct copy number changes are observed. (JPT/CHB and YRI data not shown.)

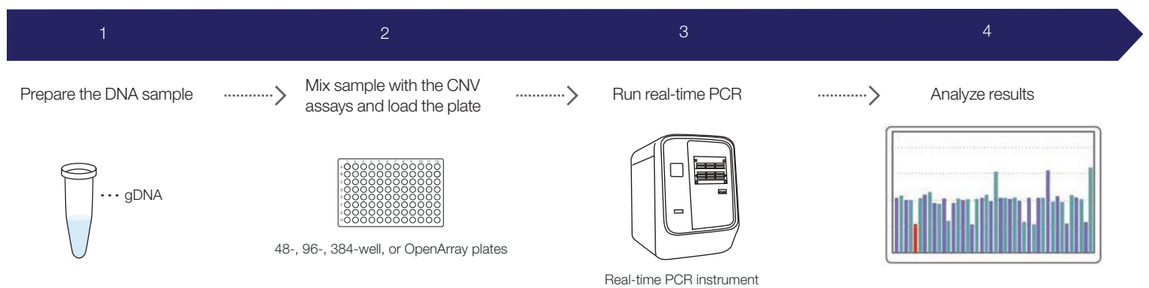


Figure 6. Workflow for TaqMan Copy Number Assays.

TaqMan Copy Number Assays

TaqMan Copy Number Assays include predesigned collections for both human and mouse genomes. The human collection includes more than 1.6 million assays for genome-wide coverage. The mouse predesigned collection includes more than 180,000 assays targeting gene exons. Predesigned assays to common vector marker and reporter genes are also available for transgenic studies.

Find predesigned assays using our online TaqMan Assay search tool at **thermofisher.com/cnv**

Applied Biosystems™ Custom Plus TaqMan™ Copy Number Assays are an optimal solution for studying variation in human and mouse genomic regions of interest for which a predesigned assay is not available. Custom Plus assays use the same bioinformatics pipeline used to manufacture predesigned TaqMan Copy Number Assays (which includes premasking of SNPs and repetitive sequences and assay genome uniqueness checks) and can be generated for high-quality genomic targets of interest using the online Applied Biosystems™ GeneAssist™ Copy Number Assay Tool. Standard Custom TaqMan Copy Number Assays are an option for additional targets of interest. Unlike Custom Plus assays, standard Custom assay designs do not go through premasking or genome quality checks, but can be compared with the human or mouse reference assays for compatibility in duplex reactions.

Two Applied Biosystems™ TaqMan™ Copy Number Reference Assays are available for copy number analysis in both human and mouse species. Note that the reference assays are species-specific.

Feature	Predesigned TaqMan Copy Number Assay	Custom Plus TaqMan Copy Number Assay	Custom TaqMan Copy Number Assay
Designed using copy number–specific algorithm optimized for performance	✓	✓	✓
Availability limited to human and mouse assays	✓	✓	
Contains TaqMan FAM dye–labeled MGB probes and two unlabeled PCR primers	✓	✓	✓
Targets undergo SNP and repetitive sequence masking	✓	✓	
Genome specificity check	✓	✓	
Reference assay compatibility check	✓	✓ (optional)	✓
Assay sequences provided			✓
Assay context sequences and genome location provided	✓	✓	

A simple CNV analysis workflow

TaqMan Copy Number Assays have one of the simplest workflows of all currently available CNV analysis methods (Figure 6). The test assay (FAM dye–labeled), the reference assay (VIC dye–labeled), your sample DNA, and TaqMan Master Mix (TaqMan Genotyping Master Mix is recommended, with TaqMan Universal Master Mix II and Applied Biosystems™ TaqMan™ Gene Expression Master Mix also being compatible) are combined and then run on an Applied Biosystems real-time PCR system using standard TaqMan Assay PCR conditions. On average, setup to primary analysis takes only 3–4 hours (including a ~2 hour PCR run).

Analysis tools and methods

TaqMan Copy Number Assays are supplied in single tubes, or the assays can be custom-plated in 96- and 384-well plates. The assay reactions are run on a real-time PCR instrument, and the data are analyzed using Applied Biosystems™ CopyCaller™ Software.

Additional information on TaqMan Copy Number Assays, as well as links to CopyCaller Software and the GeneAssist Copy Number Assay Tool, can be found at **thermofisher.com/cnv**

	Number of 10 µL rxns (384-well plate)	Number of 20 µL rxns (96-well plate)	Assay mix formulation	Assay type	Cat. No.
Predesigned TaqMan Copy Number Assays					
Small-scale	720	360	20X	Made-to-order	4400291
Medium-scale	1,500	750	20X	Made-to-order	4400292
Large-scale	5,800	2,900	60X	Made-to-order	4400293
Custom Plus TaqMan Copy Number Assays					
Small-scale	720	360	20X	Made-to-order	4442487
Medium-scale	1,500	750	20X	Made-to-order	4442520
Large-scale	5,800	2,900	60X	Made-to-order	4442488
Custom TaqMan Copy Number Assays					
Small-scale	720	360	20X	Made-to-order	4400294
Medium-scale	1,500	750	20X	Made-to-order	4400295
Large-scale	5,800	2,900	60X	Made-to-order	4400296
TaqMan Copy Number Reference Assays (Human)					
RNase P	1,500	750	20X (1 tube)	Inventoried	4403326
RNase P	6,000	3,000	20X (4 tubes)	Inventoried	4403328
TERT	1,500	750	20X (1 tube)	Inventoried	4403316
TERT	6,000	3,000	20X (4 tubes)	Inventoried	4403315
TaqMan Copy Number Reference Assays (Mouse)					
Tfrc	1,500	750	20X (1 tube)	Inventoried	4458366
Tfrc	6,000	3,000	20X (4 tubes)	Inventoried	4458367
Tert	1,500	750	20X (1 tube)	Inventoried	4458368
Tert	6,000	3,000	20X (4 tubes)	Inventoried	4458369

Looking for a different formulation, scale, or label? The TaqMan Custom Assay and Oligo Service can accommodate special requests. To learn more, email specialoligos@thermofisher.com or contact your local sales representative.

Go to **thermofisher.com/cnv** to order.

TaqMan Mutation Detection Assays for somatic mutation detection

- **High specificity**—mutant allele detection is based on an allele-specific primer, while wild type background is suppressed by the proprietary MGB blocker oligonucleotide
- **High sensitivity**—assays can detect down to 0.1% mutant molecules in a background of wild type DNA, as demonstrated in spiking experiments (Figure 8)
- **Detect multiple types of mutations**—detect single- and multiple-nucleotide mutations and insertion/deletions (indels)
- **Wide dynamic range and excellent PCR efficiency**—assays demonstrate at least 4 logs of dynamic range and an average efficiency of 100% ± 10%
- **Fast, simple workflow**—like other TaqMan Assays, typically require 3 hours from sample to results, with minimum hands-on time

Somatic mutations can be present at low levels against a high background of wild type sequences, and methods used to detect and characterize these mutations in tumor specimens need to be highly sensitive and accurate. Methods that are commonly used include gene sequencing (including pyrosequencing and traditional Sanger sequencing) and real-time PCR.

Applied Biosystems™ TaqMan™ Mutation Detection Assays were designed based on a novel competitive allele-specific Applied Biosystems™ TaqMan™ (castPCR™) technology (Figure 7), which combines allele-specific TaqMan qPCR with an allele-specific MGB blocker oligonucleotide to effectively suppress nonspecific amplification of the off-target allele. These assays target mutations in 45 genes implicated in a number of cancer models:

ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, VHL

TaqMan Mutation Detection Assays

TaqMan Mutation Detection Assays contain mutant allele assays, which specifically detect one or more mutant alleles, and corresponding gene reference assays, which detect mutation-free regions of the genes in which the target mutations reside.

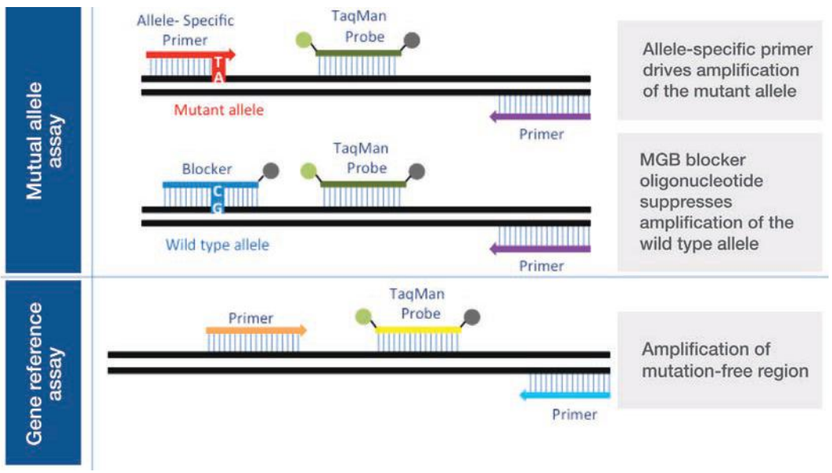


Figure 7. TaqMan Mutation Detection Assay types.

Two experiment types

Mutation detection analysis requires two types of experiments:

Detection ΔC_t cutoff determination

A mutant allele assay and corresponding gene reference assay are run on three or more wild type gDNA samples that are from the same sample type as the test samples (e.g., gDNA from FFPE tissue samples). The ΔC_t value is calculated for the mutant allele assay/gene reference assay pair, for each sample. The average ΔC_t for all samples is then calculated and is used to derive the detection ΔC_t cutoff value for the mutant allele assay.

Mutation detection

A test sample is run with one or more mutant allele assays and a corresponding gene reference assay. The ΔC_t for the mutant allele assay/gene reference assay pair is calculated, and this value is compared to the previously determined detection ΔC_t cutoff value to determine the sample's mutation status.

Simple workflow

Purified gDNA, extracted from a sample of unknown mutation status, is run with one or more mutant allele assays and corresponding gene reference assays. For each real-time PCR, the gDNA is combined with:

- A TaqMan Mutation Detection Assay
- TaqMan Genotyping Master Mix
- (Optional) Applied Biosystems™ TaqMan Mutation Detection IPC Reagent Kit—to distinguish true target negatives from PCR failure or inhibition

Reactions are run on a real-time PCR system using a universal thermal cycling protocol for mutation detection. After the run, the real-time PCR system analysis software determines the C_t for each TaqMan Mutation Detection Assay and (optional) IPC reagent reactions. Real-time results can be exported as files that can be opened in free Applied Biosystems™ Mutation Detector™ Software.

Ordering information

Product	Size	Assay type	Cat. No.
TaqMan Mutation Detection Assays	150 µL, 10X	Inventoried	4465804
TaqMan Mutation Detection Reference Assays	150 µL, 10X	Inventoried	4465807
TaqMan EGFR Exon 19 Deletions Assay	150 µL, 10X	Inventoried	4465805
TaqMan Mutation Detection IPC Reagent Kit	1 kit	Inventoried	4467538

New assays for other cancer gene mutation targets will continually be released.

Go to **thermofisher.com/castpcr** for the most current list.

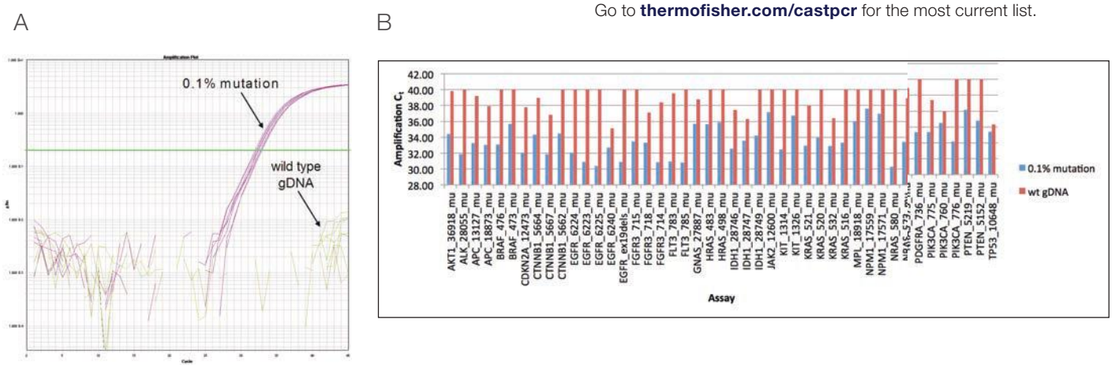


Figure 8. C_t differences between 0.1% mutation samples and wild type gDNA in TaqMan Mutation Detection Assays. For each mutant allele assay, 0.1% mutant allele samples were obtained by spiking cell line wild type gDNA (30 ng, ~10,000 copies) with 10 copies of mutant allele synthetic templates. **(A)** Example of amplification plot for KRAS_522_mu assay run on a 0.1% mutation sample and a wild type gDNA sample (30 ng gDNA). **(B)** For a majority of the assays, there is a significant difference in amplification C_t values between the 0.1% mutant allele sample and wild type gDNA (*P* value < 0.05).

TaqMan genotyping reagents for optimal performances

TaqMan Sample-to-SNP Kit
The TaqMan Sample-to-SNP Kit takes you from biological sample to results typically in less than an hour, without isolating DNA. The kit consists of two parts: the Applied Biosystems™ DNA Extract All Reagents and the TaqMan GTXpress Master Mix. The DNA All Lysis Reagents reduce prolonged procedures for the release of real-time PCR–ready DNA to a 5-minute protocol. They are compatible with a wide variety of samples ranging from blood to buccal swabs to animal and plant tissues. DNA extracted with DNA Extract All Reagents can be used with TaqMan SNP Genotyping Assays (not recommended for other TaqMan Assays).

TaqMan master mixes
TaqMan master mixes contain buffer, dNTPs, passive reference dye, thermostable hot-start DNA polymerase, and other components, and are provided in a convenient single-vial format. They are formulated to provide optimal results for TaqMan Assays.

- **TaqMan Genotyping Master Mix**—the TaqMan Genotyping Master Mix is optimized for end-point fluorescence detection in SNP genotyping applications in standard mode; the TaqMan Genotyping Master Mix provides excellent pre- and post-PCR stability for high-throughput setup and analysis
- **TaqMan GTXpress Master Mix**—the TaqMan GTXpress Master Mix is designed to deliver accurate genotyping results with robust performance in less than 50 minutes; the TaqMan GTXpress Master Mix is also available as part of the TaqMan Sample-to-SNP Kit

Ordering information and assay compatibility

	TaqMan Genotyping Master Mix	TaqMan GTXpress Master Mix
Cat. No. (size)	4371355 (10 mL)†	4401892 (10 mL)
TaqMan SNP Genotyping Assays	††	††
TaqMan Drug Metabolism Genotyping Assays	††	+
TaqMan Copy Number Assays	††	–
TaqMan Mutation Detection Assays for somatic mutation detection	††	–

†Other pack sizes are available.

††Thermo Fisher Scientific validated: We have performed extensive testing and optimization.
+Thermo Fisher Scientific demonstrated: Limited testing has been performed. We cannot guarantee optimal performance for all TaqMan Assays.
–Not recommended.

Quality service and support at every step of your workflow

From manufacturing to follow-up—consistent reliability

TaqMan Assays are designed, manufactured, packaged, tested, and shipped using the highest-quality materials and methods. Furthermore, they are backed by our worldwide technical support teams.

Quality manufacturing and stringent quality control
TaqMan Assays are manufactured in-house at our ISO 13485–certified manufacturing facilities and are never outsourced.

Comprehensive worldwide support
Whether you need help finding a TaqMan Assay for your target, deciding which format best suits your needs, placing your order through our online ordering system, or setting up your reactions, our sales and technical support staff are here to help.

Sales support
Your sales representative can help you find Web and print resources to help you choose the right TaqMan Assay products for your genetic variation research. For more demanding projects, she or he can also involve our technical sales specialists, who have more in-depth knowledge of TaqMan Assay technology and our relevant supporting reagents and instruments.

Technical support
If you have questions about how to use TaqMan Assays or how to analyze results, go to **thermofisher.com/support** to contact our technical support specialists. These agents are skilled in experimental planning and design, are expert troubleshooters, and are familiar with a wide variety of applications that use TaqMan Assays.

Rapid delivery
We continually strive to minimize delivery time on TaqMan Assay products. To that end, we have implemented streamlined order processing systems that interface with our new manufacturing facilities to help reduce delivery times.

TaqMan Assay type	Estimated delivery time (business days/weeks)
Inventoried (in stock)	1–4 days
Made-to-order/Custom TaqMan Assays (manufactured when order is placed)	5–12 days
TaqMan Custom Plating Service (configure 96- or 384-well plates with any TaqMan assays)	2–5 weeks

**The TaqMan Assays QPCR Guarantee
We stand behind every predesigned TaqMan Assay you buy. We're committed to helping you achieve your research goals and believe our predesigned TaqMan Assays establish the benchmark for high-quality and easy-to-use real-time PCR products. If you are not satisfied with the performance of a predesigned TaqMan Assay, we'll replace it at no cost or credit your account. For more information, and full terms and conditions of the guarantee, go to **thermofisher.com/taqmanguarantee**

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Find out more at **thermofisher.com/taqman**

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Company code 122351387, VAT code LT223513811

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CERTIFICATE OF ANALYSIS

4444557 TaqMan™ Fast Advanced Master Mix

Packaging Lot: **3168876**
Manufacturing Date: 05.12.2024 (DD.MM.YYYY)
Expiry Date: 31.12.2026 (DD.MM.YYYY)
Storage: at 5±3°C in the dark
Note: **For Research Use Only. Not for use in diagnostic procedures.**

Filling lots for components in package:

Lot	Quantity	Description
3168876	5 mL	TaqMan™ Fast Advanced Master Mix

QUALITY CONTROL

Parameter	Method	Requirement	Result
Functional test	Product is tested for Gene Expression Assay performance using a TaqMan™ RNaseP Assay coupled with the TaqMan™ Exogenous Internal Positive Control Reagents on Applied Biosystems Real-Time PCR System.	•PCR Efficiency is within specification limit •R^2 value ≥ 0.98 •Fold Discrimination is within specification limit • Duplex Ct is within specification limit	Pass
dNTP (dATP, dCTP, dGTP, dUTP) concentrations	Determined by HPLC.	Within range of target concentration	Pass
Mg^{2+} concentration	Determined by Ion chromatography.	Within range of target concentration	Pass
E. coli DNA Burden	Determined by analytical method.	Meets predetermined specifications	Pass
DNase level	Determined using DNase Alert QC System.	Meets predetermined specifications	Pass
RNase level	Determined using RNase Alert QC System.	Meets predetermined specifications	Pass
pH	Measured using pH meter at 25°C.	Within range of target pH	Pass

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

Real-time PCR master mixes and instrument compatibility

	TaqMan® and TaqPath real-time PCR master mixes								One-step real-time RT-PCR master mixes					
Applications	**Gene expression/ small RNA**	**Genotyping/ CNV**	**Gene expression/ genotyping**	**Gene expression/ genotyping**	**Gene expression**	**Genotyping/ SNP**	**Genotyping/ SNP**	**Gene expression**	**Gene expression/ pathogen detection**	**Gene expression/ pathogen detection**	**Gene expression/ pathogen detection**	**Gene expression for 1-step and multiplex**	**Gene expression/ pathogen detection**	**Gene expression/ pathogen detection**
	TaqMan Fast Advanced Master Mix	TaqPath ProAmp Master Mix	TaqMan Universal PCR Master Mix	TaqMan Universal Master Mix II	TaqMan Gene Expression Master Mix	TaqMan Genotyping Master Mix	TaqMan GTXpress Master Mix	TaqMan Fast Universal PCR Master Mix	TaqMan RNA-to-C_T *1-Step* Kit	*Power* SYBR Green RNA-to-C_T *1-Step* Kit	TaqMan Fast Virus 1-Step Master Mix	TaqPath 1-Step Multiplex Master Mix	SuperScript III Platinum One-Step qRT-PCR Kit	SuperScript III Platinum One-Step qRT-PCR Kit w/ ROX
Instruments	4444556 4444557 4444963 4444964 4444965 4444558	A32704, A30865, A30866, A30871, A30867, A30872, A32705, A30868, A30869, A30873, A30870, A30874	4304437, 4305719, 4318157, 4326708, 4364338, 4364340, 4324018, 4324020, 4326614, 4364341, 4364343	4440043, 4440040, 4440047, 4440048, 4440049, 4440041, 4440042, 4440038, 4440044, 4440045, 4440046, 4440039	4370048 4369016 4369514 4369510 4369542 4370074	4371353 4371355 4381656 4371357 4381657	4403311 4401892 4401890 4401857	4352042 4364103 4366072 4366073 4367846	4392653 4392938 4392656	4391178 4389986	4444432 4444434 4444436	A28521, A28522, A28523, A28525, A28526, A28527	11732020 11732088	11745500 11745100
Analytik Jena qTOWER	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems 7500	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems 7500 Fast	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems 7300	√	√	√	√	√	√	√	√	√	√	√	√*	√	√
Applied Biosystems 7900HT	√	√	√	√	√	√	√	√	√	√	√	√*	√	√
Applied Biosystems QuantStudio 12K Flex	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems QuantStudio 6	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems QuantStudio 3/5	√	√*	√	√	√	√	√	√	√	√	√	√*	√	
Applied Biosystems QuantStudio 7	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems StepOne	√	*	√	√	√	√	√	√	√	√	√	*	√	√
Applied Biosystems StepOnePlus	√	*	√	√	√	√	√	√	√	√	√	*	√	√
Applied Biosystems ViiA 7	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad CFX384	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad CFX96	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad iQ5	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad MiniOpticon	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad/MJ Chromo4	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad/MJ Opticon	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad/MJ Opticon 2	√	√	√	√	√	√	√	√	√	√	√	√	√	
Eppendorf Mastercycler ep realplex	√	√	√	√	√	√	√	√	√	√	√	√	√	
Qiagen/Corbett Rotor-Gene 6000	√	√	√	√	√	√	√	√	√	√	√	√	√	
Qiagen/Corbett Rotor-Gene Q	√	√	√	√	√	√	√	√	√	√	√	√	√	
Qiagen/Corbett Rotor-Gene 3000	√	√	√	√	√	√	√	√	√	√	√	√	√	
Roche LightCycler 480	√	√	√	√	√	√	√	√	√	√	√	√	√	
Roche LightCycler Nano	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	
Agilent/Stratagene MX3000P	√	√	√	√	√	√	√	√	√	√	√	√	√	
Agilent/Stratagene MX3005P	√	√	√	√	√	√	√	√	√	√	√	√	√	
Agilent/Stratagene MX4000	√	√	√	√	√	√	√	√	√	√	√	√	√	
TaKaRa T800	◊	◊	◊	◊	◊	◊	◊	◊	◊	√	◊	◊	◊	

√ – Indicates preferred kit and tested on this instrument.
◊ – Indicates kit should work, but has not been tested on this instrument.
* – Not compatible with multiplexing. The QuantStudio 3 system can be used for limited multiplexing; it is not compatible with Mustang Purple or Cy®5 dyes. The QuantStudio 5 system is compatible with multiplexing.

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Real-time PCR master mixes and instrument compatibility

	SYBR Green real-time PCR master mixes					
Applications	**Gene expression**	**Gene expression**	**Gene expression**	**Gene expression**	**Gene expression**	**Gene expression**
	SYBR Select Master Mix	Fast SYBR Green Master Mix	*Power* SYBR Green PCR Master Mix	SYBR Green PCR Master Mix	SYBR GreenER qPCR SuperMix Universal	PowerUp SYBR Green Master Mix
Instruments	4472903, 4472908, 4472913, 4472918, 4472919, 4472920	4385610, 4385612, 4385616, 4385617, 4385618, 4385614	4367659, 4367660, 4368577, 4368702, 4368706, 4368708	4344463, 4309155, 4364344, 4364346, 4334973, 4312704	1176202K, 11762100, 11762500	A25742, A25743, A25776, A25777, A25778, A25779, A25780, A25918
Applied Biosystems 7500	√	√	√	√	√	√
Applied Biosystems 7500 Fast	√	√	√	√	√	√
Applied Biosystems 7300		√	√	√	√	√
Applied Biosystems 7900HT		√	√	√	√	√
Applied Biosystems QuantStudio 12K Flex	√	√	√	√	√	√
Applied Biosystems QuantStudio 6	√	√	√	√	√	√
Applied Biosystems QuantStudio 3/5	√	√	√	√	√	√
Applied Biosystems QuantStudio 7	√	√	√	√	√	√
Applied Biosystems StepOne	√*	√	√	√	√	√
Applied Biosystems StepOnePlus	√*	√	√	√	√	√
Applied Biosystems ViiA 7	√	√	√	√	√	√
Bio-Rad CFX384	√	√	√	◊	◊	√
Bio-Rad CFX96	√	√	√	◊	◊	√
Bio-Rad iQ5	√	√	√	√		√
Bio-Rad MiniOpticon	√	√	√	√	√	√
Bio-Rad/MJ Chromo4	√	√	√	√	√	√
Bio-Rad/MJ Opticon	√	√	√	√	√	√
Bio-Rad/MJ Opticon 2	√	√	√	√	√	√
Eppendorf Mastercycler ep realplex		√	√	√		√
Qiagen/Corbett Rotor-Gene 6000	√*	√	√	√	√	√
Qiagen/Corbett Rotor-Gene Q	√*	√	√	√	◊	√
Qiagen/Corbett Rotor-Gene 3000	√*	√	√	√	◊	√
Roche LightCycler 480	√	√	√	√		√
Roche LightCycler Nano		◊	◊	◊		◊
Agilent/Stratagene MX3000P	√	√	√	√	√	√
Agilent/Stratagene MX3005P	√	√	√	√	√	√
Agilent/Stratagene MX4000	√	√	√	√	√	√
TaKaRa T800		√	√	√		√

√ – Indicates preferred kit and tested on this instrument.
√* – Ensure primer concentration guide is followed (<200 nM for standard mode and 300–400 nM for fast mode). For StepOne/StepOnePlus System, install software version 2.3 or higher update.
◊ – Indicates kit should work, but has not been tested, on this instrument.

Find out more at **thermofisher.com/mastermixes**

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TaqMan® Genotyping Master Mix

Catalog Numbers 4371353, 4371355, 4381656, 4371357, and 4381657

Pub. No. 4374656 **Rev.** D

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Genotyping Master Mix Protocol* (Pub. No. 4371131). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Contents and storage

Cat. No.	Number of reactions	Contents	Storage
4371353	40	1 mL	2–8°C for up to one year
4371355	400	10 mL	
4381656	800	2 × 10 mL	
4371357	2,000	50 mL	
4381657	4,000	2 × 50 mL	

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer: Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

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Revision history: Pub. No. 4374656

Revision	Date	Description
D	04 September 2018	Updated manufacturing address, branding, licensing, trademarks, general style and format.
C	September 2011	Baseline for this revision.

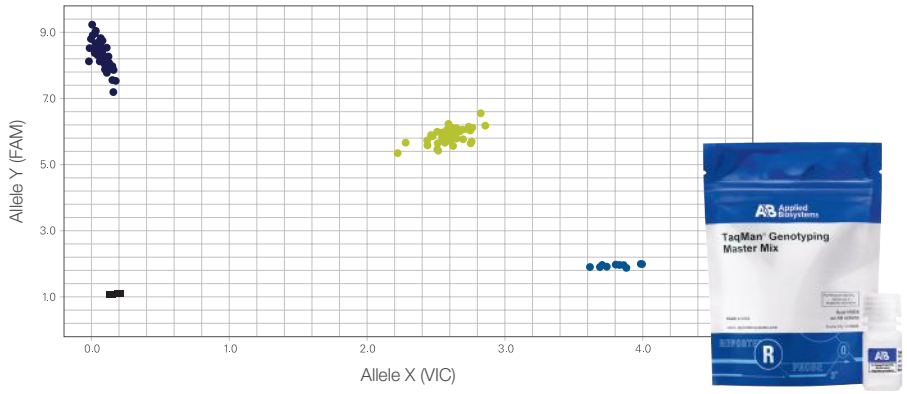
Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

TaqMan Genotyping Master Mix

Real-time PCR master mix tailored for SNP genotyping studies

Tailored for unrivaled cluster resolution for unambiguous single-nucleotide polymorphism (SNP) allelic discrimination, the Applied Biosystems™ TaqMan™ Genotyping Master Mix is optimized for genotyping applications, including:

- Candidate gene studies
- Drug target validation
- Disease association studies
- Population genetics
- Linkage mapping
- Agricultural applications
- Copy number variation analysis



Introduction

TaqMan Genotyping Master Mix is designed to deliver reliable, cost-effective SNP detection for accurate and reproducible allelic discrimination. The master mix optimizes the preferential binding of the allele-specific probe, providing exceptional separation and clustering of alleles and consistently strong fluorescent signals. Powered with the highly purified Applied Biosystems™ AmpliTaq Gold™ DNA Polymerase, UP (Ultra Pure), TaqMan Genotyping Master Mix can replace Applied Biosystems™ TaqMan™ Universal PCR Master Mix in existing SNP genotyping protocols using the same reaction setup and thermal cycling conditions.

Benefits

- Specifically formulated for endpoint fluorescent detection of SNPs and insertions/deletions
- Discrete clusters and high call rates for accurate and reproducible allelic discrimination
- Reliable discrimination of SNPs in difficult targets
- Excellent room-temperature stability for flexible pre- and post-PCR setup and analysis
- Universal thermal cycling conditions for consistent results
- Validated for use with Applied Biosystems™ TaqMan™ SNP Genotyping Assays, TaqMan™ Copy Number Assays, and TaqMan™ Mutation Detection Assays

Optimized formulation for exceptional performance

TaqMan Genotyping Master Mix is a convenient 2X mix for TaqMan probe–based genotyping reactions. It includes the following components:

- AmpliTaq Gold DNA Polymerase, UP (Ultra Pure), a highly purified DNA polymerase. This hot-start enzyme is inactive at room temperature, so reactions can be set up on the benchtop. The enzyme is activated during thermal cycling.
- Optimized components including buffer and dNTPs for consistent, reliable genotypes
- Passive internal reference based on proprietary ROX™ dye for precise data analysis

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Setting a new standard for allelic discrimination

For clear genotyping results, each allele-specific TaqMan™ probe must yield bright and consistent fluorescent signals to provide discrete clusters that are widely separated, indicating excellent specificity. The performance of TaqMan Genotyping Master Mix was tested using 3 ng samples of human genomic DNA (gDNA) and a validated SNP assay to genotype dbSNP rs2293052 in the gene *NOS1*. The resulting cluster plot (Figure 1) shows strong fluorescent signals for each allele and clear separation between the three clusters—easily discriminating the two homozygous and one heterozygous genotypes. In a comparison against five commercially available mixes, TaqMan Genotyping Master Mix shows the highest average call rate (Figure 2). Tight, well-separated clusters for each genotype provide exceptional call rates and, most importantly, accurate and efficient SNP analysis.

Consistent performance—even with difficult templates

TaqMan Genotyping Master Mix offers unambiguous allelic discrimination even for the most challenging assays. For example, GC-rich targets can present amplification challenges that reduce SNP detection because of persistent secondary structure. Human gDNA samples were genotyped for a SNP in a GC-rich region using a TaqMan SNP Assay to genotype dbSNP rs12214 in the cathepsin D gene. As shown in Figure 3, TaqMan Genotyping Master Mix yields brighter fluorescent signals, tighter clusters, and more accurate allele calling compared to a mix from supplier "S". These data demonstrate that TaqMan Genotyping Master Mix provides higher call rates for reliable SNP genotyping in difficult targets, eliminating the need to retest uncalled samples.

Copy number variation applications

Copy number variation is an important polymorphism in the human genome that can be associated with certain genomic disorders as well as some simple genetic and complex diseases. TaqMan Genotyping Master Mix, used with TaqMan Copy Number Assays, provides relative quantitation of an experimental gene compared to a reference gene in a duplex PCR. Between 1 and 3 copies of *CYP2D6, the* gene for a drug-metabolizing enzyme, were detected for 92 human gDNA samples when the samples were amplified using TaqMan Genotyping Master Mix (Figure 4).

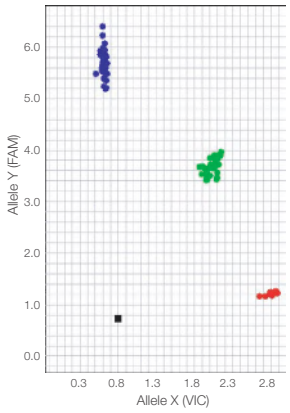

Figure 1. TaqMan Genotyping Master Mix provides bright fluorescence signals for discrete, well-separated allelic clusters. Cluster plot of 94 gDNA samples and two no-template controls genotyped using Applied Biosystems™ TaqMan™ SNP Genotyping Assay C__15969983_10, with PCR performed on the Applied Biosystems™ GeneAmp™ PCR System 9700 and allelic discrimination on the 7900HT Fast Real-Time PCR System.

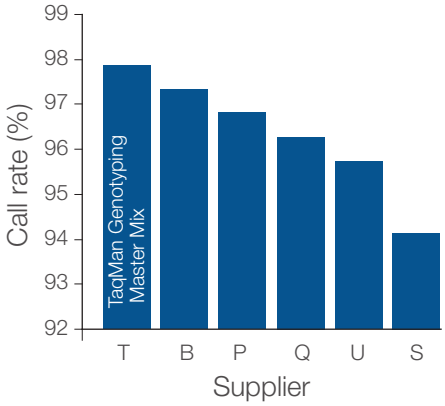

Figure 2. TaqMan Genotyping Master Mix provides the highest SNP call rates, outperforming other master mixes. Average call rates for 94 gDNA samples and two no-template controls genotyped using Applied Biosystems™ TaqMan™ SNP Genotyping Assay C__27102425_10, with PCR performed on the GeneAmp PCR System 9700 and allelic discrimination on the 7900HT Fast Real-Time PCR System.

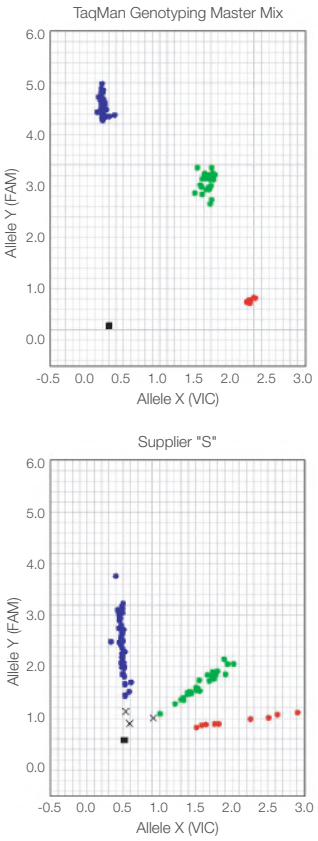

Figure 3. Consistent, reliable SNP detection in a GC-rich region using TaqMan Genotyping Master Mix. Genotyping assays were compared using TaqMan Genotyping Master Mix and a PCR master mix from supplier "S" on a set of 94 human gDNA samples (3 ng) and two no-template controls, using Applied Biosystems™ TaqMan™ SNP Assay C__12050942_10. PCR was performed on the GeneAmp PCR System 9700 and allelic discrimination on the 7900HT Fast Real-Time PCR System.

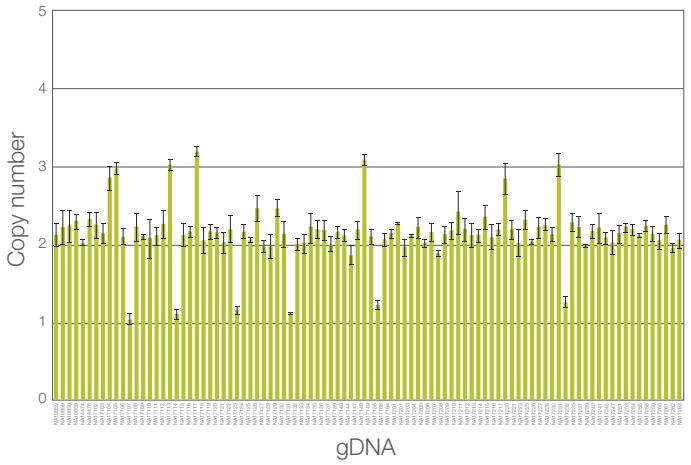

Figure 4. TaqMan Genotyping Master Mix is used for amplification in TaqMan Copy Number Assays. TaqMan Genotyping Master Mix is used with a TaqMan Copy Number Assay designed to target the *CYP2D6* gene, determining the copy number for this target in 92 gDNA samples. The RNase P reference gene is present in two copies per diploid genome or one copy per haploid genome.

Somatic mutation detection applications

TaqMan Mutation Detection Assays can detect somatic mutations in genes that are associated with cancer from different sample types, such as cell lines, formalin-fixed, paraffin-embedded tissue samples, and fresh frozen tissue samples. TaqMan Genotyping Master Mix, combined with TaqMan Mutation Detection Assays, which use competitive allele-specific Applied Biosystems™ TaqMan™ PCR (castPCR™) technology, can help detect rare amounts of mutated DNA in a sample that contains large amounts of normal, wild type DNA.

Pre- and post-PCR stability

Benchtop stability of real-time PCR mixes provides the flexibility to perform experiments over multiple days. To demonstrate the stability of TaqMan Genotyping Master Mix, both pre- and post-PCR storage conditions were tested to determine the effects on genotyping data. To evaluate pre-PCR stability, reactions were set up at room temperature (24°C), stored in the dark for up to three days, thermal-cycled for PCR, and read for endpoint fluorescence to assign alleles. To assess post-PCR stability, PCR was conducted immediately after reaction setup, but reactions were left on the bench for up to three days before measuring endpoint fluorescence for allelic discrimination. Even after three days at room temperature, either before or after PCR, TaqMan Genotyping Master Mix yielded tight clusters and reproducible results (Figure 5). The excellent benchtop stability of TaqMan Genotyping Master Mix gives ample flexibility for experimental setup and sample processing.

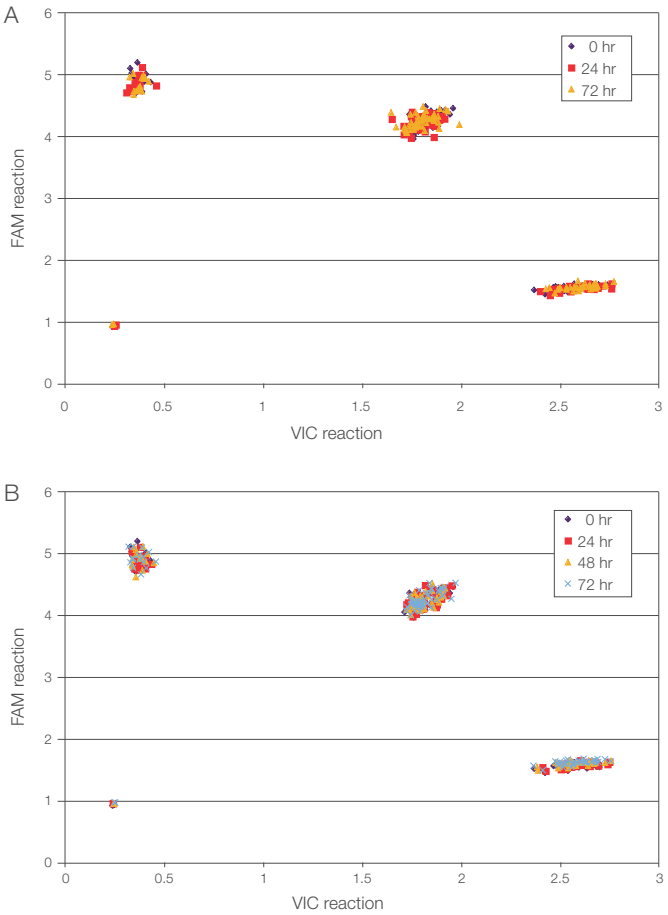

Figure 5. TaqMan Genotyping Master Mix provides pre- and post-PCR stability for up to 3 days. Genotyping reactions were set up using 94 gDNA samples and two no-template controls with TaqMan Genotyping Master Mix and TaqMan SNP Assay C___2188620_10. Reactions were left on the bench either **(A)** before or **(B)** after thermal cycling for the indicated amounts of time. PCR was conducted on the GeneAmp PCR System 9700 and allelic discrimination on the 7900HT Fast Real-Time PCR System.

Conclusion

TaqMan Genotyping Master Mix:

- Demonstrates extremely reliable allelic discrimination for SNP genotyping, with discrete clusters for high call rates even with challenging targets
- Provides reliable quantitation of DNA copy number when used with TaqMan Copy Number Assays
- Offers robust benchtop stability at room temperature, pre- and post-PCR, and consistent results across multiple instruments over multiple days to meet all throughput needs
- Complements TaqMan Mutation Detection Assays to provide high specificity and sensitivity for mutant allele detection

applied biosystems

Instruments and assays compatible with TaqMan Genotyping Master Mix (standard thermal cycling mode)

Instruments and assays
QuantStudio 3/5/6/7/12 Real-Time PCR Systems
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems
Applied Biosystems™ 7000, 7300, 7500, 7500 Fast, and 7900HT Fast Real-Time PCR Systems
Applied Biosystems™ Veriti™ Thermal Cyclers
GeneAmp PCR System 9700
Applied Biosystems™ 9800 Fast Thermal Cycler
TaqMan SNP Genotyping Assays
TaqMan Drug Metabolism Genotyping Assays
TaqMan Copy Number Assays
TaqMan Mutation Detection Assays
Applied Biosystems™ Custom TaqMan™ SNP Genotyping Assays
21 CFR Part 11 compliance module

Ordering information

Product	Unit size	Reactions*	Cat. No.
TaqMan Genotyping Master Mix			
Mini pack	1 mL tube	40	4371353
1-pack	10 mL bottle	400	4371355
2-pack	2 x 10 mL bottles	800	4381656
Single bulk pack	50 mL bottle	2,000	4371357
Multi-bulk pack	2 x 50 mL bottles	4,000	4381657
Quick Reference Card	1 card	—	4371130
Protocol	1 protocol	—	4371131

* Assumes 50 µL reaction volume; consult protocol for other recommended reaction volumes.

Find out more at **thermofisher.com/taqmanmm**

ThermoFisher SCIENTIFIC

applied biosystems
by life technologies

TaqMan® Universal PCR Master Mix

Pub. no. 4480831 **Rev.** A

Catalog No.	Quantity	Storage condition
4304437, 1-Pack	1 × 5 mL	Store at 2°C to 8°C.
4364338, 2-Pack	2 × 5 mL	
4364340, 5-Pack	5 × 5 mL	
4305719, 10-Pack	10 × 5 mL	
4318157, 10 Unit Pack	10 × 5 mL	
4326708, 1 Bulk Pack	1 × 50 mL	

Product Use

Research Use Only. Not for use in diagnostic procedures.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Procedure for use

Note: For detailed procedures using the TaqMan® Universal PCR Master Mix refer to the *TaqMan® Universal PCR Master Mix User Guide* (Pub. no. 4304449) available from **www.lifetechnologies.com/support**.

Limited Use Label License No. 358: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or OutLicensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Use Label License No. 413: PCR Enzymes

Notice to Purchaser: For research purposes only. Diagnostic uses require a separate license from Roche.

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30 May 2013

WHITE PAPER

Modifications to TaqMan® Universal PCR Master Mix have no effect on functional performance or stability

Abstract

Beginning in April 2014, minor modifications will be made to the TaqMan® Universal PCR Master Mix and TaqMan® Universal PCR Master Mix, No AmpErase® UNG. These modifications include transfer of manufacturing from Branchburg, NJ to Warrington, UK and changes in source/vendor for some raw material components. In recognition of the importance of these products in existing protocols, we have conducted extensive studies to demonstrate functional equivalency. These changes have been shown to have no effect on the overall functional characteristics of the master mixes. This paper describes the testing process and test results for dynamic range, sensitivity, specificity, discrimination, C_t and ΔRn comparisons, and pre-PCR stability. The results show no differences between the current TaqMan® Universal PCR Master Mix manufactured in Branchburg, NJ and validation lots manufactured in Warrington, UK with the described changes.

Introduction

As part of the ongoing efforts at Life Technologies to provide the highest quality qPCR master mixes, modifications will be made to the TaqMan® Universal PCR Master Mix and TaqMan® Universal PCR Master Mix, No AmpErase® UNG, beginning in April 2014. The impacted catalog numbers are listed in the Appendix (Table 4). These modifications include the transfer of manufacturing from Branchburg, NJ (Roche Molecular Systems, third party manufacturer) to Warrington, UK (Thermo Fisher Scientific Inc., formerly Life Technologies). The formulation remains unchanged; however, there is a change in source/vendor for some raw material components such as dNTPs and AmpliTaq Gold® DNA Polymerase.

The execution of the transfer is tightly controlled in a vigilant manner to minimize disruption to the many laboratories that rely on these reagents every day. The most important aspect of this process is to maintain consistent performance and reliability. This paper describes the testing process, involving multiple lots, with a number of gene expression assays, to assess dynamic range, sensitivity, specificity, discrimination, and pre-PCR stability. Results show no functional difference between current TaqMan® Universal PCR Master Mix manufactured in Branchburg, NJ and validation lots manufactured in Warrington, UK with the implemented changes. A separate document is available describing testing and results for TaqMan® Universal PCR Master Mix, No AmpErase® UNG.

Materials and methods

Material lots tested

For performance comparisons, four unique lots of TaqMan® Universal PCR Master Mix made in Branchburg, NJ (designated R1, R2, R3, and R4) were ordered in 50 mL kits directly from the Life Technologies website.

life technologies™

Three validation lots (designated L1, L2, and L3) were formulated in full-scale volumes at the Warrington, UK manufacturing site.

Validation material QC

Validation lots passed analytical QC specifications set for Mg^{2+} concentration (HPIC), dNTP concentrations (HPLC), DNase/RNase activity, *E. coli* contamination, and pH. They also passed functional tests with RNase P and ß-actin gene expression assays.

Functional performance

Dynamic range

Four TaqMan® Gene Expression Assays (FAM™-MGB, 20X) were tested across a 5-log concentration dynamic range: FN1 (Hs00277509_m1), PGK1 (Hs99999906_m1), RPLP0 (Hs99999902_m1), and B2M (Hs00187842_m1). The serial dilution spanned final concentrations of 100 ng to 1 pg of cDNA per reaction. The cDNA template was synthesized from Universal Human RNA (Stratagene) and the SuperScript® VILO™ cDNA Synthesis Kit (Cat. No. 11754250). Additionally, duplex performance was tested with B2M and an exogenous internal positive control (IPC) (VIC®-TAMRA™ probe; Cat. No. 4308323). Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (50°C, 2 min; 95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with six technical replicates and was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to generate amplification plots and determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Gene expression panel

138 TaqMan® Gene Expression Assays (FAM™-MGB, 20X) and six TaqMan® endogenous controls were functionally tested. Refer to Table 3 in the Appendix for assay information. The six endogenous controls were primer-limited (150 nM instead of 900 nM) and had VIC®-TAMRA™ probes. Reactions followed the standard product protocol for a 10 µL reaction volume. 1 ng of cDNA synthesized from Universal Human RNA (Stratagene) and the SuperScript® VILO™ cDNA Synthesis Kit was used as the final template amount for all reactions. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (50°C, 2 min; 95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with six technical replicates. ViiA™ 7 software v1.2.2 was used to generate amplification plots and determine C_t values (analysis settings: auto-baseline; threshold: set at 0.1).

Pre-PCR stability

The 144-assay gene expression panel was also tested for pre-PCR stability of 24 and 72 hours. Reactions were assembled as described above and the sealed 384-well plates were stored on the benchtop at room temperature, exposed to intermittent light before being run on the ViiA™ 7 Real-Time PCR System. Thermal cycling conditions and analysis settings were identical to those previously described.

Specificity

The 144-assay gene expression panel was also tested for specificity by running "no-template control" (NTC) reactions. Reactions were assembled as described above, except that water replaced the sample volume. Thermal cycling conditions and analysis settings were identical to those previously described.

Sensitivity

The RNase P assay from the TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831) was tested with CEPH gDNA (Cat. No. 403062) diluted to a final concentration of 2 copies per 20 µL reaction. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (50°C, 2 min; 95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with 20 technical replicates to overcome sampling error at the low concentration. NTC reactions were tested with six technical replicates. Each set of replicates was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Discrimination

The RNase P assay from the TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831) was tested with CEPH gDNA (Cat. No. 403062) diluted to final concentrations of either 1,600 or 800 copies per 20 µL reaction. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR

System using universal cycling conditions (50°C, 2 min; 95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with 10 technical replicates. NTC reactions were tested with six technical replicates. Each set of replicates was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Results

Dynamic range

With each of the four TaqMan® Gene Expression Assays—FN1, PGK1, RPLP0, and B2M—no difference was observed between master mix lots for the mean C_t values calculated from the 18 data points at each dilution point. Three validation lots (UMM L1–L3) were compared against four lots of the current product (UMM R1–R4).

The following graphs depict the mean C_t vs. log concentration for the four tested assays (Figures 1A–4A) and a representation of the amplification plots for each assay (Figures 1B–4B), showing strong linearity (R^2 values ≥ 0.998), tight clustering across the series, and clean amplification curves.

Figures 5A and 5B depict the dilution plot and amplification curve, respectively, of the duplex B2M assay with IPC, once again displaying strong linearity (R^2 ≥ 0.999) and amplification.

PCR efficiency values were determined for the three tested lots using the slope of the mean C_t values for each of the four assays and the duplex assay (see Figure 6). Efficiencies across lots were consistent (< 2% difference) for each tested assay.

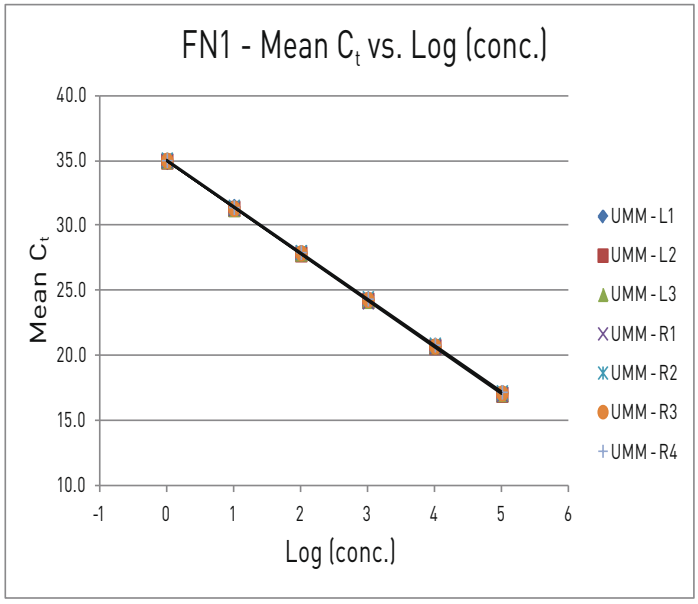


Figure 1A. FN1 (Hs00277509_m1) mean C_t values plotted across 5 logs of cDNA sample for all seven lots of Universal PCR Master Mix.

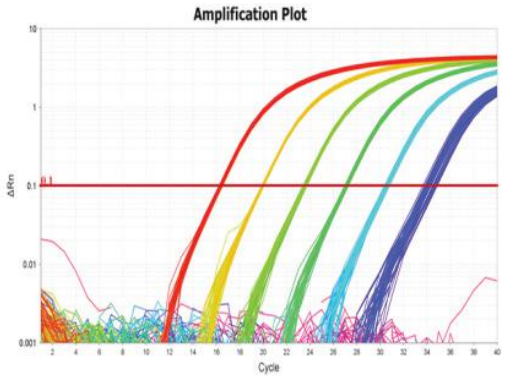


Figure 1B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point.

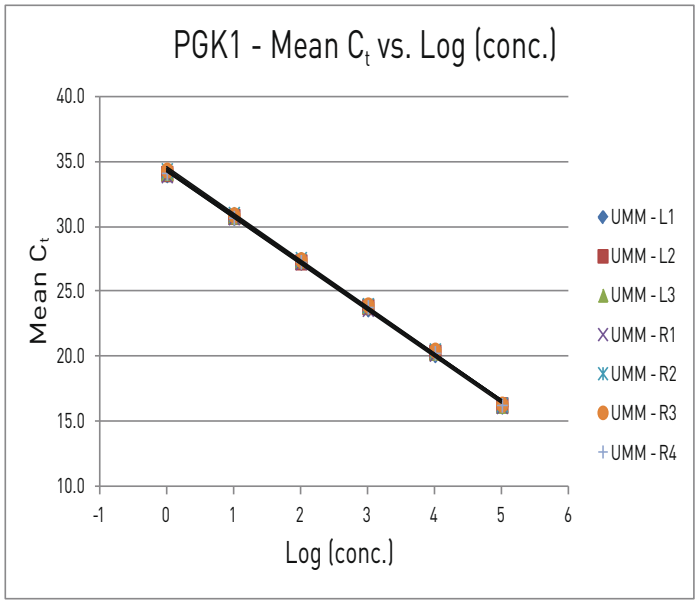


Figure 2A. PGK1 (Hs99999906_m1) mean C_t values plotted across 5 logs of cDNA sample for all seven lots of Universal PCR Master Mix.

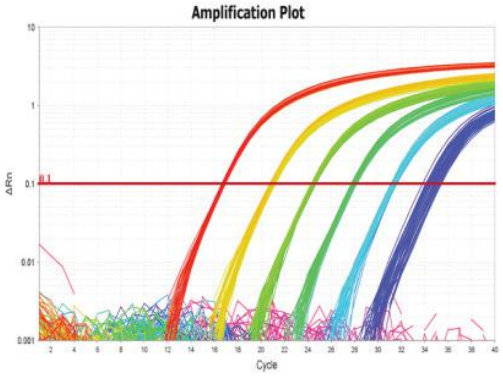


Figure 2B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point.

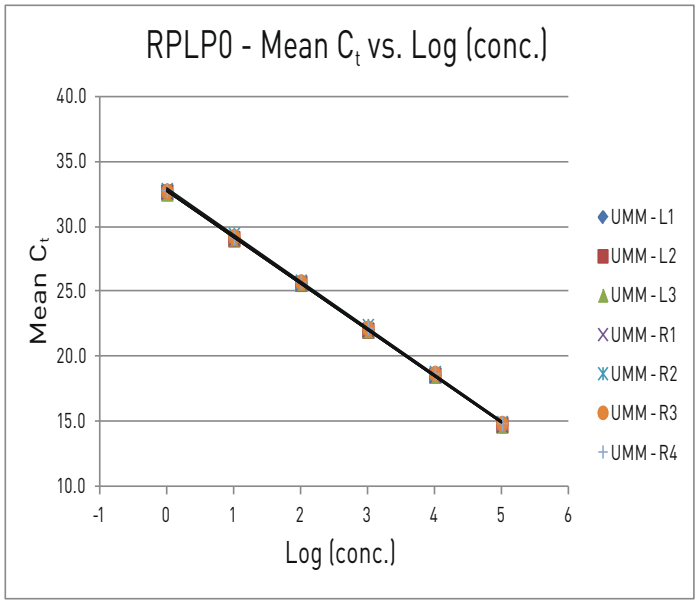


Figure 3A. RPLP0 (Hs99999902_m1) mean C_t values plotted across 5 logs of cDNA sample for all seven lots of Universal PCR Master Mix.

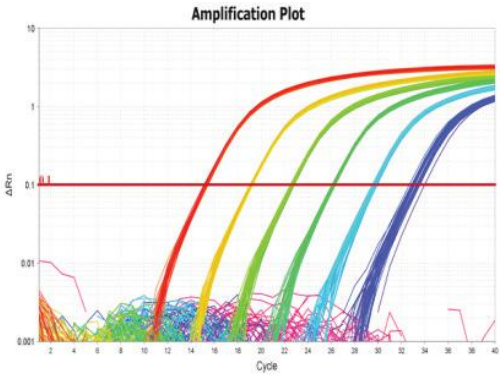


Figure 3B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point.

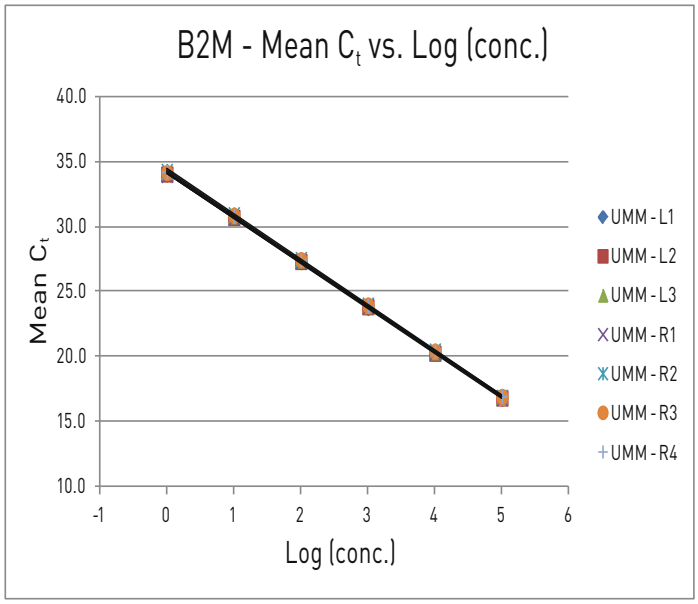


Figure 4A. B2M (Hs00363670_m1) mean C_t values plotted across 5 logs of cDNA sample for all seven lots of Universal Master Mix.

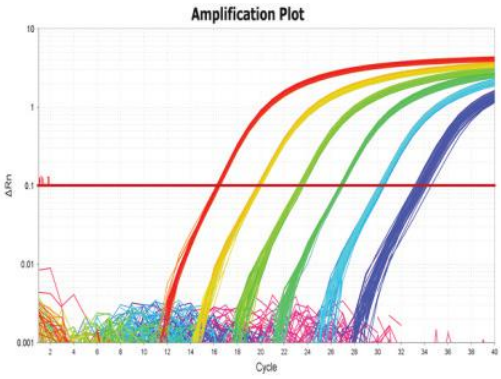


Figure 4B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point.

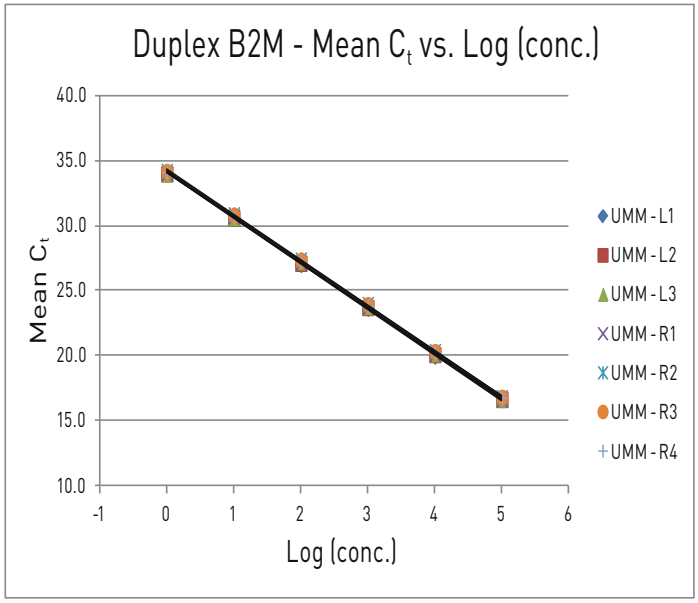


Figure 5A. B2M (Hs00363670_m1) mean C_t values when duplexed with an exogenous IPC (4308323); plotted across 5 logs of cDNA sample for all seven lots of Universal PCR Master Mix.

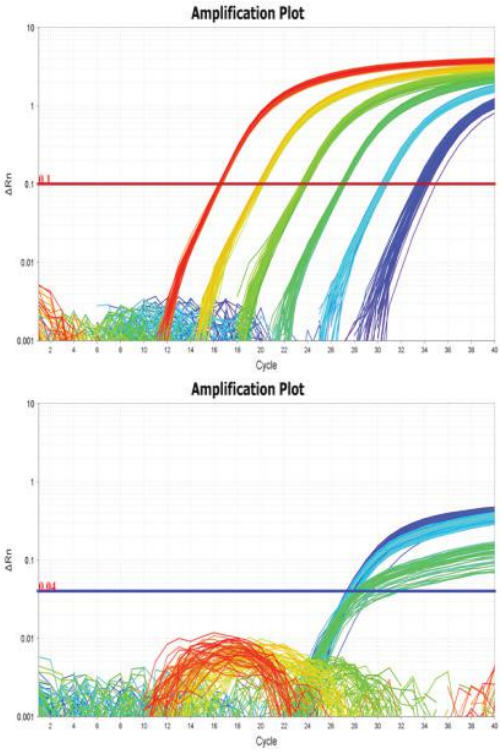


Figure 5B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point and for the exogenous IPC (bottom). Note that at high concentrations the B2M assay out-competes the exogenous IPC, accounting for the observed variability in the IPC amplification plot.

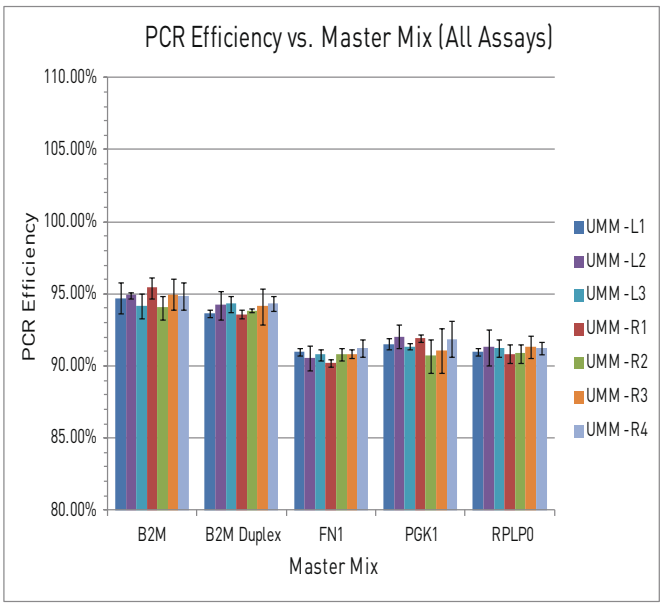
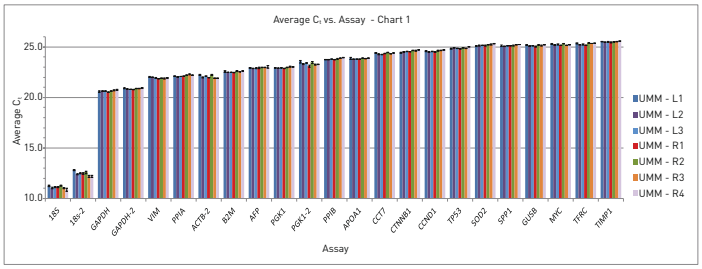


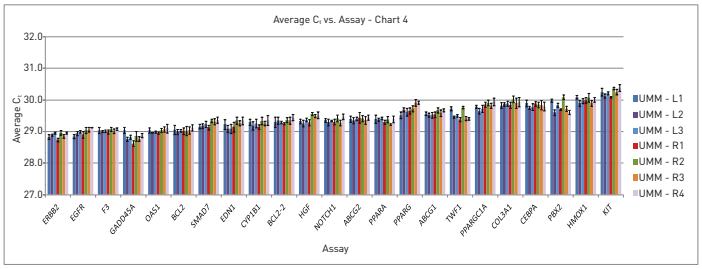
Figure 6. PCR efficiency values for each gene expression assay, calculated from the slope of the mean C_t vs. log(conc.) plot. Each assay exhibited consistent efficiencies between lots of Universal PCR Master Mix (differences <2%).

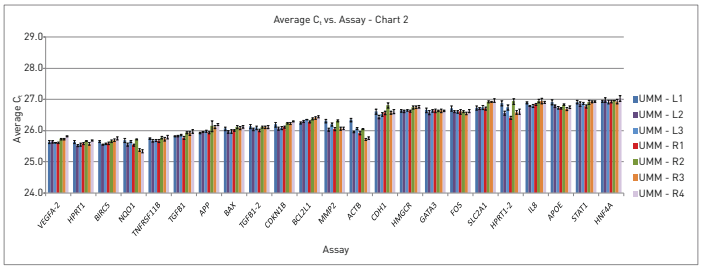
Gene expression panel

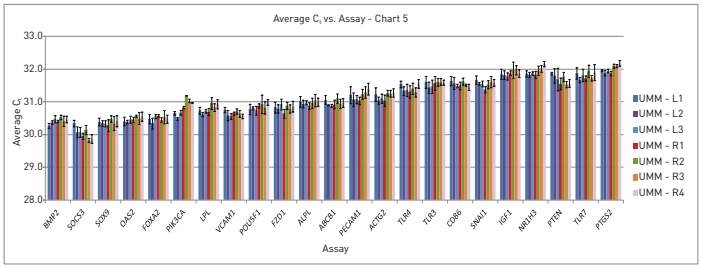
Performance in gene expression assays was tested across the seven lots using 138 TaqMan® Gene Expression Assays and six endogenous control assays with average C_t values calculated from six technical replicates. The plots in Figure 7 show clear consistency of C_t values (average difference: <0.4%) within each assay across the seven lots from low-, medium-, and high-expressing genes.

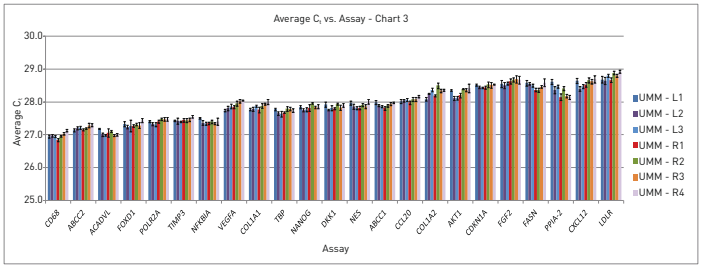
Figure 8 depicts the mean ΔRn values for the same 144 TaqMan® Gene Expression Assays. No real differences are observed, with 99% of assays showing between-lot differences of <20% (calculated with the mean ΔRn of each population for each assay).











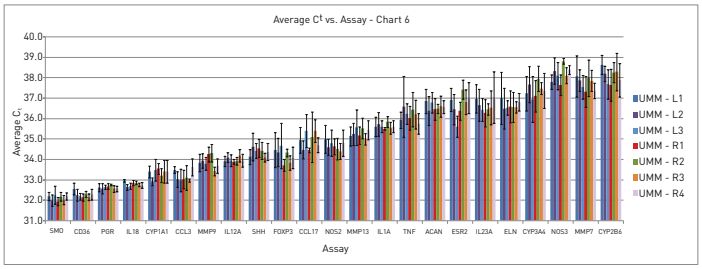


Figure 7. Mean C_t values obtained from each master mix (L1–L3 of Universal Master Mix and current lots R1–R4 Universal Master Mix) across all 138 TaqMan® Gene Expression Assays and 6 endogenous control assays. Six assays were excluded because of no amplification with any lot groups: ADIPOQ, CYP2C9, IL12B, IL2, FABP4, and FASLG. Six technical replicates per assay were run with each master mix on the same PCR plate (n = 6; error bars are ±1σ).

Figure 8. Mean ΔRn values obtained from each master mix (L1–L3 of Universal Master Mix and current lots R1–R4 Universal Master Mix) across all 138 TaqMan® Gene Expression Assays and 6 endogenous control assays. Six assays were excluded because of no amplification with any lot groups: ADIPOQ, CYP2C9, IL12B, IL2, FABP4, and FASLG. Six technical replicates per assay were run with each master mix on the same PCR plate (n = 6; error bars are ±1σ).

Pre-PCR stability

Extensive stability testing was performed on all 144 assays used for the performance testing above. Assembled reaction plates were sealed and left at room temperature for 24 and 72 hours, and all results calculated and collated. Figure 9 is a representation from a single validation lot (left) and current lot (right), showing mean C_t values for each assay with clear correlation across time points (Pearson's value correlation scores were ≥0.998 for validation lots and current lots). Similarly, Figure 10 shows data for mean ΔRn values for the same two lots (Pearson's r-value correlation scores were ≥ 0.98 for validation lots and ≥0.97 for current lots).

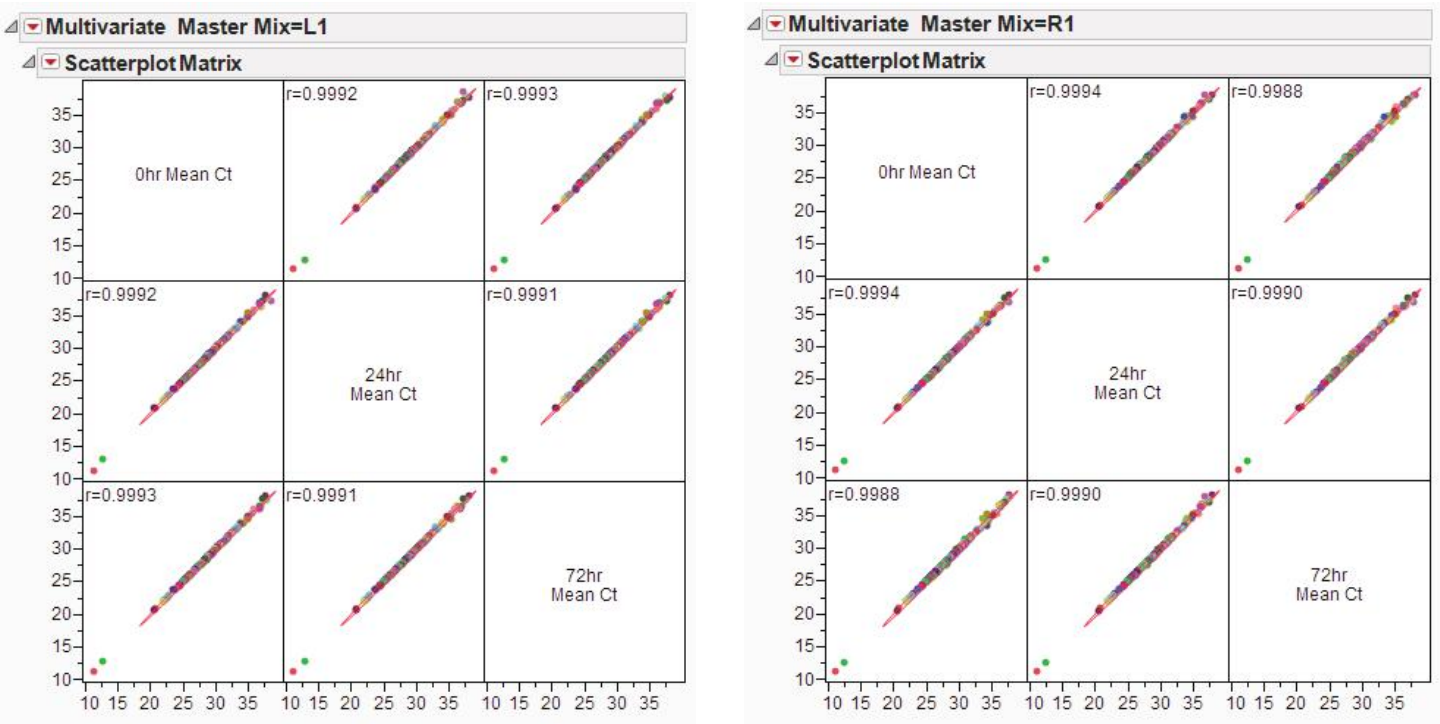


Figure 9. Mean C_t values compared between benchtop stability time points of 0, 24, and 72 hours (JMP 10, SAS Inc.). Each point represents a single assay out of the 144 gene expression assay panel. Data are displayed for validation lot L1 (left) and a representative lot of current UMM (right). Correlation values were ≥0.998, with red oval boundaries representing the 95% confidence curves (α = 0.05).

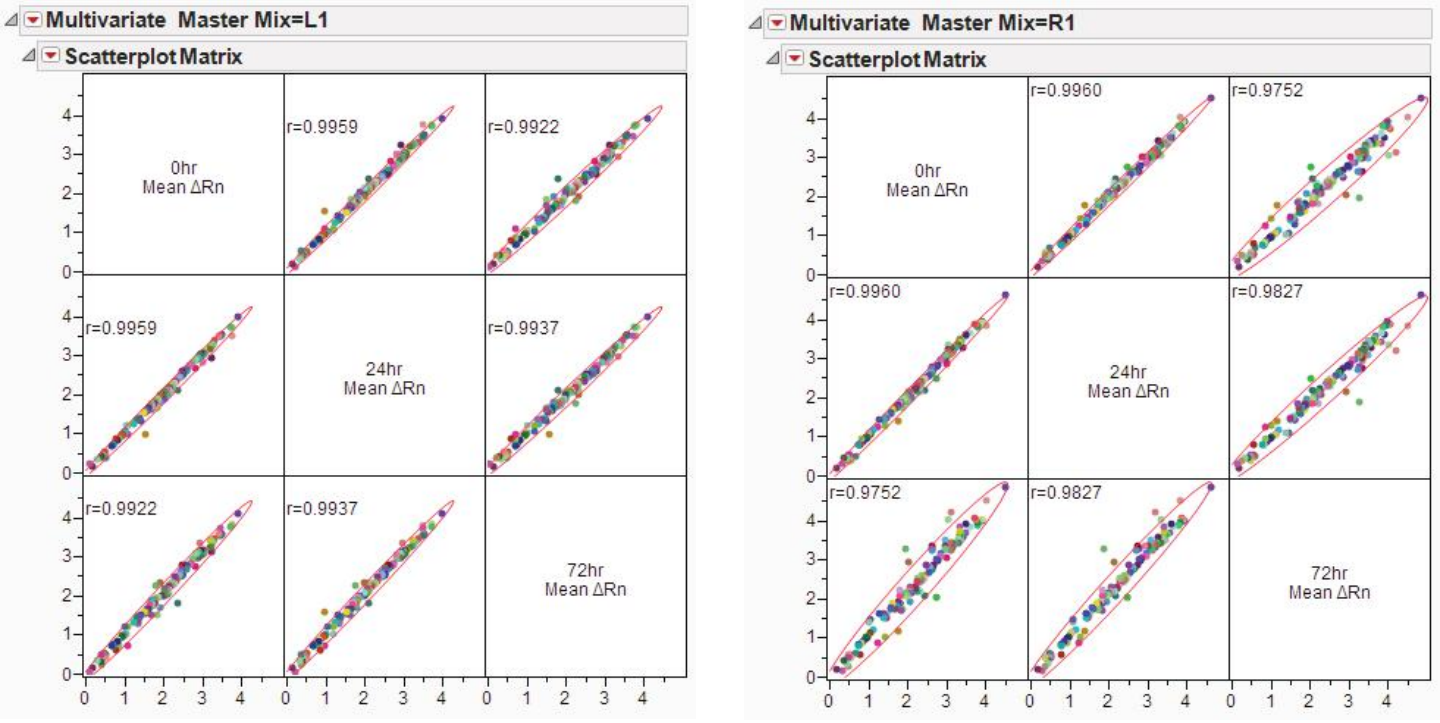


Figure 10. Mean ΔRn values compared between benchtop stability time points of 0, 24, and 72 hours (JMP 10, SAS Inc.). Each point represents a single assay out of the 144 gene expression assay panel. Data are displayed for validation lot L1 (left) and a representative lot of Branchburg, NJ-made UMM (right). Correlation values were ≥0.97, with red oval boundaries representing the 95% confidence curves (α = 0.05).

Specificity

To test performance with regards to nonspecific amplification, primer-dimer formation, and other specificity concerns, the same 144 TaqMan® Gene Expression Assays were run using water in place of template. Greater than 98% of NTC reactions across all assays exhibited no amplification. Table 1 shows the NTC amplification percentage statistics (C_t values <40) for the current and validation lots, with no statistical difference (p = 0.781) observed between the two populations.

Sensitivity

Sensitivity performance was tested using the TaqMan® RNase P assay on CEPH gDNA diluted to a 2-copy final dilution. Figure 11 shows distribution graphs for a total of 60 data points (collected across three runs of 20 replicates) for a single, representative master mix lot. Fitted normal distribution curves are shown in red.

NTC Amplification Percentage.

	Mean	St. Dev.	95% Conf.	Interval
Validation	1.33%	0.10%	1.22%	1.44%
Current	1.39%	0.32%	1.08%	1.70%

Table 1. Percentage of NTC reactions containing positive amplification (nonspecific amplification) vs. master mix (three validation lots (n = 3) and four current lots (n = 4)). All 144 gene expression assays were run with four technical replicates each (total n = 576), and the percentage was calculated for each master mix lot. Positive amplifications were largely from both 18S assays (Hs99999901_s1; 4310893E).

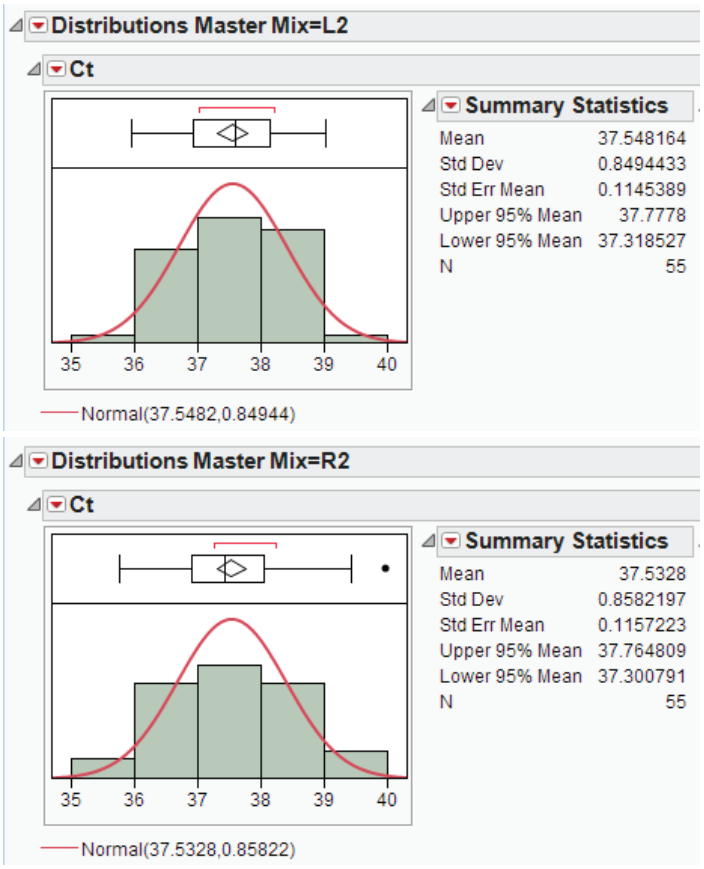


Figure 11. Distribution of RNase P C_t values obtained from 2 copies/rxn of CEPH gDNA. Data from three PCR setups were combined (20 technical replicates each, total n = 60). Data are displayed using the JMP 10 Statistical Software package (SAS Institute Inc.) for a single, representative master mix lot. Reactions that did not amplify were considered to have 0 copies of CEPH gDNA, a result of sampling error near the digital concentration range.

Discrimination

Two-fold discrimination performance was tested using the TaqMan® RNase P assay on CEPH gDNA diluted to either 1,600 or 800 copies per reaction. Figure 12 shows distribution graphs for a total of 30 data points (collected across three runs of 10 replicates) for a single, representative master mix lot. Fitted normal distribution curves are shown in red for each mean copy number. Mean C_t values for the two concentrations were separated by >6 SD, providing sufficient separation for 2-fold copy number resolution.

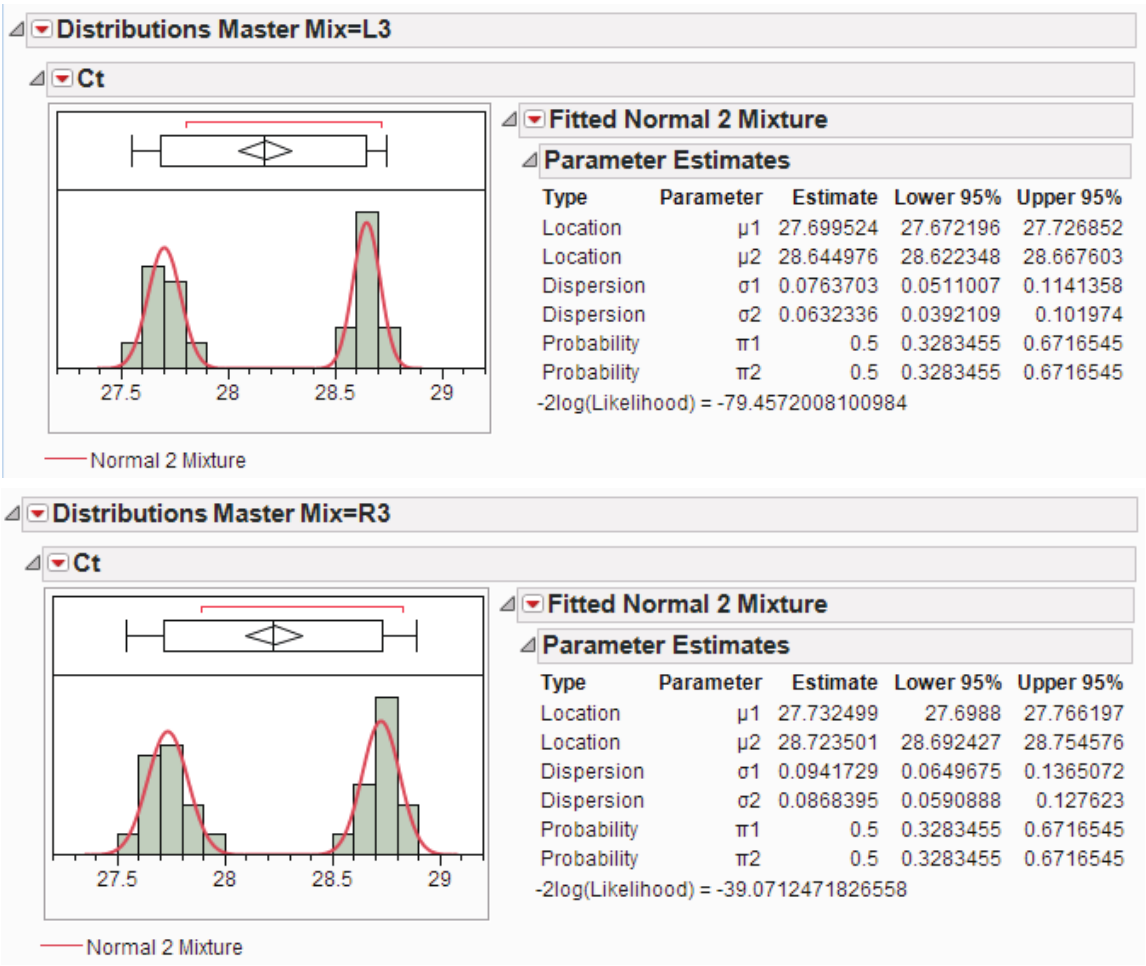


Figure 12. Distributions of RNase P C_t values obtained from 1,600 and 800 copies/rxn of CEPH gDNA. Data from three PCR setups were combined (10 technical replicates for each concentration, total n = 30). Data are displayed using the JMP 10 Statistical Software package (SAS Institute Inc.) for a single, representative master mix lot.

Conclusion

Validation lots incorporating the manufacturing changes were extensively tested against current lots of TaqMan® Universal PCR Master Mix to ascertain whether the changes would impact the functionality of the reagents. Tests showed no discernible differences in dynamic range, sensitivity, specificity, discrimination, and pre-PCR stability as well as general assay performance for gene expression assays selected to cover a representative range.

We are confident that the TaqMan® Universal PCR Master Mix offered after April 2014 will continue to perform with the same quality, integrity, and functional performance as exists today.

Appendix

Table 2. Linear dynamic range assays.

Number	Assay ID	Gene symbol
1	Hs00277509_m1	FN1
2	Hs99999906_m1	PGK1
3	Hs99999902_m1	RPLP0
4	Hs00187842_m1	B2M
Duplex	4308323	Exo IPC

Table 3. Gene expression assays.

Number	Assay ID	Gene symbol
1	Hs99999901_s1	18S
2	Hs00184500_m1	ABCB1
3	Hs00219905_m1	ABCC1
4	Hs00245154_m1	ABCG1
5	Hs00153936_m1	ACAN
6	Hs99999903_m1	ACTB
7	Hs00242273_m1	ACTG2
8	Hs00173490_m1	AFP
9	Hs00178289_m1	AKT1
10	Hs00163641_m1	APOA1
11	Hs00171168_m1	APOE
12	Hs99999907_m1	B2M
13	Hs00180269_m1	BAX
14	Hs00153353_m1	BIRC5
15	Hs00277039_m1	CCND1
16	Hs00154355_m1	CD68
17	Hs00170423_m1	CDH1
18	Hs00355782_m1	CDKN1A
19	Hs00153277_m1	CDKN1B
20	Hs00269972_s1	CEBPA
21	Hs00164004_m1	COL1A1
22	Hs00171022_m1	CXCL12
23	Hs00164383_m1	CYP1B1
24	Hs00604506_m1	CYP3A4
25	Hs00183740_m1	DKK1
26	Hs00174961_m1	EDN1
27	Hs00230957_m1	ESR2
28	Hs00266645_m1	FGF2
29	Hs00170630_m1	FOS
30	Hs00232764_m1	FOXA2
31	Hs00203958_m1	FOXP3
32	Hs00268943_s1	FZD1
33	Hs00169255_m1	GADD45A
34	Hs99999908_m1	GUSB
35	Hs00168352_m1	HMGCR
36	Hs00157965_m1	HMOX1
37	Hs00168405_m1	IL12A

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
38	Hs00155517_m1	IL18
39	Hs00174092_m1	IL1A
40	Hs00174103_m1	IL8
41	Hs00174029_m1	KIT
42	Hs00234422_m1	MMP2
43	Hs00159163_m1	MMP7
44	Hs02387400_g1	NANOG
45	Hs00707120_s1	NES
46	Hs00167166_m1	NOS3
47	Hs00242943_m1	OAS1
48	Hs00855025_s1	PBX2
49	Hs99999906_m1	PGK1
50	Hs00172183_m1	PGR
51	Hs00180679_m1	PIK3CA
52	Hs00172187_m1	POLR2A
53	Hs00742896_s1	POU5F1
54	Hs00173304_m1	PPARGC1A
55	Hs99999904_m1	PPIA
56	Hs00168719_m1	PPIB
57	Hs00197884_m1	SLC2A1
58	Hs00170665_m1	SMO
59	Hs00269575_s1	SOCS3
60	Hs00167093_m1	SPP1
61	Hs00234829_m1	STAT1
62	Hs00427620_m1	TBP
63	Hs99999911_m1	TFRC
64	Hs00171257_m1	TGFB1
65	Hs99999918_m1	TGFB1
66	Hs00171558_m1	TIMP1
67	Hs00174128_m1	TNF
68	Hs00171068_m1	TNFRSF11B
69	Hs00173626_m1	VEGFA
70*	4310893E	18s-2
71*	4310881E	ACTB-2
72*	4310884E	GAPDH-2
73	Hs00166123_m1	ABCC2
74	Hs00184979_m1	ABCG2
75	Hs00817723_g1	ACADVL
76	Hs00605917_m1	ADIPOQ
77	Hs00758162_m1	ALPL
78	Hs00169098_m1	APP
79	Hs00153350_m1	BCL2
80	Hs00608023_m1	BCL2
81	Hs00236329_m1	BCL2L1
82	Hs00154192_m1	BMP2
83	Hs00171074_m1	CCL17
84	Hs00171125_m1	CCL20
85	Hs00234142_m1	CCL3

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
86	Hs00362446_m1	CCT7
87	Hs00169627_m1	CD36
88	Hs00199349_m1	CD86
89	Hs00164099_m1	COL1A2
90	Hs00164103_m1	COL3A1
91	Hs00170025_m1	CTNNB1
92	Hs00153120_m1	CYP1A1
93	Hs00167937_g1	CYP2B6
94	Hs00426397_m1	CYP2C9
95	Hs00193306_m1	EGFR
96	Hs00355783_m1	ELN
97	Hs00170433_m1	ERBB2
98	Hs00175225_m1	F3
99	Hs00609791_m1	FABP4
100	Hs00181225_m1	FASLG
101	Hs00188012_m1	FASN
102	Hs00270117_s1	FOXD1
103	Hs02758991_g1	GAPDH
104	Hs00231122_m1	GATA3
105	Hs00300159_m1	HGF
106	Hs00230853_m1	HNF4A
107	Hs99999909_m1	HPRT1
108	Hs00153126_m1	IGF1
109	Hs00233688_m1	IL12B
110	Hs00174114_m1	IL2
111	Hs00372324_m1	IL23A
112	Hs00181192_m1	LDLR
113	Hs00173425_m1	LPL
114	Hs00233992_m1	MMP13
115	Hs00234579_m1	MMP9
116	Hs00153408_m1	MYC
117	Hs00153283_m1	NFKBIA
118	Hs00167248_m1	NOS2
119	Hs00413187_m1	NOTCH1
120	Hs00168547_m1	NQO1
121	Hs00172885_m1	NR1H3
122	Hs00159719_m1	OAS2
123	Hs00169777_m1	PECAM1
124	Hs00231882_m1	PPARA
125	Hs00234592_m1	PPARG
126	Hs00829813_s1	PTEN
127	Hs00153133_m1	PTGS2
128	Hs00179843_m1	SHH
129	Hs00178696_m1	SMAD7
130	Hs00195591_m1	SNAI1

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
131	Hs00167309_m1	SOD2
132	Hs00165814_m1	SOX9
133	Hs00165949_m1	TIMP3
134	Hs00152933_m1	TLR3
135	Hs00152939_m1	TLR4
136	Hs00152971_m1	TLR7
137	Hs00153340_m1	TP53
138	Hs00702289_s1	TWF1
139	Hs00365486_m1	VCAM1
140	Hs00900054_m1	VEGFA
141	Hs00185584_m1	VIM
142*	4310890E	HPRT1-2
143*	4310885E	PGK1-2
144*	4310883E	PPIA-2

* TaqMan® endogenous control assays; primer-limited (150 nM instead of 900 nM) with VIC-TAMRA probes.

Table 4. Affected catalog numbers.

List of all affected catalog numbers of TaqMan® Universal PCR Master Mix and TaqMan® Universal Master Mix, No AmpErase® UNG impacted by the manufacturing site change.

Product name	Cat. No.
TaqMan® Universal PCR Master Mix, 1-Pack (1 x 5 mL)	4304437
TaqMan® Universal PCR Master Mix, 10-Pack (10 x 5 mL)	4305719
TaqMan® Universal PCR Master Mix, 10 Unit Pack (10 x 5 mL)	4318157
TaqMan® Universal PCR Master Mix, 1 Bulk Pack (1 x 50 mL)	4326708
TaqMan® Universal PCR Master Mix, 2-Pack (2 x 5 mL)	4364338
TaqMan® Universal PCR Master Mix, 5-Pack (5 x 5 mL)	4364340
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 1-Pack (1 x 5 mL)	4324018
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 10 Unit Pack (10 x 5 mL)	4324020
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 1 Bulk Pack (1 x 50 mL)	4326614
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 2-Pack (2 x 5 mL)	4364341
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 5-Pack (5 x 5 mL)	4364343

First Strand cDNA Synthesis Kit

Catalog Number K1612

Pub. No. MAN0012612 **Rev.** B00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

Contents

Cat. No.	Contents	Amount	Storage
K1612 (100 rxns)	M-MuLV Reverse Transcriptase (20 U*/µL)	240 µL	-25 °C to -15 °C
	RiboLock RNase Inhibitor (20 U**/µL)	120 µL	
	5X Reaction Buffer 250 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT	500 µL	
	10mM dNTP Mix	250 µL	
	Oligo$(dT)_{18}$ Primer 100 µM, 0.5 µg/µL	120 µL	
	Random Hexamer Primer 100 µM, 0.2 µg/µL	120 µL	
	Forward GAPDH Primer, 10 µM 5' – CAAGGTCATCCATGACAACTTTG – 3'	20 µL	
	Reverse GAPDH Primer, 10 µM 5' – GTCCACCACCCTGTTGCTGTAG - 3'	20 µL	
	Control GAPDH RNA 1.3 kb 3'-poly(A) tailed RNA transcript, 0.05 µg/µL	20 µL	
	Water, nuclease-free	2 × 1.25 mL	

* One unit of M-MuLV RT incorporates 1 nmol of dTMP into a polynucleotide fraction in 10 min at 37 °C.
** One unit of RiboLock RNase Inhibitor inhibits the activity of 5 ng RNase A by 50 %.

Description

Thermo Scientific™ First Strand cDNA Synthesis Kit is a complete system for efficient synthesis of first strand cDNA from mRNA or total RNA templates. The kit uses M-MuLV Reverse Transcriptase, which has lower RNase H activity compared to AMW reverse transcriptase. The enzyme maintains activity at 37 °C and is suitable for synthesis of cDNA up to 9 kb.
The recombinant Thermo Scientific™ RiboLock™ RNase Inhibitor, supplied with the kit, effectively protects RNA from degradation at temperatures up to 55 °C.
The kit is supplied with both oligo$(dT)_{18}$ and random hexamer primers. Random hexamer primers bind non-specifically and are used to synthesize cDNA from all RNAs in total RNA population. The oligo$(dT)_{18}$ primer selectively anneals to the 3'-end of poly(A) RNA, synthesizing cDNA only from poly(A) tailed mRNA. Gene-specific primer may also be used with the kit to prime synthesis from a specified sequence.
First strand cDNA synthesized with this system can be directly used as a template in PCR or real-time PCR. It is also ideal for second strand cDNA synthesis or linear RNA amplification. Radioactively and non-radioactively labeled nucleotides can be incorporated into first strand cDNA for use as a probe in hybridization experiments, including microarrays.

Storage

All components of the kit should be stored at -20 °C. Keep control RNA at -70 °C for longer storage.

Important Notes

Avoiding ribonuclease contamination

RNA purity and integrity is essential for synthesis of full-length cDNA. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. All components of the kit have been rigorously tested to ensure that they are RNase free. To prevent contamination both the laboratory environment and all prepared solutions must be free of RNases.

General recommendations to avoid RNase contamination:

- DEPC-treat all tubes and pipette tips to be used in cDNA synthesis or use certified nuclease-free labware.
- Wear gloves when handling RNA and all reagents, as skin is a common source of RNases. Change gloves frequently.
- Use RNase-free reagents, including high quality water (e.g., Water, nuclease-free (#R0581).
- Use an RNase inhibitor, such as RiboLock™ RNase Inhibitor (provided with the kit) to protect RNA from the activity of RNases.
- Keep all kit components tightly sealed when not in use. Keep all tubes tightly closed during the reverse transcription reaction.

Template RNA

Total cellular RNA isolated by standard methods is suitable for use with the kit. Purified RNA must be free of salts, metal ions, ethanol and phenol to avoid inhibiting the cDNA synthesis reaction. Trace contaminants can be removed by ethanol precipitation of the RNA followed by two washes of the pellet with cold 75% ethanol.

For RT-PCR applications, template RNA must be free of DNA contamination. Prior to cDNA synthesis, RNA can be treated with DNase I, RNase-free (#EN0521) to remove trace amounts of DNA. Always perform a control (RT-minus) reaction which includes all components for RT-PCR except for the reverse transcriptase enzyme.

Removal of genomic DNA from RNA preparations

1. Add to an RNase-free tube:

Component	Volume
RNA	1 µg
10X Reaction Buffer with $MgCl_2$	1 µL
DNase I, RNase-free (#EN0521)*	1 µL (1 U)
Water, nuclease-free	to 10 µL

*Do not use more than 1 U of DNase I, RNase-free per 1 µg of RNA.

2. Incubate at 37 °C for 30 min.
3. Add 1 µL 50 mM EDTA and incubate at 65 °C for 10 min. RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent (1). Alternatively, use phenol/chloroform extraction.
4. Use the prepared RNA as a template for reverse transcriptase.

RNA sample quality

Assess RNA integrity prior to cDNA synthesis. The most common method is denaturing agarose gel electrophoresis followed by ethidium bromide staining. If both 18S and 28S rRNA appear as sharp bands after electrophoresis of total eukaryotic RNA, the RNA is considered to be intact. The 28S rRNA band should be approximately twice as intense as the 18S rRNA. Any smearing of rRNA bands is an indication of degraded mRNA. If this occurs, a new sample of total RNA should be prepared.

To evaluate the suitability of purified RNA (human, mouse or rat) for RT-PCR applications a control RT-PCR can be performed using template RNA and the control GAPDH primers provided in the kit. The GAPDH-specific control PCR primers are designed to be complementary to human, mouse and rat GAPDH genes and generate a 496 bp RT-PCR product.

RNA quantity

- Use 100 ng - 5 µg of total RNA or 10 ng - 500 ng of poly(A) mRNA to generate first strand cDNA as the initial step of a two-step RT-PCR protocol.
- Use 1 µg of isolated mRNA to generate first strand cDNA for second-strand synthesis and subsequent cloning reactions.

Primers
Synthesis of first strand cDNA can be primed with either oligo(dT)$_{18}$ primer, random primers or gene-specific primers. Oligo(dT)$_{18}$ primes cDNA synthesis from the poly(A) tail present at the 3'-end of eukaryotic mRNA. Random primers initiate cDNA synthesis from the total RNA population (rRNA and mRNA). Therefore, using random primers for first strand synthesis results in a greater complexity of the generated cDNA compared with the oligo(dT)$_{18}$ primer. As a consequence, the sensitivity and specificity of subsequent PCR reactions may be reduced. However, there are several applications where it is beneficial to use random primers, such as cDNA synthesis using mRNAs without a poly(A) tail, or cDNA synthesis using poly(A)-enriched RNA samples.
Gene-specific primers are used to synthesize specific cDNA from a pool of total RNA or mRNA and must be obtained by the user.

First Strand cDNA synthesis procedure
The first strand cDNA reaction can be performed as an individual reaction or as a series of parallel reactions with different RNA templates. Therefore, the reaction mixture can be prepared by combining reagents individually or a master mix containing all of the components except template RNA can be prepared. Depending on the structure of the RNA template, separate steps for RNA denaturation and primer annealing may improve RT-PCR results.

Protocols
Please read the Important Notes section before starting.

RT-PCR
I. First Strand cDNA Synthesis
After thawing, mix and briefly centrifuge the components of the kit. Store on ice.
1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Component	Volume
Template RNA total RNA *or* poly(A) mRNA *or* specific RNA	 0.1 - 5 µg 10 ng - 0.5 µg 0.01 pg - 0.5 µg
Primer oligo (dT)$_{18}$ primer *or* random hexamer primer *or* gene-specific primer	 1 µL 1 µL 15-20 pmol
Water, nuclease-free	to 11 µL
Total volume	**11 µL**

2. *Optional.* If the RNA template is GC-rich or contains secondary structures, mix gently, centrifuge briefly and incubate at 65°C for 5 min. Chill on ice, spin down and place the vial back on ice.
3. Add the following components in the indicated order:

Component	Volume
5X Reaction Buffer	4 µL
RiboLock RNase Inhibitor (20 U/µL)	1 µL
10 mM dNTP Mix	2 µL
M-MuLV Reverse Transcriptase (20 U/µL)	2 µL
Total volume	**20 µL**

4. Mix gently and centrifuge.
5. For oligo(dT)$_{18}$ or gene-specific primed cDNA synthesis, incubate for 60 min at 37 °C.
For random hexamer primed synthesis, incubate for 5 min at 25 °C followed by 60 min at 37 °C.
Note. For GC-rich RNA templates the reaction temperature can be increased up to 45 °C.
6. Terminate the reaction by heating at 70 °C for 5 min.
The reverse transcription reaction product can be directly used in PCR applications or stored at -20 °C for less than one week. For longer storage, -70 °C is recommended.

II. PCR Amplification of First Strand cDNA

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. The volume of first strand cDNA synthesis reaction mixture should not comprise more than 1/10 of the total PCR reaction volume. Normally, 2 µL of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50 µL total volume. *Taq* DNA polymerase (#EP0401) or 2X PCR Master Mix (#K0171) can be used to amplify fragments less than 3 kb. Thermo Scientific™ DreamTaq™ DNA polymerase (#EP0701) is suitable for amplification of longer fragments up to 6 kb. Phusion High Fidelity DNA Polymerases are recommended to generate amplicons up to 20 kb.

Control Reactions

Positive and negative control reactions should be used to verify the results of the first strand cDNA synthesis steps.

- **Reverse transcriptase minus (RT-) negative control** is important in RT-PCR or RT-qPCR reactions to assess for genomic DNA contamination of the RNA sample. The control RT- reaction contains every reagent for the reverse transcription reaction except for the RT enzyme.
- **No template negative control (NTC)** is important to assess for reagent contamination. The NTC reaction contains every reagent for the reverse transcription reaction except for RNA template.
- **Positive control** RNA template and gene-specific primers are supplied with the kit. The human GAPDH control RNA (1.3 kb) was produced by *in vitro* transcription. The GAPDH-specific control PCR primers are designed to be complementary to human, mouse and rat GAPDH genes and generate 496 bp RT-PCR product. The protocol for the positive control RT-PCR is provided below.

I. Positive control first strand cDNA synthesis reaction

Mix and briefly centrifuge all components after thawing, keep on ice.

1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Component	Volume
Control GAPDH RNA (50 ng/µL)	2 µL
Oligo $(dT)_{18}$ Primer *or* Random Hexamer Primer *or* Reverse GAPDH Primer	1 µL
5X Reaction Buffer	4 µL
RiboLock RNase Inhibitor (20 U/µL)	1 µL
10 mM dNTP Mix	2 µL
M-MuLV Reverse Transcriptase (20 U/µL)	2 µL
Water, nuclease-free	8 µL
Total volume	**20 µL**

2. Mix gently and centrifuge.
3. For oligo$(dT)_{18}$ or gene-specific primed cDNA synthesis, incubate for 60 min at 37 °C.

For random hexamer primed synthesis, incubate for 5 min at 25 °C followed by 60 min at 37 °C.

4. Terminate the reaction by heating at 70 °C for 5 min.
5. Briefly centrifuge and proceed with control PCR amplification.

II. Control PCR amplification

1. Dilute the cDNA generated with the control first strand cDNA reaction 1:1000 in Water, nuclease-free.
2. Gently vortex and briefly centrifuge all PCR reagents after thawing.
3. Place a thin-walled PCR tube on ice and add the following reagents:

Component	Volume
cDNA from control RT reaction (1:1000 dilution)	2 µL
10X PCR Buffer	5 µL
10 mM dNTP Mix	1 µL (0.2 mM each)
25 mM $MgCl_2$	3 µL
Forward GAPDH Primer	1.5 µL
Reverse GAPDH Primer	1.5 µL
Taq DNA Polymerase (5 U/µL)	0.5 µL
Water, nuclease-free	35.5 µL
Total volume	**50 µL**

4. Perform PCR in a thermal cycler with a heated lid.

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	94	3 min	1
Denaturation	94	30 s	35
Annealing	58	30 s	
Extension	72	45 s	

5. Load 5-10 µL of the RT-PCR product on 1 % agarose gel. A distinct 496 bp PCR product should be visible after ethidium bromide staining.

Troubleshooting

Low yield or no RT-PCR product
Degraded RNA template. RNA purity and integrity is essential for synthesis of full-length cDNA. Always assess the integrity of RNA prior to cDNA synthesis. Sharp 18S and 28S RNA bands should be visible after denaturing agarose gel electrophoresis of total eukaryotic RNA. Follow general recommendations to avoid RNase contamination. **Low template purity.** Trace amounts of agents used in RNA purification protocols may remain in solution and inhibit first strand synthesis, e.g., SDS, EDTA, guanidine salts, phosphate, pyrophosphate, polyamines, spermidine. To remove trace contaminants, re-precipitate the RNA with ethanol and wash the pellet with 75 % ethanol. **Insufficient template quantity.** Increase the amount of template to the recommended level. Following DNase I treatment, terminate the reaction by heat inactivation in the presence of EDTA (to bind magnesium ions). RNA hydrolyzes during heating in the absence of a chelating agent (1). **Incorrect primer choice.** Use the correct primer for the RNA template. Use the random hexamer primer instead of the oligo(dT)18 primer with bacterial RNA or RNA without a poly(A) tail. Ensure sequence-specific primers are complementary to 3'-end of the template RNA. GC rich template. If the RNA template is GC rich or is known to contain secondary structures, increase the temperature of the reverse transcription reaction up to 45 °C. However, the yield will be compromised.
RT-PCR product longer than expected
RNA template is contaminated with DNA. Amplification of genomic DNA containing introns. Perform DNase I digestion prior reverse transcription. To avoid amplification of genomic DNA, design PCR primers on exon-intron boundaries.
RT-PCR product in negative control
RNA template is contaminated with DNA. PCR product in the negative control (RT-) indicates the reaction is contaminated with DNA. Perform DNase I digestion prior reverse transcription.

Reference

1. Wiame, I., et al., Irreversible heat inactivation of DNaseI without RNA degradation, BioTechniques, 29, 252-256, 2000.

Revision history: Pub. No. MAN0012612

Revision	Date	Description
B00	2024-04-08	Revized user guide template, removed COA content and updated related products

Limited product warranty

Life Technologies Corporation and/or it affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html.** If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

7500 Real-Time PCR Systems Spectral Calibration Kit II

Catalog Number 4351151

Pub. No. 4351155 **Rev.** B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Contents and storage

Contents	Amount	Storage
Spectral Calibration Plates sealed with optical covers	3	–25°C to –15°C

Related documentation

For detailed information on instrument setup and the calibration process, refer to the *Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide* (Pub. no. 4347828).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com**

1 December 2015

ThermoFisher SCIENTIFIC

applied biosystems™

Certificate of Analysis

Real-Time PCR Systems Spectral Calibration Kit II, 96-Well

Product No. **4351151**
Lot No. **2407580**

TEST	SPECIFICATION	RESULT

Material Test

Applied Biosystems performs spectrofluorimetric analysis using a calibrated PerkinElmer LS55 Fluorescence Spectrometer to test fluorescence emission wavelength maximum in each lot of component bulk material used in the Spectral Calibration Kit. The PerkinElmer LS55 is calibrated using a mercury arc lamp which verifies the emission monochrometer wavelength accuracy and then the excitation wavelength accuracy is verified against the emission wavelength as a reference.

Component	Part Number	Lot Number			
Cy3 Dye	4349412	2407270	562 ± 5 nm	**560 nm**	**Pass**
Cy5 Dye	4349413	2407278	659 ± 8 nm	**660 nm**	**Pass**
Texas Red® Dye	4349414	2407275	608 ± 5 nm	**608 nm**	**Pass**

For Research Use Only. Not for use in diagnostic procedures.

ISO13485 REGISTERED

Singapore

Farah Dina
18 JUL 2024
Quality Assurance

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To obtain a Certificate of Analysis on-line go to www.appliedbiosystems.com or email us at cofarequest@appliedbiosystems.com

Doc p/n: 100030349 Rev B

TaqMan®
life technologies™

7300 and 7500 Real-Time PCR Systems TaqMan® RNase P 96-Well Instrument Verification Plate

Publication Part Number 4350620D **Revision Date** 17 December 2011

Part Number	Part	Storage Conditions
4350584	TaqMan® RNase P 96-Well Instrument Verification Plate with optical cover	Store at –20°C. **Do not store in a frost-free freezer**.

Note: For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product information

The 7300 and 7500 Real-Time PCR Systems TaqMan® RNase P 96-Well Instrument Verification Plate (Part no. 4350584) is used to verify the performance of the 7300 and 7500 Real-Time PCR Systems.

Note: Do not use a compression pad when running this plate.

The TaqMan RNase P Instrument Verification Plate is a 96-well MicroAmp® PCR tray pre-loaded and sealed with complete TaqMan reagents to detect and quantitate genomic copies of the human RNase P gene. Each well contains 50 µL of reaction mix consisting of human genomic DNA template at a defined copy number, 1X TaqMan® PCR Master Mix and RNase P primers and probe (FAM™ dye-labeled). The RNase P gene is a single-copy gene encoding the RNA moiety of the RNase P enzyme.

A standard curve is generated from the Ct values obtain from a set of replicate standards. The standard curve (1250, 2500, 5000, 10,000, and 20,000 copies) is then used to determine the copy number of the two sets of unknown templates (5000 and 10,000 replicate populations).

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K
B	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K
C	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K	UNKN 5K
D	NTC	NTC	NTC	NTC	STND 1.25K	STND 1.25K	STND 1.25K	STND 1.25K	STND 2.5K	STND 2.5K	STND 2.5K	STND 2.5K
E	STND 5K	STND 5K	STND 5K	STND 5K	STND 10K	STND 10K	STND 10K	STND 10K	STND 20K	STND 20K	STND 20K	STND 20K
F	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K
G	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K
H	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K	UNKN 10K

Refer to the *Installation and Maintenance User Guide for the Applied Biosystems 7300/7500 Real-Time PCR System* (Part no. 4347828) for detailed instructions on performing the run and data analysis.

Procedure notes

- You must perform both a background run and a pure-dye run before running an RNase P plate.
- Always wear talc-free gloves when handling the RNase P plate.
- Remove the RNase P plate from the freezer and remove the foil packaging.

  Note: Remove the plate from the foil packaging immediately prior to use to minimize exposure to light.
- Briefly mix the contents by inverting the plate several times, then centrifuge the plate to force all reagent to the bottom of the wells and eliminate any air bubbles from the mixture.
- Transfer the sealed plate to the real-time PCR instrument sample block. Do not use a compression pad.
- Run the RNase P plate according to the *Applied Biosystems 7300/7500 Real-Time PCR Systems Installation and Maintenance User Guide* (Part no. 4347828).

Data analysis notes

- Adjust the threshold value to the point within the exponential phase of the logarithmic scale amplification plot where the least variability is observed within the replicate populations. The exponential phase occurs within the range of the data points that increase linearly when graphed in this plot.

  Note: To evaluate the threshold value where the least variability is observed, look at each replicate population individually. Viewing all 96 data points at the same time may make it difficult to accurately adjust the threshold value.

  For more information on data analysis, refer to the appropriate Real-Time PCR Systems instrumentation User Guide.
- The install specification of the 7300 and 7500 systems demonstrates the ability to distinguish between 5000 and 10,000 genomic equivalents with a 99.7% confidence level for a subsequent sample run in a single well. The following equation verifies the install specifications:

  [(CopyUnk.1)–3(STDev.CopyUnk.1)] > [(CopyUnk.2) + 3(STDev.CopyUnk.2)] where:
  - CopyUnk.1 = The Average Copy Number of Unknown #1 (10K replicate population)
  - STDev.Unk1 = The Standard Deviation of Unknown #1 (10K replicate population)
  - CopyUnk.2 = The average Copy Number of Unknown #2 (5K replicate population)
  - STDev.Unk.2 = The Standard Deviation of Unknown #2 (5K replicate population)

  Note: Up to 6 wells from each replicate group can be ignored to meet specification.

  Note: The aforementioned values can be obtained from the experimental report window.

For more information go to: **www.lifetechnologies.com**

7500 Real-Time PCR Systems Spectral Calibration Kit I

Catalog Number 4349180

Pub. No. 4350071 **Rev.** D

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Contents and storage

Contents	Amount	Storage
Background Plate sealed with an optical cover	1	–25°C to –15°C
Spectral Calibration Plates sealed with optical covers	7	
Region of Interest (ROI) Calibration Plate sealed with an optical cover	1	

Related Documentation

For detailed information on instrument setup and the calibration process, refer to the *Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide* (Pub. no. 4347828).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com**

1 December 2015

ThermoFisher SCIENTIFIC

applied biosystems
by Thermo Fisher Scientific

Certificate of Analysis

Real-Time PCR Systems Spectral Calibration Kit I, 96-Well

Product No. **4349180**
Lot No. **2407239**

TEST	SPECIFICATION	RESULT

Material Test

Applied Biosystems performs spectrofluorimetric analysis using a calibrated PerkinElmer LS55 Fluorescence Spectrometer to test fluorescence emission wavelength maximum in each lot of component bulk material used in the Spectral Calibration Kit. The PerkinElmer LS55 is calibrated using a mercury arc lamp which verifies the emission monochrometer wavelength accuracy and then the excitation wavelength accuracy is verified against the emission wavelength as a reference.

Component	Part Number	Lot Number			
SYBR® GREEN Dye	4349763	2407302	522 ± 5 nm	**524 nm**	**Pass**
ROX™ Dye	4349411	2407352	601 ± 5 nm	**603 nm**	**Pass**
TAMRA™ Dye	4349410	2407351	578 ± 4 nm	**578 nm**	**Pass**
NED™ Dye	4349408	2407361	573 ± 4nm	**571 nm**	**Pass**
JOE™ Dye	4349409	2407353	548 ± 4 nm	**549 nm**	**Pass**
VIC® Dye	4349764	2407340	548 ± 4 nm	**549 nm**	**Pass**
FAM™ Dye	4349762	2407331	518 ± 4 nm	**518 nm**	**Pass**
ROI™ Dye	4349415	2407423	Visual Inspection	**Pass**	
Background	4330124	2407449	Visual Inspection	**Pass**	

For Research Use Only. Not for use in diagnostic procedures.

ISO13485 REGISTERED FACILITY

Singapore

Farah Dina
26 JUL 2024

Quality Assurance
(Name, Signature & Date)

Life Technologies Holdings Pte Ltd, Block 33, Marsiling Industrial Estate Road 3, #07-06, Singapore 739256 Tel: (65) 6362 9300
To obtain a Certificate of Analysis on-line go to www.thermofisher.com or email us at QA-SG_COA_Request@lifetech.com

Doc p/n: 100030347 Rev C

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CERTIFICATE OF ANALYSIS

K0171 **PCR Master Mix (2X)**

Packaging Lot: **3167297**

Expiry Date: 31.01.2027 (DD.MM.YYYY)

Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
3144577	4 × 1.25 mL	2X PCR Master Mix
3140056	4 × 1.25 mL	Water, Nuclease-free

QUALITY CONTROL

Parameter	Method	Requirement	Result
Endodeoxyribonucleases (nicking activity)	Incubation of supercoiled plasmid DNA with PCR Master Mix (2X).	Not detectable	Conforms
Exodeoxyribonuclease	Incubation of Lambda DNA/HindIII fragments with PCR Master Mix (2X).	Not detectable	Conforms
Ribonucleases	Incubation of [3H]-RNA with PCR Master Mix.	Not detectable	Conforms
Functional testing	PCR amplification of 956 bp single copy gene from human genomic DNA and analysis on agarose gel.	Reaction produces specific PCR product	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

Thermo SCIENTIFIC

PRODUCT INFORMATION

PCR Master Mix (2X)

#K0172 for 1000 rxns

Lot: __ Expiry Date: __

Store at -20°C

Ordering Information

Component	#K0171 200 rxns of 50 µL	#K0172 1000 rxns of 50 µL
PCR Master Mix (2X)	4 × 1.25 mL	20 × 1.25 mL
Water, nuclease-free	4 × 1.25 mL	20 × 1.25 mL

www.thermoscientific.com/onebio

Rev.11

Description

PCR Master Mix is a 2X concentrated solution of *Taq* DNA polymerase, dNTPs and all other components required for PCR, except DNA template and primers. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. The mix is optimized for efficient and reproducible PCR.

Applications

- High throughput PCR.
- Routine PCR with high reproducibility.
- Generation of PCR products for TA. cloning.
- RT-PCR.

Composition of the PCR Master Mix (2X)

0.05 U/µL *Taq* DNA polymerase, reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP).

PROTOCOL

1. Gently vortex and briefly centrifuge PCR Master Mix (2X) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 µL reaction:

PCR Master Mix (2X)	25 µL
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	10 pg - 1 µg
Water, nuclease-free	to 50 µL
Total volume	50 µL

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	25-40
Annealing	Tm-5	30 s	
Extension	72	1 min/kb	
Final Extension	72	5-15 min	1

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform "no template control" (NTC) reactions to check for contamination.

GUIDELINES FOR PRIMER DESIGN

Use the Thermo Scientific REviewer primer design software at www.thermoscientific.com/reviewer or follow the general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3'-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- When introducing restriction enzyme sites into primers, refer to the table "Cleavage efficiency close to the termini of PCR fragments" located on www.thermoscientific.com/onebio to determine the number of extra bases required for efficient cleavage.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

$$Tm = 4 (G + C) + 2 (A + T),$$

where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides we recommend using specialized computer programs e.g., REviewer™ (www.thermoscientific.com/reviewer) to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

All routine DNA purification methods can be used to prepare the template, e.g. Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0721) or GeneJET™ Plasmid Miniprep Kit (#K0502). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, may inhibit DNA polymerase. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.

For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3-1 µM.

(continued on reverse page)

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension

The optimal extension temperature for *Taq* DNA polymerase is 70-75°C. The recommended extension step is 1 min/kb at 72° for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb.

Number of cycles

If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for an additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product has to be cloned into a TA vector, e.g. using Thermo Scientific InsTAclone PCR Cloning Kit (#K1213), the final extension step may be prolonged to 30 min to ensure the highest efficiency of 3'-dA tailing of the PCR product. If the PCR product has to be used for cloning using Thermo Scientific CloneJET PCR Cloning Kit (#K1231), the final extension step can be omitted.

Troubleshooting

For troubleshooting please visit www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation of DNA was observed after incubation of 1 µg of pUC19 DNA with 25 µL of PCR Master Mix (2X) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of DNA was observed after incubation of 1 µg of lambda DNA/HindIII fragments with 25 µL of PCR Master Mix (2X) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 1 µg of [^{3}H]-RNA with 25 µL PCR Master Mix (2X) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.

Functional Assay

PCR Master Mix (2X) was tested for amplification of 956 bp single copy gene from human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene