ThermoFisher SCIENTIFIC

Life Technologies CERTIFICATE OF ANALYSIS

Catalog Number Q32854
Product Name Qubit™ dsDNA HS Assay Kit, 500 assays
Lot Number 2585794

	LOT DATA	SPECIFICATION
ABSORPTION[1] OD ± 0.05 at lambda max ± 5 nm	meets specification	OD ± 0.05 at lambda max ± 5 nm
ABSORPTION Optical Density of Component D[2]	0.203 at 260 nm	0.2 ± 0.008 at 260 nm

1. Solvent: 50 mM potassium phosphate buffer, pH 7. Method: Optical Density: 20X dilution.

2. Solvent: Buffer, TE Buffer. Method: Optical Density. undiluted

Zach Luedtke, Quality Assurance Manager
3-Apr-2023

Life Technologies Corporation certifies on the date above that this is an accurate record of the analysis of the subject lot, and that the data conform to the specifications in effect for this product at the time of analysis.

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Printed Apr 04, 2023

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invitrogen

Qubit fluorometers and assays

Accurate, specific, and sensitive quantification of DNA, RNA, and protein samples

ThermoFisher SCIENTIFIC

Qubit fluorometers

Intuitive user interface coupled with accurate measurements

Invitrogen™ Qubit™ 4 and Qubit™ Flex fluorometers are benchtop microvolume fluorometers designed to accurately measure DNA, RNA, or protein quantity. Whether you are an expert or a novice, the easy-to-use touchscreen menus make it easy to perform assays, with accurate and reliable results displayed in just a few seconds. Both instruments provide flexible data exportation using a USB drive, Wi-Fi cloud connectivity, or direct USB cable connection so your quantification data is easily accessed.

Figure 1. Qubit Flex and Qubit 4 fluorometers with intuitive touchscreens and applications for specific assays.

Key benefits of Qubit fluorometers

- **High sensitivity**—more sensitive than UV absorbance–based quantification
- **Accuracy and speed**—accurately quantifies DNA, RNA, or protein in less than 3 seconds
- **Ideal for precious samples**—requires as little as 1 µL of sample
- **Optimized reagents and tubes**—Invitrogen™ Qubit™ reagents and assay tubes work best with Qubit fluorometers

Qubit fluorescence technology

Qubit fluorometers and assay kits are designed to measure the intensity of the signal from fluorescent dyes bound to specific biological molecules. These optimized dyes bind selectively to DNA, RNA, or protein and only emit a fluorescent signal when bound to the target.

Qubit fluorometers use specialized curve-fitting algorithms to develop a calibration curve using standard samples with a known concentration. An unknown sample concentration of DNA, RNA, or protein is calculated by comparing the relative fluorescence units (RFUs) of the sample to the RFUs of the standards used in calibration. The detection limits of the measurements are specific to each assay.

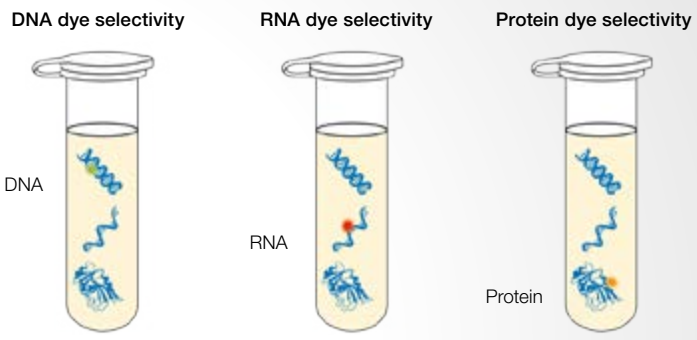


Figure 2. Fluorescent dyes selectively bind to DNA, RNA, or protein. Dyes only emit signal when bound to the target.

Convenient, easy-to-use onboard calculators

Reagent calculator

The reagent calculator conveniently calculates how much working solution to prepare based on the number of samples to quantify. Available in both the Qubit 4 and Qubit Flex fluorometer models.

Assay range calculator

The assay range calculator displays the core sample concentration range based on the sample volume, as well as the extended low and high ranges. This aids in the selection of the appropriate Qubit assay for the most accurate quantification based on your sample volume and estimated sample concentration. This calculator is only available with the Qubit Flex Fluorometer.

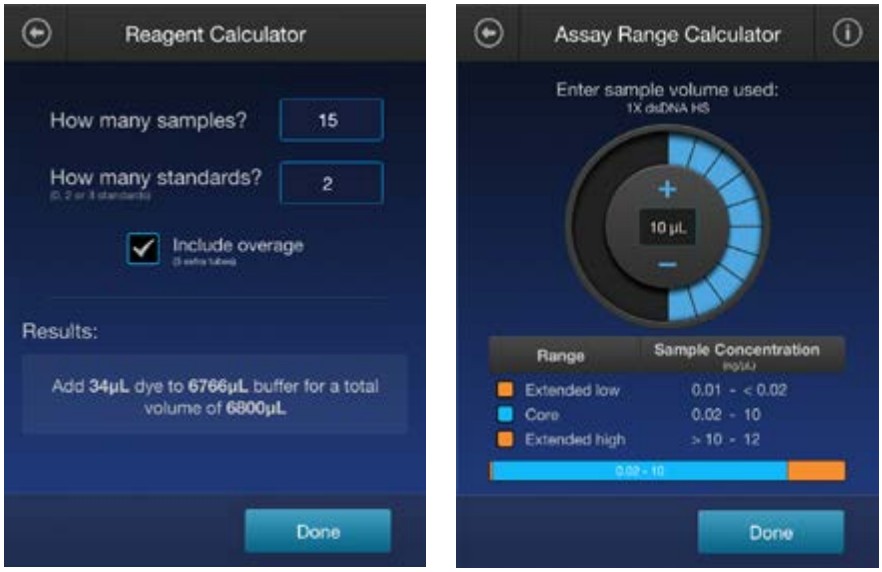


Figure 3. Reagent and assay range calculators. Easily make working solutions for all assays that are not in 1X format using the reagent calculator. The assay range calculator aids in the determination of sample volume requirements based on required accuracy.

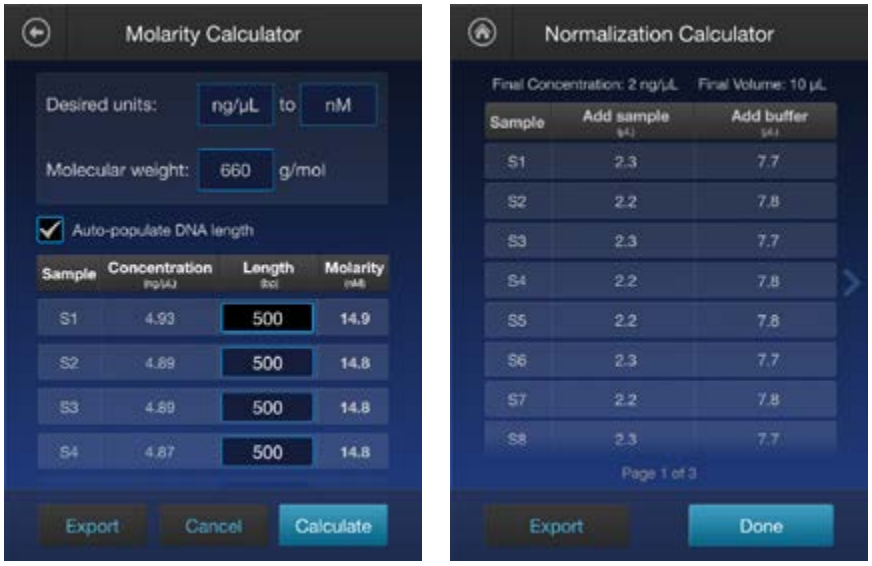


Figure 4. Integrated post-results molarity and normalization calculators. Use the molarity calculator to convert values to molarity based on nucleic acid length. Use the normalization calculator to determine how to dilute the samples to the same concentration.

Calculators for next-generation sequencing (NGS) workflows

Molarity calculator

Quickly calculate the molarity of your samples based on nucleic acid length and the measured concentration. The molarity calculator is only available on the Qubit Flex Fluorometer.

Normalization calculator

Easily normalize to a desired mass, concentration, or molarity with the normalization calculator. This replaces spreadsheet calculations for standard normalization during library preparation for sequencing. The normalization calculator is only available on the Qubit Flex Fluorometer.

Personalized workflows

Envision and create custom assays for the Qubit 4 Fluorometer

MyQubit functionality brings your favorite fluorescence assays right to your benchtop, providing a reliable platform for many quantitation needs—from laboratory research and quality control to process monitoring and beyond. Any fluorescent reagent or assay that is spectrally compatible with the Qubit hardware can be adapted for use with the Qubit 4 Fluorometer.

Compare Qubit fluorometers

	Qubit 4 Fluorometer	Qubit Flex Fluorometer
Sample throughput	1 sample in 3 seconds	1 to 8 samples in 3 seconds
User interface	5.7 in. color touchscreen	8 in. color touchscreen
Onboard calculators	Reagent calculator	Reagent calculator Assay range calculator Molarity calculator Normalization calculator
Informs where the sample concentration resides within the assay range	Provides quantification data for samples that are within the core and the extended range of the standard curve. Sample concentrations that are out of range are not given a measurement.	
System check	Qubit 4 System Verification Assay Kit	Qubit Flex System Verification Assay Kit
Fluorometer mode	Yes	No
Programable open format	Yes—MyQubit	No
Instrument footprint (W x L x H)	13.6 x 25 x 5.5 cm 5.4 x 10 x 2.2 in.	1.86 x 28.2 x 10.3 cm 7.3 x 11.1 x 4.1 in.
Sample data storage	1,000 samples	10,000 samples
Data export	Wi-Fi USB drive Direct to computer via USB or ethernet cable	Wi-Fi USB drive Direct to computer via USB or ethernet cable
Light sources	Blue LED (peak ~470 nm), Red LED (peak ~635 nm)	Blue LED (peak ~460–480 nm), Red LED (peak ~620–640 nm)
Excitation filters	Blue LED (430–495 nm), Red LED (600–645 nm)	Blue LED (456–484 nm), Red LED (612–644 nm)
Emission filters	Green (510–580 nm), Red (665–720 nm)	Green (513–563 nm), Far-red (671–693 nm)

Qubit assays

Qubit assays are designed to work with Qubit fluorometers. Common contaminants such as salts, free nucleotides, RNA, solvents, detergents, and proteins are well tolerated in Qubit assays.

Qubit 4 and Qubit Flex kits for system verification

The Invitrogen™ Qubit™ 4 System Verification Assay Kit and the Invitrogen™ Qubit™ Flex System Verification Assay Kit are fast, easy-to-use, reagent-based assays that test the performance of Qubit fluorometers. Each kit consists of three components: a blank reagent solution, a green fluorescent reagent, and a far-red fluorescent reagent. Paired with a hardware functionality test, the assay is designed to test the internal components of the instrument to help ensure proper functionality.

Qubit RNA quantification assays

There are three RNA assay kits, which offer differing detection ranges, and one microRNA assay kit:

- **Invitrogen™ Qubit™ RNA HS Assay Kit**—high sensitivity
- **Invitrogen™ Qubit™ RNA BR Assay Kit**—broad range
- **Invitrogen™ Qubit™ RNA XR Assay Kit**—extended range
- **Invitrogen™ Qubit™ microRNA Assay Kit**—highly selective for miRNA over rRNA or large mRNA (>1,000 bp)

The RNA assays are accurate for initial sample concentrations from as little as 250 pg/µL to 10,000 ng/µL. These kits are highly selective for RNA over dsDNA. Unlike other RNA assays, they do not require DNase if DNA is present in the sample for an accurate measurement.

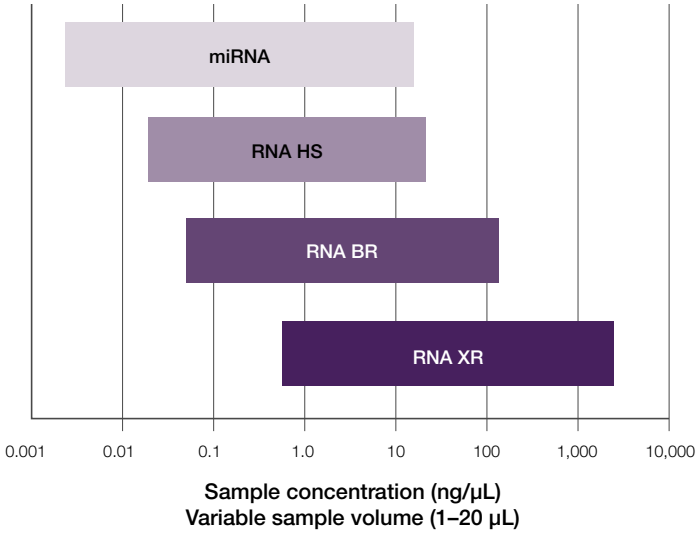


Figure 5. Quantification ranges of Qubit RNA assay kits.

Qubit RNA integrity and quality (IQ) assay

The Invitrogen™ Qubit™ RNA IQ Assay was developed to quickly assess the quality and integrity of an RNA sample. This assay allows assessment of RNA quality at a lower cost and with an easier, simpler, and faster workflow than other solutions currently on the market.

The Qubit RNA IQ Assay utilizes two unique dyes—one that binds to large, intact, and/or structured RNA, and another that selectively binds to small, degraded RNA. Together, they are able to quickly assess the quality and integrity of an RNA sample. To use, simply add your samples to the Qubit RNA IQ working solution, then measure on the Qubit 4 or Qubit Flex Fluorometer.

Results are presented as a total value for the RNA sample integrity and quality, or RNA IQ number, and as the calculated percentage of large and small RNA in the sample. The RNA IQ number is based on a scale of 1 to 10, wherein a high IQ number indicates the majority of the sample consists of large and/or structured RNA. Conversely, a small IQ number indicates the sample comprises mainly small RNA with limited tertiary structure.

Figure 6. A proprietary algorithm is used to report a quality score representative of the ratio of small and large and/or structured RNA in the sample.

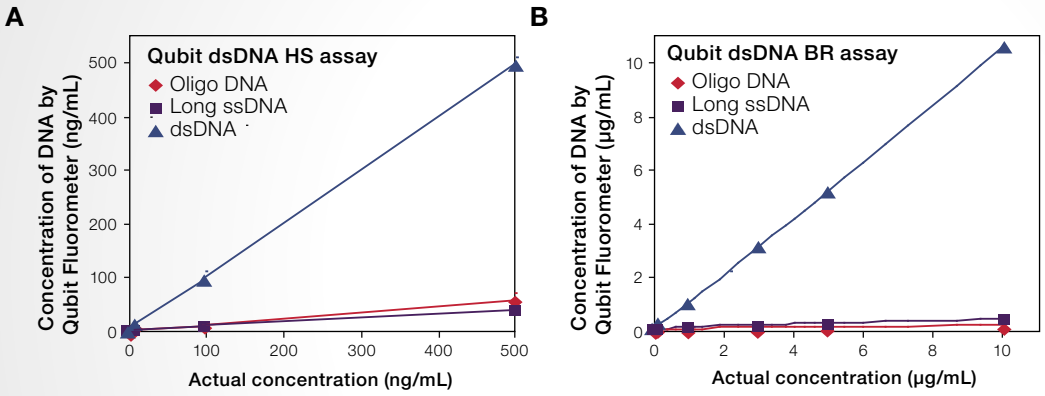
Qubit DNA quantification assays

Invitrogen™ Qubit™ DNA assay kits are broadly categorized as double-stranded DNA (dsDNA) assays or single-stranded DNA (ssDNA) assays.

Qubit dsDNA assay kits—available in two detection ranges and two formats

Detection ranges: high-sensitivity and broad-range assays

- The Invitrogen™ Qubit™ dsDNA High-Sensitivity (HS) Assay Kit is for samples with a low concentration of dsDNA, making it ideal for precious samples. It has a detection range between 0.1 and 120 ng.
- The Invitrogen™ Qubit™ dsDNA Broad-Range (BR) Assay Kit is ideal for a broad range of DNA concentrations and applications. It can detect between 4 and 4,000 ng.



Use high-sensitivity (HS) assays for low concentrations and broad-range (BR) assays for high concentrations of dsDNA

Figure 7. Detection of double-stranded DNA by the Qubit dsDNA HS (A) and BR (B) assay kits. Duplicate samples of long ssDNA, oligo DNA, or lambda dsDNA at concentrations of 0.5 to 500 ng/mL in the assay tube were quantified using the Qubit dsDNA HS assay, and at concentrations of 0.01 to 10 µg/mL in the assay tube using the Qubit dsDNA BR assay according to kit protocols.

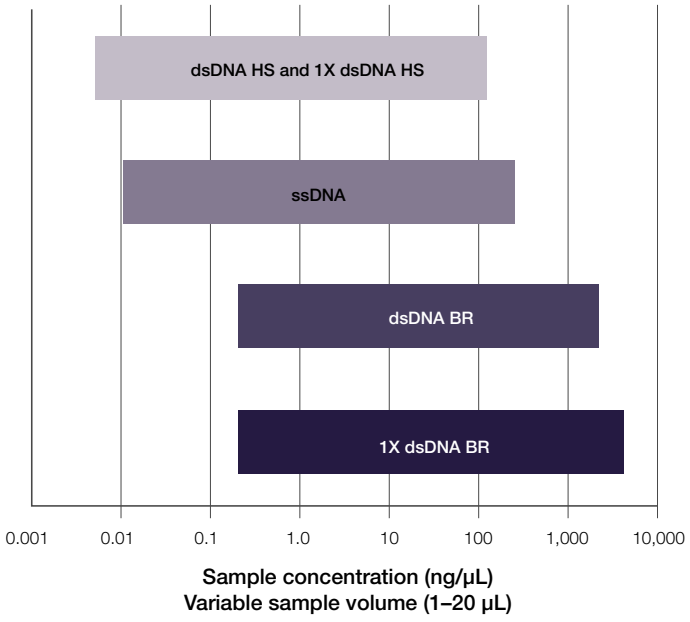


Figure 8. Quantification ranges of Qubit DNA assay kits.

Formats: standard assay and 1X assay

- Invitrogen™ Qubit™ standard assays require same-day mixing of the buffer with the reagent to create the working solution prior to preparing standards and samples for quantification.
- Invitrogen™ Qubit™ 1X assays eliminate the step of preparing the working solution.
  - The Invitrogen™ Qubit™ 1X dsDNA HS Assay Kit provides the same dynamic range and limit of detection as the standard assay, while the Invitrogen™ Qubit™ 1X dsDNA BR Assay Kit has a wider dynamic range than the standard assay, achieving 4,000 ng/µL in the extended range.
  - This format offers a simplified workflow while reducing the tubes in the kit, therefore reducing the amount of plastic used.
  - Simply add your sample or standard to the premixed solution, incubate, and read your results.

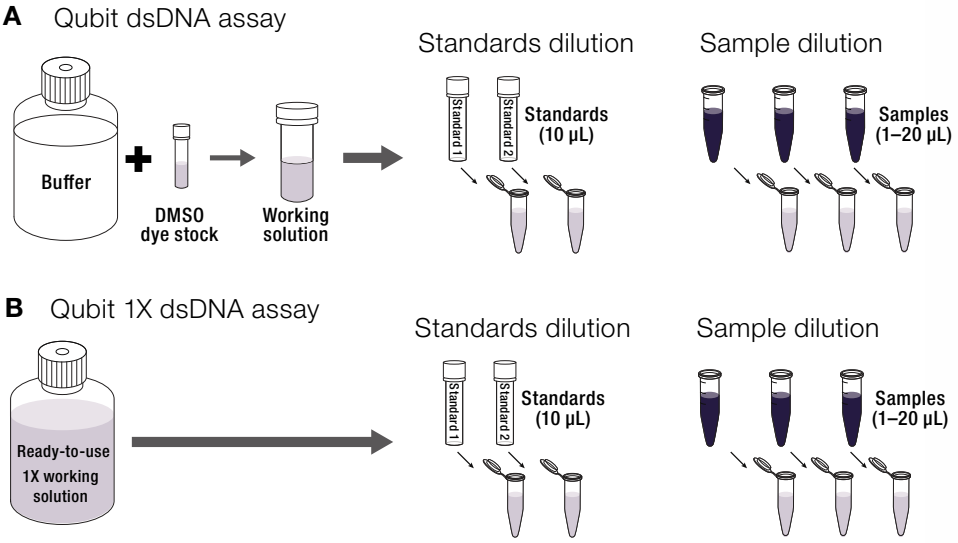


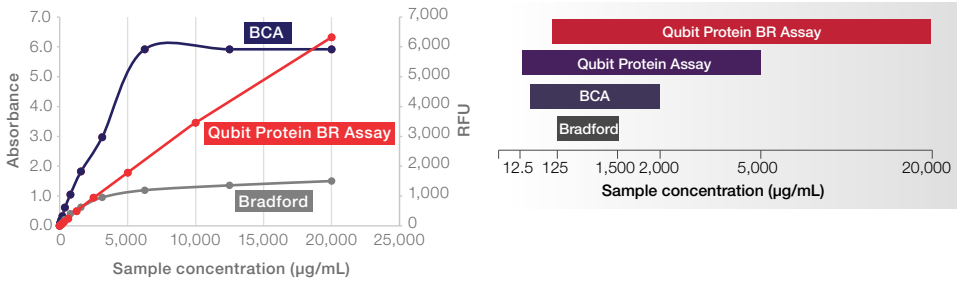
Figure 9. Workflow comparison for the (A) Qubit dsDNA and (B) Qubit 1X dsDNA assays. Standard Qubit dsDNA High Sensitivity (HS) and Qubit dsDNA Broad Range (BR) assay kits include a fluorogenic dye, buffer, and dsDNA standards. Prior to each assay, a fresh aqueous working solution needs to be prepared by diluting the dye stock in the provided buffer in a 1:200 ratio. Qubit 1X dsDNA assay kits eliminate this step by providing a ready-to-use working solution.

Qubit ssDNA and oligos quantification assays

The Invitrogen™ Qubit™ ssDNA Assay Kit is ideal for quantifying single-stranded DNA or oligonucleotides. It is accurate for initial sample concentrations from 50 pg/µL to 200 ng/µL, providing an assay range of 1–200 ng.

Qubit protein quantification assays

Invitrogen™ Qubit™ protein assay kits are designed to make protein quantification easy and fast. The assays provide low protein variability, rapid quantitation, and high sensitivity. Common contaminants, such as reducing reagents (DTT, β-mercaptoethanol), salts, free nucleotides, amino acids, solvents, DNA, and detergents (Invitrogen™ Qubit™ Protein BR Assay only), are well tolerated in the assays. The assays' wide dynamic ranges make it easy to determine the concentrations of a wide range of samples compared to standard colorimetric protein assays.



	Qubit Protein BR Assay	Qubit Protein Assay
Platform	Qubit 4	Qubit 4, Qubit Flex
Compatibility	Detergents, reducing agents	Reducing agents
Quantitation range	100 µg/mL to 20 mg/mL	12.5 µg/mL to 5 mg/mL

Figure 10. Quantification ranges of protein assays.

Need higher throughput for your nucleic acid or protein samples?

Qubit assays are ideal when the number of samples you measure at one time is low enough not to warrant a microplate reader. With larger sample batches requiring a fluorescence microplate reader, use Invitrogen™ Quant-iT™ assay kits and reagents, which are designed to use with microplate readers for nucleic acid or protein quantification.

Learn more about Quant-iT assays at **thermofisher.com/quantit**

Learn more about microplate readers at **thermofisher.com/platereaders**

Frequently asked questions

Q. I already have a Thermo Scientific™ NanoDrop™ instrument; why should I use a Qubit fluorometer?

A. NanoDrop instruments use UV absorbance to measure DNA and RNA concentrations. Absorbance-based measurements have limitations in distinguishing between DNA, RNA, and free nucleotides, which absorb at 260 nm.

Qubit assays are fluorescence-based. They are designed to only quantify the target analyte. Additionally, fluorescence-based nucleic acid quantification provides a more sensitive dynamic range than absorbance-based instruments.

When used with Qubit assays, Qubit fluorometers can accurately measure low concentrations of sample, while NanoDrop spectrophotometers can detect the presence of common contaminants.

Q. Do I have to use new standards every time?

A. For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you can determine the level of comfort you have using the calibration data stored from the last time the instrument was calibrated. We do recommend running a new calibration curve every time you prepare a new working solution.

Q. Is there a difference in signal between supercoiled and relaxed plasmid DNA when using a Qubit fluorometer?

A. Yes, we have seen a 20–30% difference. For the different forms of plasmid DNA, we recommend using a standard that more closely represents the composition of the plasmid DNA in the sample.

Q. Does the Qubit Protein Assay or Qubit Protein BR Assay work well in the presence of detergents?

A. The Qubit Protein BR Assay is compatible with samples that contain up to 5% detergents. The Qubit Protein Assay is not recommended if detergent is present.

Q. Why are some of the instrument setting menu options not available on my Qubit 4 or Qubit Flex device?

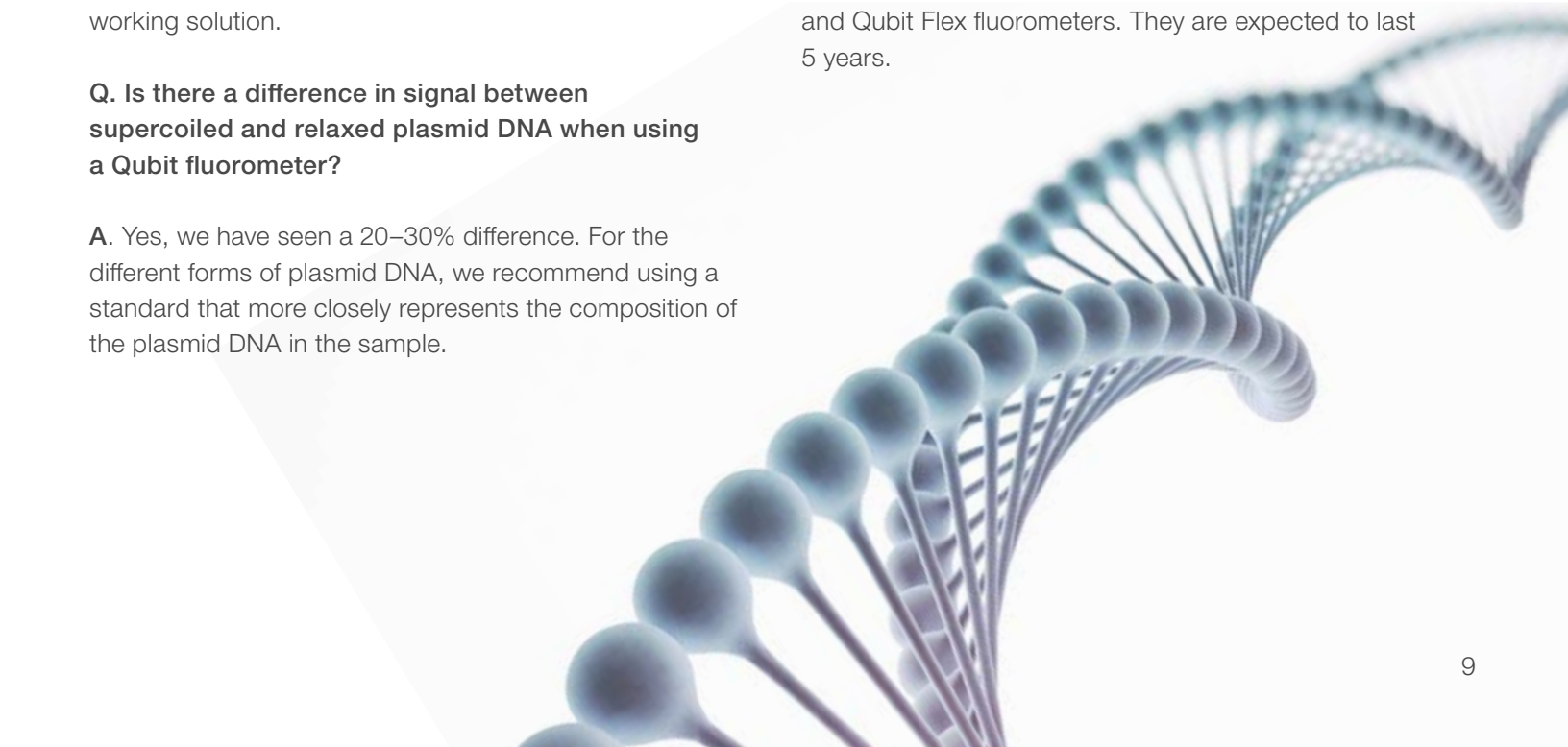
A. To adhere to cybersecurity legal standards, the latest firmware for Qubit 4 and Qubit Flex fluorometers will require users to log in to access certain menu options (such as instrument settings, software update, and system verification). When not logged into a user profile, these menu options will appear to be inactive.

Q. Is there a way to verify that my Qubit 4 or Qubit Flex fluorometer is functioning properly?

A. Qubit 4 and Qubit Flex System Verification Assay Kits offer a fast, easy-to-use, reagent-based method to test the internal components of a Qubit fluorometer. Perform the system verification when a problem with the instrument is suspected. It is not necessary to perform verification regularly.

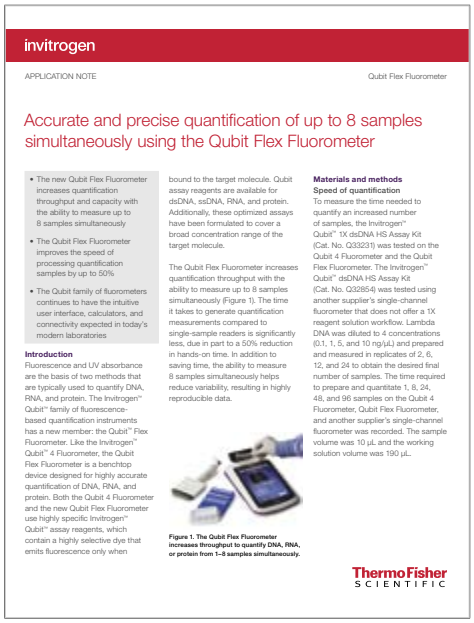
Q. How long does the lamp last?

A. There are two LED light sources in the Qubit 4 and Qubit Flex fluorometers. They are expected to last 5 years.

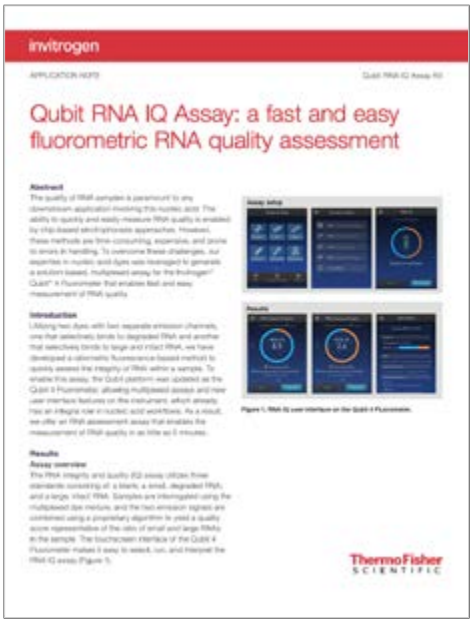


Support documents

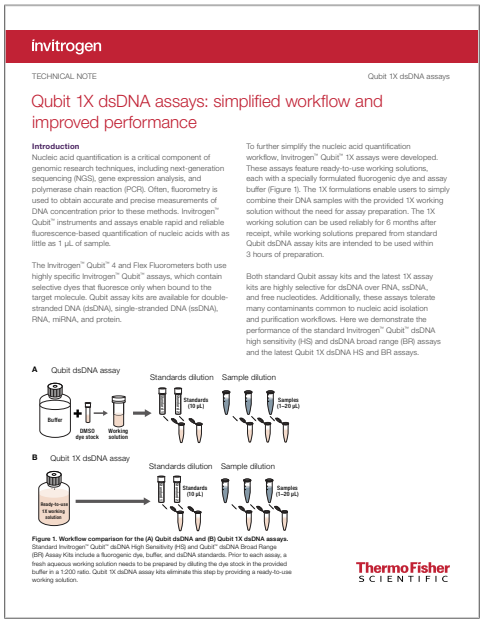
Qubit fluorometer technical resources are below—gain quick access to user guides, technical and application notes, and citations. Learn more at **thermofisher.com/qubitresources**.



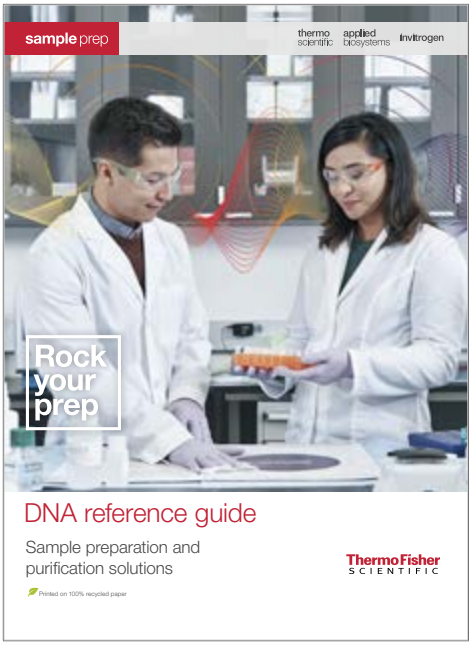
Application note: Accurate and precise quantification of up to 8 samples simultaneously using the Qubit Flex Fluorometer >



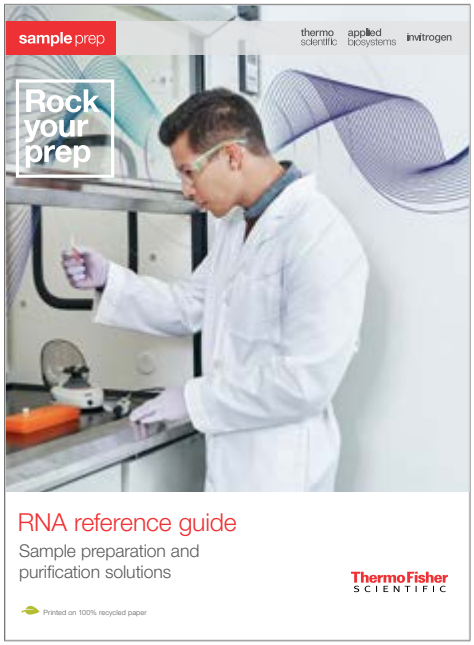
Application note: Qubit RNA IQ Assay: a fast and easy fluorometric RNA quality assessment >



Technical note: Qubit 1X dsDNA assays: simplified workflow and improved performance >



DNA reference guide: Sample preparation and purification solutions >



RNA reference guide: Sample preparation and purification solutions >

Technical note: Understanding library quantification assays for next-generation sequencing applications >

Ordering information

Instruments and accessories	Unit size	Cat. No.
Qubit 4 Fluorometer		
Qubit 4 Fluorometer (w/ Wi-Fi)	1 instrument	**Q33238**
Qubit 4 Quantitation Starter Kit (w/ Wi-Fi)	1 kit	**Q33239**
Qubit 4 NGS Starter Kit (w/ Wi-Fi)	1 kit	**Q33240**
Qubit 4 RNA IQ Starter Kit (w/ Wi-Fi)	1 kit	**Q33241**
Qubit 4 Protein BR Assay Starter Kit (w/ Wi-Fi)	1 kit	**A51292**
Qubit Assay Tubes	500 tubes	**Q32856**
Qubit 4 System Verification Assay Kit	50 assays	**Q33237**
Qubit Flex Fluorometer		
Qubit Flex Fluorometer	1 instrument	**Q33327**
Qubit Flex NGS Starter Kit	1 kit	**Q45893**
Qubit Flex Quantitation Starter Kit	1 kit	**Q45894**
Qubit Flex Assay Tube Strips	125 tube strips	**Q33252**
Qubit Flex Assay Reservoirs	100 reservoirs	**Q33253**
Qubit Flex System Verification Assay Kit	50 assays	**Q33254**

Product	Initial sample concentration (ng/µL)	Quantitation range (ng)	No. of assays	Cat. No.
DNA quantification assays				
dsDNA HS assays				
Qubit 1X dsDNA HS Assay Kit	0.005–120	0.1–120	100	**Q33230**
			500	**Q33231**
Qubit dsDNA HS Assay Kit	0.005–120	0.1–120	100	**Q32851**
			500	**Q32854**
Qubit 1X dsDNA HS Assay Lambda Standard	-	-	-	**Q33233**
dsDNA BR assays				
Qubit 1X dsDNA BR Assay Kit	0.2–4,000	4–4,000	100	**Q33265**
			500	**Q33266**
Qubit dsDNA BR Assay Kit	0.2–2,000	4–2,000	100	**Q32850**
			500	**Q32853**
Qubit 1X dsDNA BR Assay Lambda Standards	-	-	-	**Q33263**
ssDNA and oligos assay				
Qubit ssDNA Assay Kit	0.05–0.2	1–200	100	**Q10212**

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Product name	Initial sample concentration	Quantification range	No. of assays	Cat. No.
RNA quantification assays				
Qubit RNA HS Assay Kit	250 pg/µL and 100 ng/µL	5–100 ng	100	**Q32852**
			500	**Q32855**
Qubit RNA BR Assay Kit	1 ng/µL to 1 µg/µL	20–1,000 ng	100	**Q10210**
			500	**Q10211**
Qubit RNA XR Assay Kit	10 ng/µL and 10,000 ng/µL	200–10,000 ng	100	**Q33223**
			500	**Q33224**
Qubit microRNA Assay Kit	50 ng/mL to 100 µg/mL	1–1,000 ng	100	**Q32880**
			500	**Q32881**

Product name	Size	Cat. No.
RNA IQ assays		
Qubit RNA IQ Assay Kit	75 assays	**Q33221**
	275 assays	**Q33222**
Qubit RNA IQ Standards	1 set	**Q33235**

Product name	Instrument	Initial sample concentration	Size	Cat. No.
Protein assays				
Qubit Protein Assay Kit	Qubit Flex, Qubit 4	12.5 µg/mL to 5 mg/mL	100	**Q33211**
			500	**Q33212**
Qubit Protein BR Assay Kit	Qubit 4	100 µg/mL to 20 mg/mL	100	**A50668**
			500	**A50669**

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

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Certificate of Analysis

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TaqMan Universal PCR Master Mix, 10 Unit Pack (10 x 5 mL)

Product No.	**4318157**
Lot No.	**2303078**
Date of Manufacture	**05APR2023**
Expiration Date	**28JUL2024**

TEST	SPECIFICATION	RESULT
ANALYTICAL TESTS		
Deoxyribonucleoside triphosphate (dNTP) concentrations (measured individually)		Pass
Magnesium ion (Mg2+) concentration		Pass
pH at 25℃		Pass
NUCLEASE TESTS		
RNase - RNase equivalents per 5 µL	< = 1.15 picogr	Pass
DNase - DNase equivalents per 5 µL	< = 155 picogr	Pass
E.COLI DNA CARRYOVER TEST		
E.coli DNA contamination test - E.coli DNA per 25 µL	< = 4 copy	Pass
FUNCTIONAL TEST (RNASE P ASSAY) Functional Test performed on Applied Biosystems ViiA 7 Real-Time PCR System with RNase P assay		
Average NTC (No Template Control)	> = 38.0 CT	Pass

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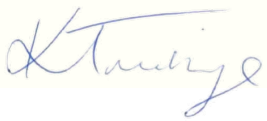
Site: Warrington, UK



Quality Assurance
Issued 11APR2023

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5781 Van Allen Way
Carlsbad, CA, USA 92008
www.thermofisher.com
For inquiries, contact us at
cofarequests@thermofisher.com

Certificate of Analysis

ThermoFisher SCIENTIFIC

TaqMan Universal PCR Master Mix, 10 Unit Pack (10 x 5 mL)

Product No.	**4318157**
Lot No.	**2303078**
Date of Manufacture	**05APR2023**
Expiration Date	**28JUL2024**

TEST	SPECIFICATION	RESULT
Average Ct for 22.2 ng/µL of human (CEPH) DNA	19.0 - 23.0 CT	Pass
PCR efficiency	85 - 115 %	Pass
R2 (correlation coefficient) for the standard curve	> = 0.98	Pass
FUNCTIONAL TEST (B-ACTIN ASSAY)		
Functional Test performed on Applied Biosystems ViiA 7 Real-Time PCR System with B-actin assay		
Positive result achieved when analyzed using 50 ng of human (Raji) DNA		Pass
Average NTC (No Template Control)	> = 38.0 CT	Pass
PHYSICAL INSPECTION		
Labels Correct and Intact		Pass
Caps Sealed		Pass

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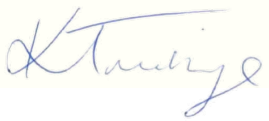
Manufactured in compliance with our ISO 13485 certified quality management system.
Site: Warrington, UK



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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**.

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30 May 2013

thermo scientific

PRODUCT INFORMATION

Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721, #K0722

Pub. No. MAN0012663
Rev. Date 12 October 2016 (Rev. B.00)


Read Storage information (p. 2) before first use!

www.thermofisher.com

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

#_
Lot _
Exp. _

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Genomic DNA Purification Kit is qualified by isolating genomic DNA from 200 µL of blood and 5 mg of mammalian tissue following described protocols. The purified genomic DNA has an $A_{260/280}$ ratio of ≥1.7. A single band of more than 30 kb is seen after agarose gel electrophoresis and ethidium bromide staining. Functional quality of genomic DNA is evaluated by PCR amplification of a single-copy gene and digestion with restriction enzymes.

Quality authorized by:  Jurgita Zilinskiene

CONTENTS page

COMPONENTS OF THE KIT

GeneJET Genomic DNA Purification Kit	#K0721 50 preps	#K0722 250 preps
Proteinase K Solution	1.2 mL	5 × 1.2 mL
RNase A Solution	1 mL	5 × 1 mL
Digestion Solution	11 mL	55 mL
Lysis Solution	24 mL	2 × 60 mL
Wash Buffer I (concentrated)	10 mL	40 mL
Wash Buffer II (concentrated)	10 mL	40 mL
Elution Buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA)	30 mL	150 mL
GeneJET Genomic DNA Purification Columns pre-assembled with Collection Tubes	50	250
Collection Tubes	50	250

STORAGE

Proteinase K and RNase A solutions are stable at room temperature as long as not opened. After being opened they should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!

DESCRIPTION

The 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. *See* Table 1 for typical genomic DNA yields from various sources.

PRINCIPLE

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

IMPORTANT NOTES

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

	#K0721 50 preps		**#K0722** 250 preps	
	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

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

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

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipets and pipet tips
- Vortex
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Thermomixer, shaking water bath or rocking platform capable of heating up to 56 °C
- Disposable gloves

Buffers

For mammalian cell lysate preparation:

- PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)
- TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)

For 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)

For yeast lysate preparation:

- Yeast lysis buffer (5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA)

GENOMIC DNA PURIFICATION PROTOCOLS

Protocols for genomic DNA purification from mammalian tissue and rodent tail, cultured mammalian cells, mammalian blood, gram-negative, gram-positive bacteria, yeast and buccal swabs are described on p.4-11.

A. Mammalian Tissue and Rodent Tail Genomic DNA Purification Protocol

<table>
<tr><th>Step</th><th>Procedure</th></tr>
<tr><td>1</td><td>Grind up to 20 mg of mammalian tissue (use up to 10 mg of spleen tissue), 0.6 cm (rat) or 0.5 cm (mouse) tail clip in liquid nitrogen using a mortar and pestle. Alternatively, cut the tissue into small pieces or disrupt it using a homogenizer.</td></tr>
<tr><td>2</td><td>Collect the material into a 1.5 mL microcentrifuge tube (not provided) and resuspend in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.</td></tr>
<tr><td>3</td><td>Incubate the sample at 56 °C until the tissue is completely lysed and no particles remain. During incubation vortex the vial occasionally or use a shaking water bath, rocking platform or thermomixer.
Suggested incubation times:
<table>
<tr><th>Quantity</th><th>Suggested incubation time</th></tr>
<tr><td>5 mg of tissue (except spleen)</td><td>1 hour</td></tr>
<tr><td>10 mg of tissue (except spleen)</td><td>2 hours</td></tr>
<tr><td>20 mg of tissue (except spleen)</td><td>3 hours</td></tr>
<tr><td>5 mg of spleen tissue</td><td>2 hours</td></tr>
<tr><td>10 mg of spleen tissue</td><td>3 hours</td></tr>
<tr><td>Mouse tail (0.5 cm), rat tail (0.6 cm)</td><td>6 hours</td></tr>
</table>
Note. Lysis time varies on the type and amount of tissue processed. In some cases incubation time should be prolonged to 6-8 hours or overnight (for rodent tail) until complete lysis occurs.</td></tr>
<tr><td>4</td><td>Add 20 µL of RNase A Solution, mix by vortexing then incubate for 10 min at room temperature.</td></tr>
<tr><td>5</td><td>Add 200 µL of Lysis Solution. Mix thoroughly by vortexing for 15 s until a homogeneous mixture is obtained.</td></tr>
<tr><td>6</td><td>Add 400 µL of 50% ethanol and mix by pipetting or vortexing.</td></tr>
<tr><td>7</td><td>Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included).
Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!</td></tr>
<tr><td>8</td><td>Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.</td></tr>
</table>

Step	Procedure
9	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **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., <5 mg of tissue) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

B. Cultured Mammalian Cells Genomic DNA Purification Protocol

Step	Procedure
1	a) Suspension cells Collect up to 5×10^6 cells in a centrifuge tube. Pellet cells by centrifugation for 5 min at 250 × g. Discard the supernatant. Rinse cells once with PBS to remove residual medium and repeat the centrifugation step. Discard the supernatant. b) 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 and pellet them by centrifugation for 5 minutes at 250 × g. Discard supernatant.
2	Resuspend the cells collected in step 1a or 1b in 200 µL of TE buffer or PBS. 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.
3	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (10 min).
4	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
5	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
6	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
7	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
8	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
9	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **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., $\leq1\times10^6$ of cultured mammalian cells) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
10	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

C. Mammalian Blood Genomic DNA Purification Protocol

Step	Procedure
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 while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (10 min).
3	Add 200 µL of ethanol (96-100%) and mix by pipetting or vortexing.
4	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
5	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
6	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
7	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **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., 50 µL) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
8	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

D. Gram-Negative Bacteria Genomic DNA Purification Protocol

Step	Procedure
1	Harvest up to 2×10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 10 min at 5000 × g. Discard the supernatant.
2	Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~30 min).
4	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
5	Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 s until a homogeneous mixture is obtained.
6	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
7	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
8	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and 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 for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **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 the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

E. Gram-Positive Bacteria Genomic DNA Purification Protocol

Before starting

Prepare 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.

Step	Procedure
1	Harvest up to 2×10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 10 min at 5000 × g. Discard the supernatant.
2	Resuspend the pellet in 180 µL of Gram-positive bacteria lysis buffer. Incubate for 30 min at 37 °C.
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 while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~30 min).
5	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
6	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
7	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
8	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and 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 for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **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 the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

F. Yeast Genomic DNA Purification Protocol

Before starting

Prepare Yeast lysis buffer: 5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA.

Step	Procedure
1	Harvest up to 1×10^8 yeast cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 5-10 s at maximum speed ≥12000 × g. Discard the supernatant.
2	Resuspend the pellet in 500 µL of Yeast lysis buffer. Incubate for 1 hour at 37 °C.
3	Centrifuge cells for 10 min at 3000 × g. Discard the supernatant.
4	Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
5	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~45 min).
6	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
7	Add 200 µL of Lysis Solution. Mix thoroughly by vortexing for 15 s until a homogeneous mixture is obtained.
8	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
9	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
10	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. 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 for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
12	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **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 the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
13	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

G. DNA Purification from Buccal Swabs

Step	Procedure
1	To collect a sample, scrape the swab 5-6 times against the inside cheek.
2	Swirl the swab for 30-60 s in 200 µL of 1 × PBS.
3	Go to step 1 of the standard Mammalian Blood Genomic DNA Purification Protocol (p. 7).

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocols. **Starting material was not completely digested.** Extend the Proteinase K digestion at 56 °C until complete lysis occurs and no particles remain. **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 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.** Make sure that ethanol was added to Wash Buffer I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation on p.3.
Purified DNA is degraded	**Sample was frozen and thawed repeatedly.** Avoid repeated freeze / thaw cycles of the samples. Use a new 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, $5x10^6$ 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 and re-spin the column for an additional 1 min. at maximum speed (≥12000 × g). **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.

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Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.

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Company code 122351387, VAT code LT223513811

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CERTIFICATE OF ANALYSIS

K0721 GeneJET Genomic DNA Purification Kit

Packaging Lot: **2701272**
Expiry Date: 22.12.2024 (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
91298422	1.2 mL	Proteinase K Solution, 20mg/ml
2682137	1 mL	RNase A Solution, 10 mg/ml
2639871	30 mL	Elution Buffer
2667167	11 mL	Digestion Solution
2700544	24 mL	Lysis Solution
2693027	10 mL	Wash Solution I (concentrated)
2700485	10 mL	Wash Solution II (concentrated)
2689585	1 pack	GeneJET DNA Purification Columns & collection Tubes
2684497	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 

Certificate of Analysis

TAQMAN® GENE EXPRESSION ASSAYS

Sales Order **54786235 (7189900)**
Storage Conditions **-15 °C to -25 °C**

PART NO.	PART DESCRIPTION	ASSAY ID	LOT NO.	GENE SYMBOL	MFG START DATE	USE BY DATE	MASS SPEC-TROMETRY [Target calculated mass +/- 0.3%]	NTC (C_T > 38)
4331182	TaqMan® GEx Assays (Inventoried)	Pa03453409_s1	1815900	HIV1-LTR	10-Sep-2019	10-Sep-2024	Pass	Pass
4331182	TaqMan® GEx Assays (Inventoried)	Pa03453398_s1	1511231	HTLV2-LTR	28-Apr-2016	28-Apr-2021	Pass	Pass

Applied Biosystems' oligo manufacturing process requires a mass spectrometry test for each component of the Taqman ® assay to verify that the identity of each oligo meets set specifications. A no template control (NTC) test is conducted on the assay level with a pass = Ct > 38 (16S and 18S rRNA assays are an exception). A "+" sign appears for Assay IDs and Gene Symbols greater than 20 characters. Full entry recorded in Assay Information File (AIF).

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

Manufactured in compliance with our ISO 9001 and ISO 13485 certified quality management system.
Site: Pleasanton, CA, USA

Quality Assurance
Issued 10Mar2020

Thermo Fisher Scientific
Life Sciences Solutions
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Pleasanton, CA 94566
www.thermofisher.com
For inquiries, contact us at cofarequests@thermofisher.com .

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

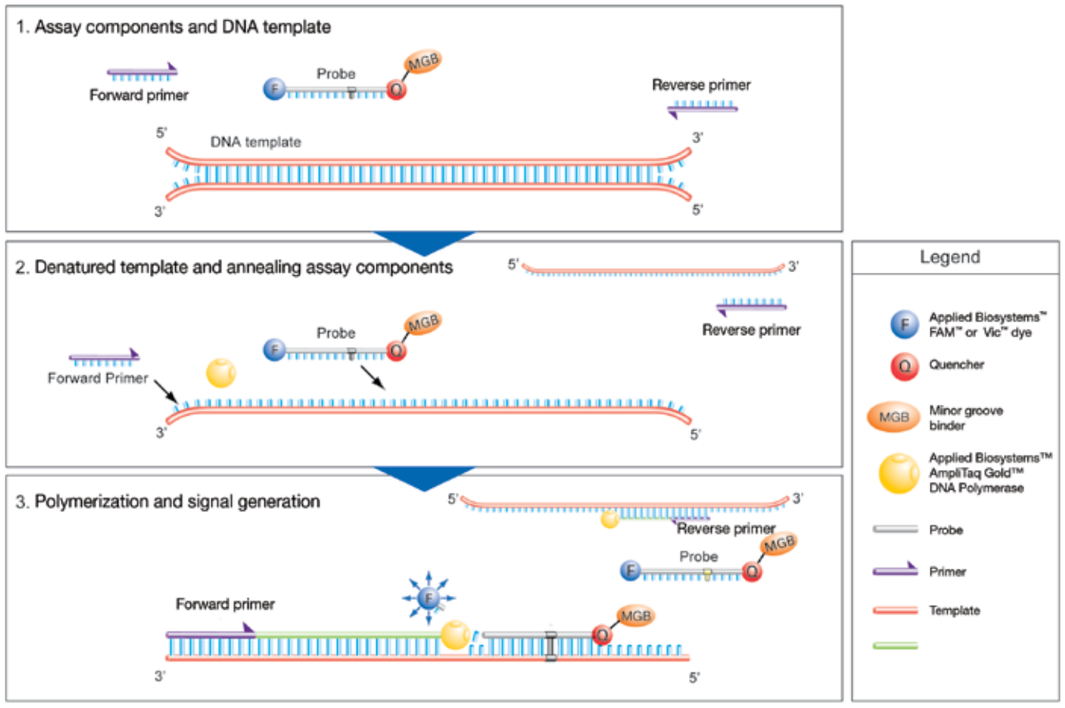
Get results you can trust

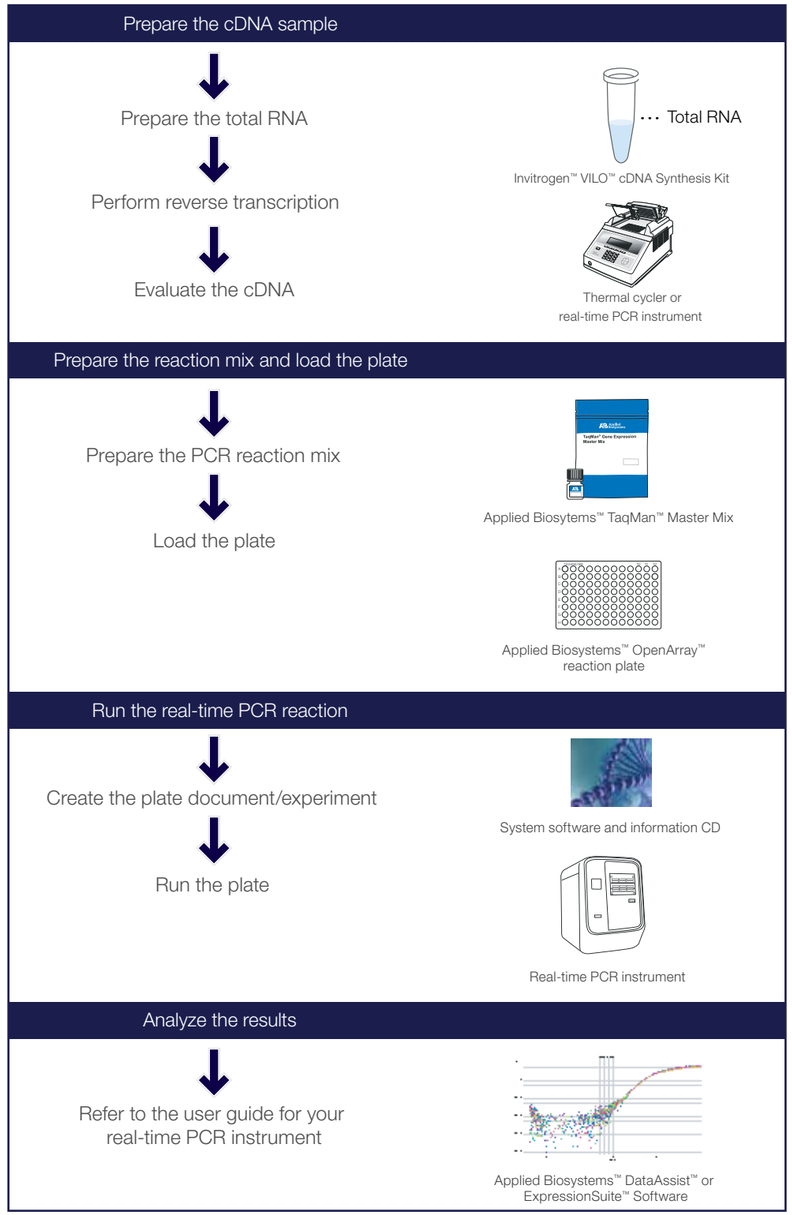
TaqMan Gene Expression Assays are referenced in tens of thousands of publications and are considered the gold standard for gene expression quantification by scientists around the world.

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.

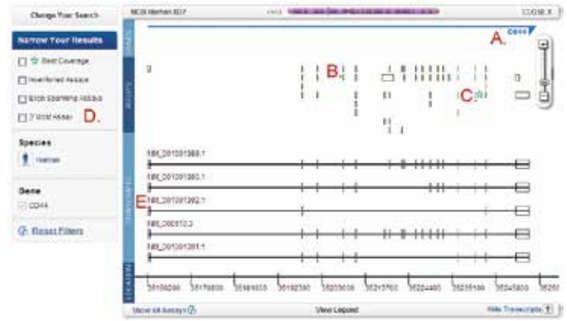
Predesigned TaqMan Gene Expression Assays
(as of November 2015)

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

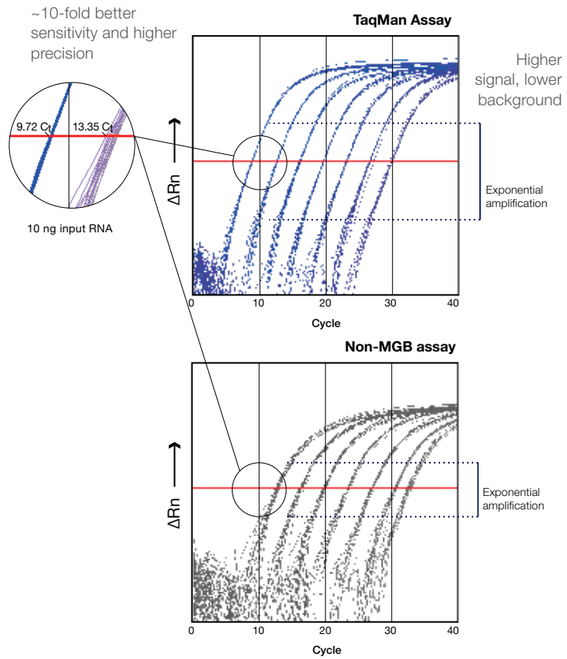


Figure 2. TaqMan probes provide better sensitivity and precision. Comparison of two 5′ nuclease PCR assays for 18S rRNA. Ten-fold dilutions of Universal Human Reference RNA (10–10^{-5} ng) were prepared and analyzed in 11 replicate real-time PCR reactions using either the TaqMan Gene Expression Assay (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

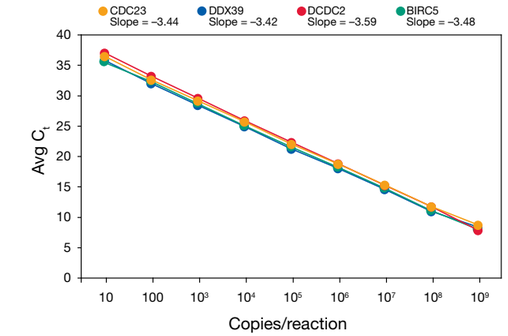


Figure 3. Sensitivity and wide dynamic range. Sequential 10-fold dilutions of synthetic sense RNA corresponding to 4 gene products—CDC23, DDX39, DCDC2, and BIRC5—were added to a background of yeast RNA to evaluate the sensitivity and dynamic range of TaqMan Gene Expression Assays. Samples containing 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

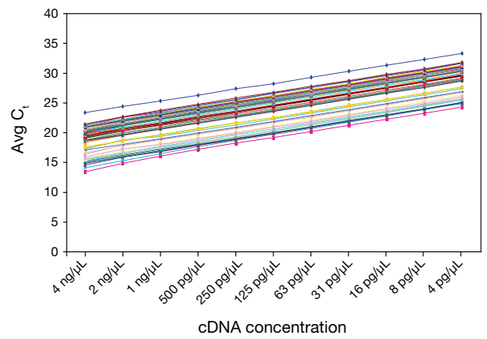


Figure 4. Reliable performance and wide dynamic range. TaqMan Gene Expression Assays were used to analyze expression of 60 targets across a 2-fold dilution series of universal reference cDNA, from 4 ng/µL to 4 pg/µL. The average slope of the lines is 1.02. TaqMan Assays exhibit virtually 100% amplification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

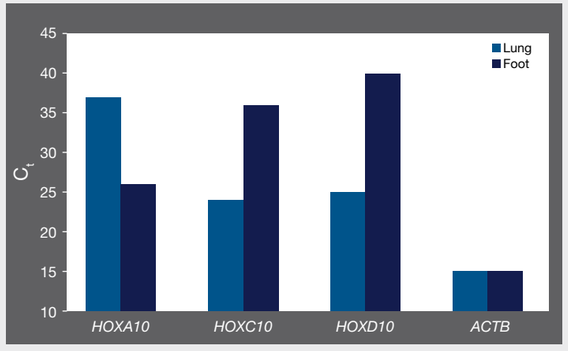


Figure 5. TaqMan Gene Expression Assays detect only their intended targets, even among the highly homologous HOX gene family members. In vertebrates, as in *Drosophila*, location-appropriate expression of members of the HOX gene family is essential for normal embryogenesis. Tissue-specific expression 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.

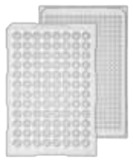
TaqMan Gene Expression Assay formats

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

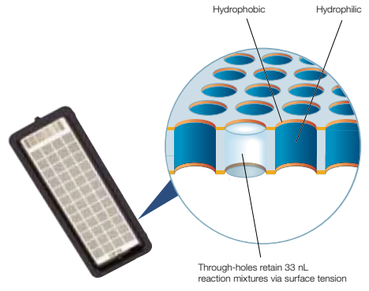
To see the complete collection of 96-well gene signature plates, go to **thermofisher.com/signatureplates**
To see the collection of 384-well gene signature microfluidic cards, go to **thermofisher.com/signaturecards**

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

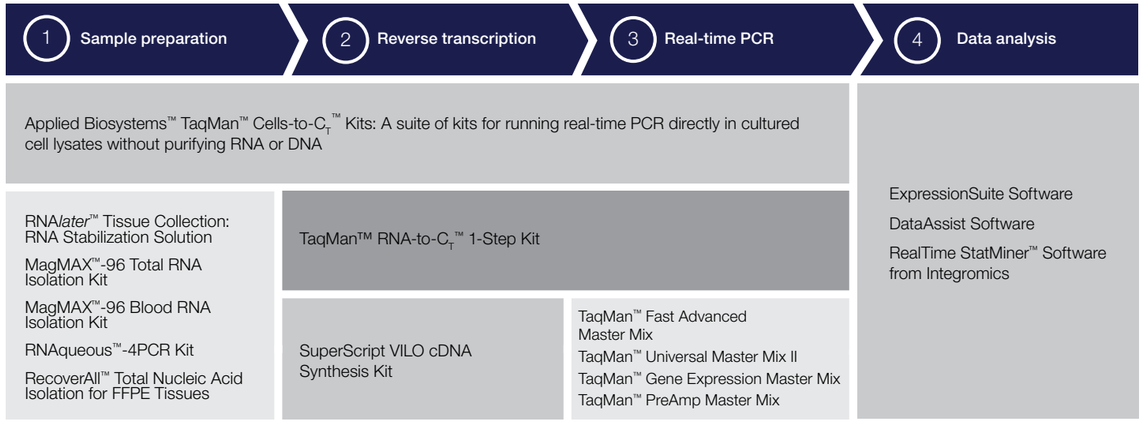
Configure 96- or 384-well plates with any TaqMan Gene Expression Assays, including custom assays designed to your target sequences and made-to-order assays.

- 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.

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Find out more at **thermofisher/allgenes**

ThermoFisher
SCIENTIFIC

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.

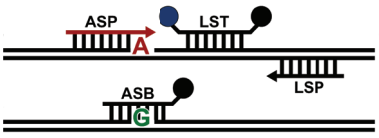
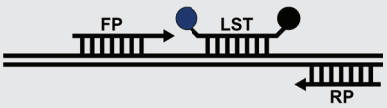
Assay type	Description	Schematic
Mutant allele assay	• Detects specific or multiple mutant alleles • An allele-specific primer detects the mutant allele • An MGB blocker oligonucleotide suppresses the wild type allele	 ASP = Allele-specific primer ASB = Allele-specific blocker (MGB) LST = Locus-specific 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.

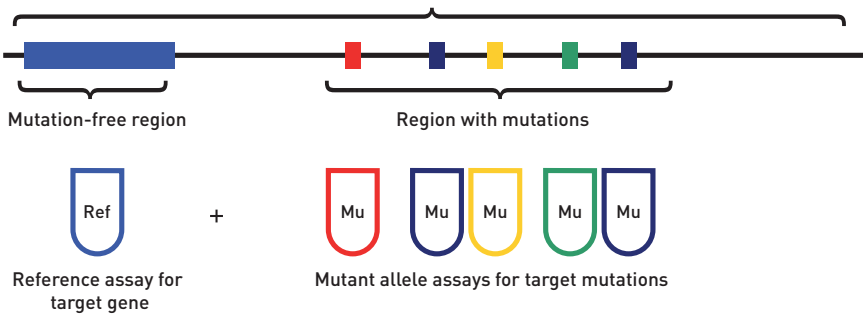
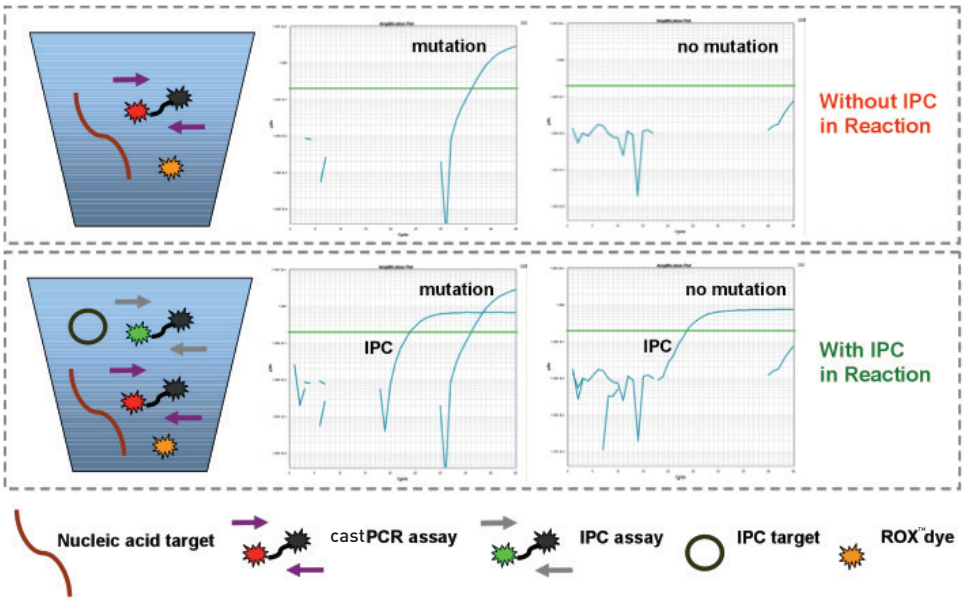


Figure 3. Internal positive controls. The TaqMan® Mutation Detection IPC Reagent Kit is a set of optional internal positive control reagents that can be duplexed with any TaqMan® Mutation Detection Assay to provide a positive PCR control result. The IPC reagents can distinguish a mutation target negative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

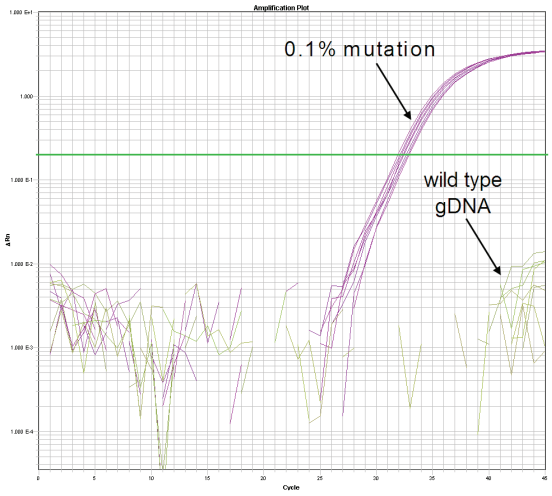
TaqMan® Mutation Detection Assays can detect as few as 1–5 mutant copies in up to one million copies of wild type background. Assay sensitivity is demonstrated using synthetic template spiking experiments (Figure 5 and 6).

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

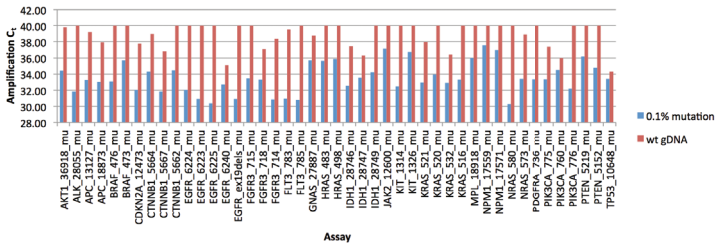


Figure 5. Assay sensitivity and selectivity. For every single assay, the sensitivity and selectivity were analyzed through synthetic template spiking experiments. 10 copies to 10^5 copies of mutant allele synthetic template were spiked into a constant background of 10^5 copies of wild type cell line 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.

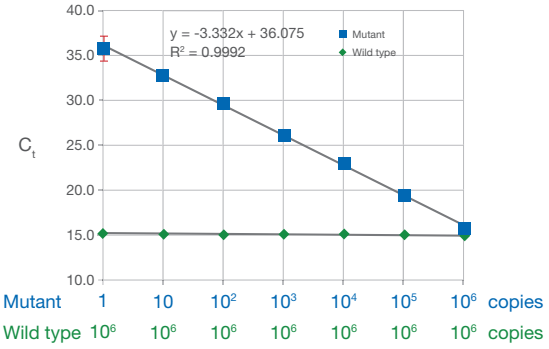
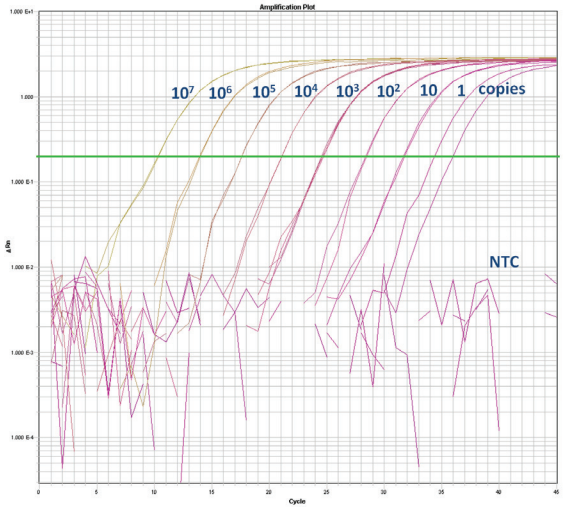


Figure 6. Assay dynamic range. Each assay was tested with 10^5 copies to 10 copies of synthetic template within a constant background of 10^5 copies of wild type genomic DNA. A subset of the assays was tested with 10^7 copies to 1 copy of synthetic template within a constant background of 10^7 copies 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**

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A Thermo Fisher Scientific Brand

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.

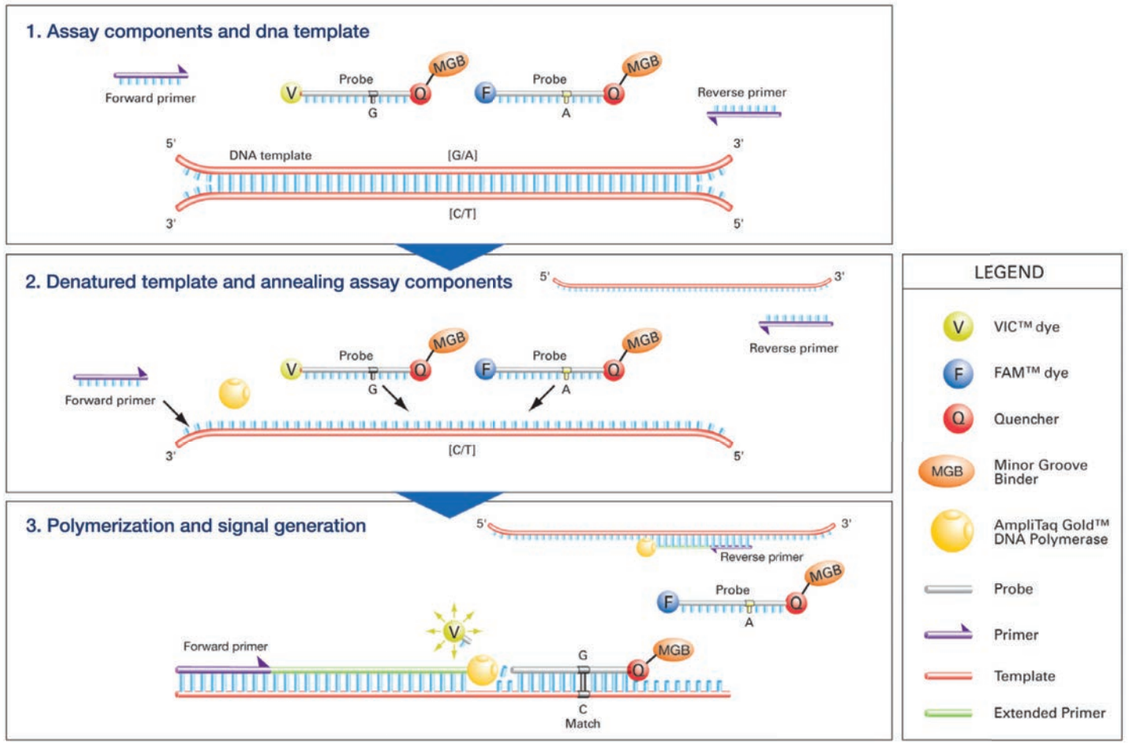


Figure 1. The TaqMan SNP Genotyping Assay. (1) The four TaqMan SNP Genotyping Assay components and the target DNA template with the SNP alleles (in brackets). **(2)** The denatured DNA target and annealing of the assay components. **(3)** Signal generation leading to specific allele detection.

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.

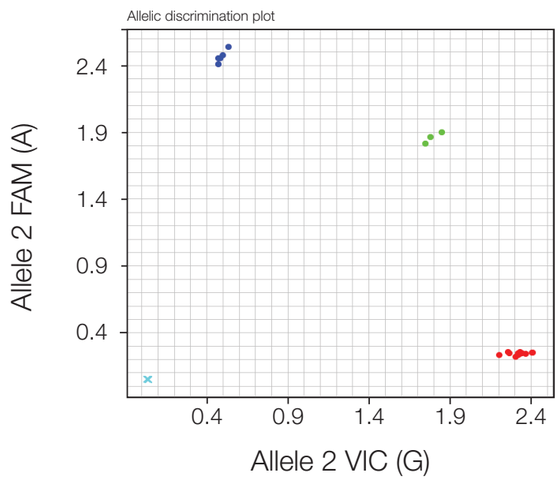


Figure 2. A three-cluster allelic discrimination plot generated with TaqMan SNP Genotyping Assay, C___1202883_20 (rs1801133) for *MTHFR* gene.

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**

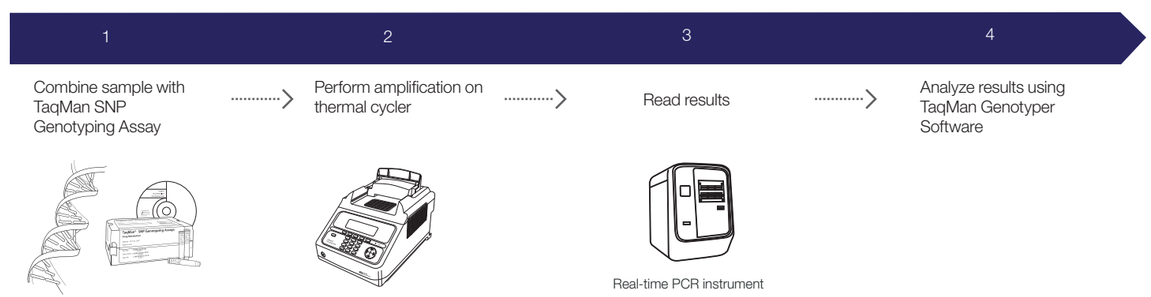


Figure 3. Workflow for TaqMan SNP Genotyping Assays.

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.

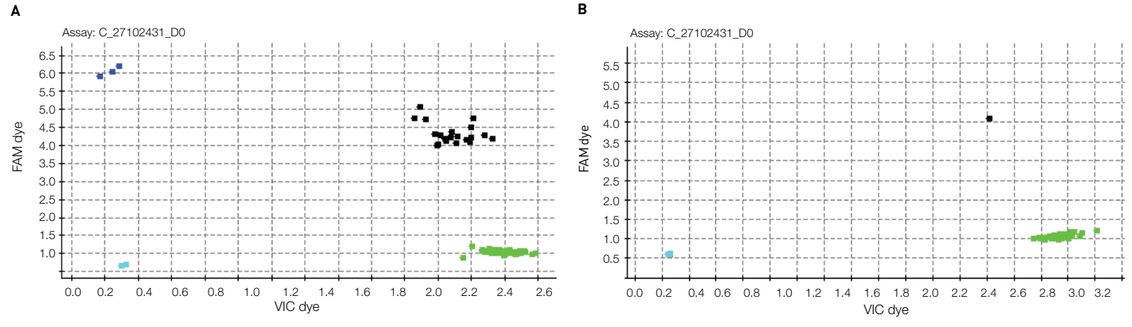


Figure 4. Allelic discrimination plots for the C__27102431_D0 assay run on (A) 45 each African-American and Caucasian, and (B) 45 each Chinese and Japanese gDNA samples. C__27102431_D0 targets the CYP2D6*4,g.1846G>A polymorphism, which encodes an mRNA splicing defect that results in a nonfunctional CYP2D6 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

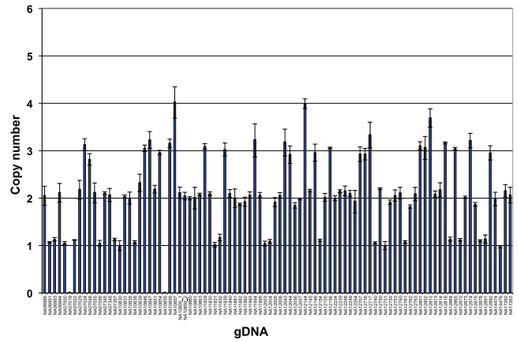
CNV, initially defined as variation in copy number of segments of DNA ≥1 kb in size, between individuals, is found in all humans as well as other animals and plants.

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

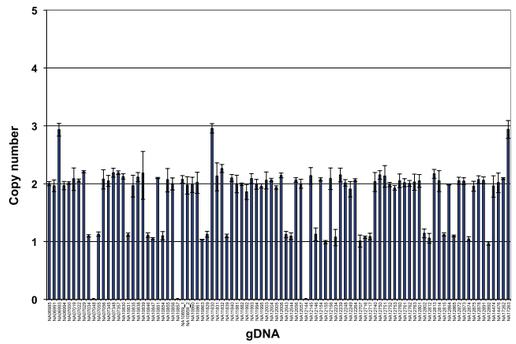


Figure 5. High specificity of TaqMan Copy Number Assays. C4A and C4B represent two isoforms of the C4 gene family. The sequences for these two genes differ in only 5 bases, but the encoded C4A and C4B proteins are functionally different. Differential detection of **(A)** C4A and **(B)** C4B is very challenging. 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.)

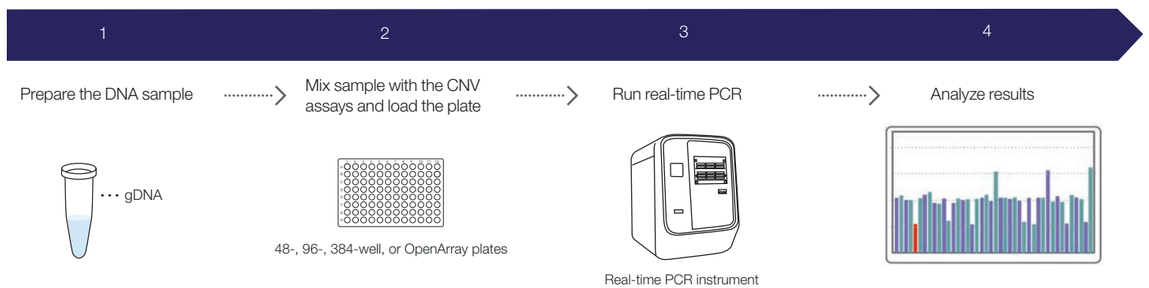


Figure 6. Workflow for TaqMan Copy Number Assays.

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.

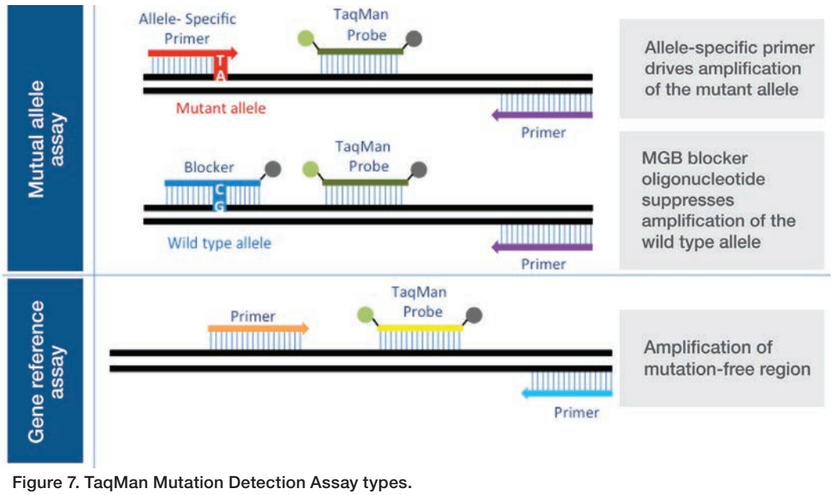


Figure 7. TaqMan Mutation Detection Assay types.

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.

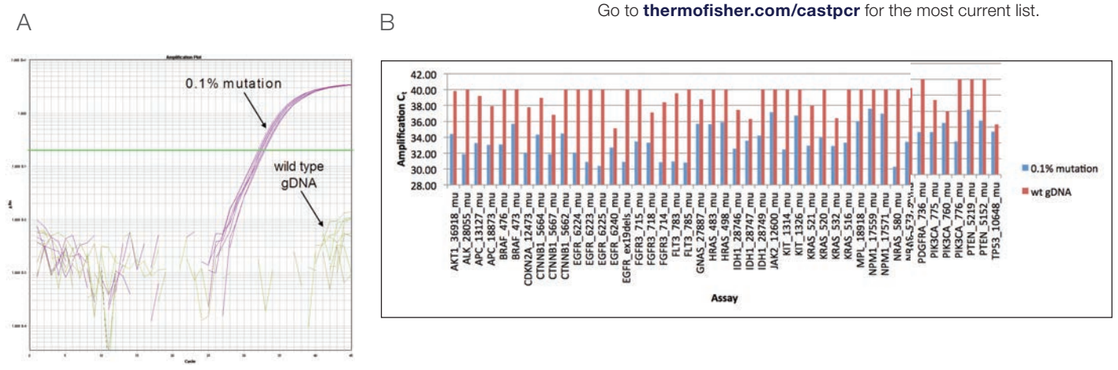


Figure 8. C_t differences between 0.1% mutation samples and wild type gDNA in TaqMan Mutation Detection Assays. For each mutant allele assay, 0.1% mutant allele samples were obtained by spiking cell line wild type gDNA (30 ng, ~10,000 copies) with 10 copies of mutant allele synthetic templates. **(A)** 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**

applied biosystems

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

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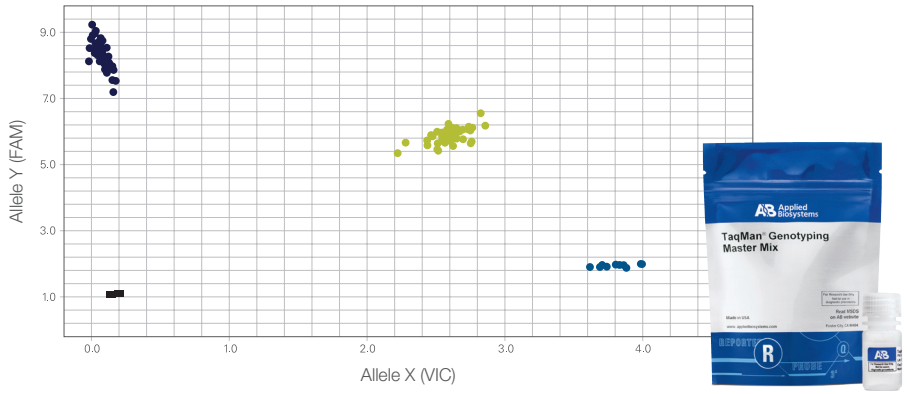
TaqMan Genotyping Master Mix

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

ThermoFisher SCIENTIFIC

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).

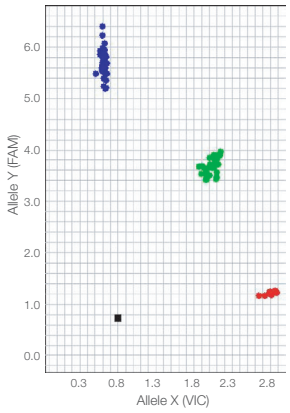


Figure 1. TaqMan Genotyping Master Mix provides bright fluorescence signals for discrete, well-separated allelic clusters. Cluster plot of 94 gDNA samples and two no-template controls genotyped using Applied Biosystems™ TaqMan™ SNP Genotyping Assay C__15969983_10, with PCR performed on the Applied Biosystems™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

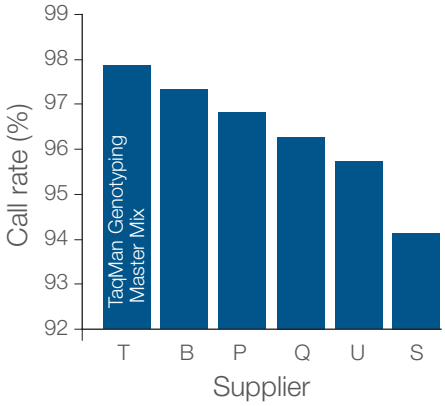


Figure 2. TaqMan Genotyping Master Mix provides the highest SNP call rates, outperforming other master mixes. Average call rates for 94 gDNA samples and two no-template controls genotyped using Applied Biosystems™ TaqMan™ SNP Genotyping Assay C__27102425_10, with PCR performed on the GeneAmp PCR System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

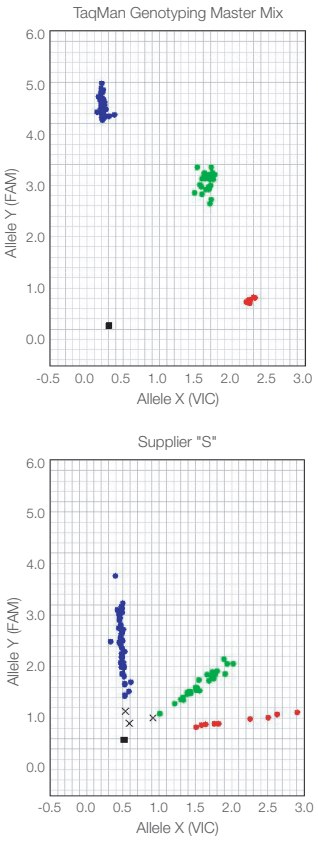


Figure 3. Consistent, reliable SNP detection in a GC-rich region using TaqMan Genotyping Master Mix. Genotyping assays were compared using TaqMan Genotyping Master Mix and a PCR master mix from supplier "S" on a set of 94 human gDNA samples (3 ng) and two no-template controls, using Applied Biosystems™ 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.

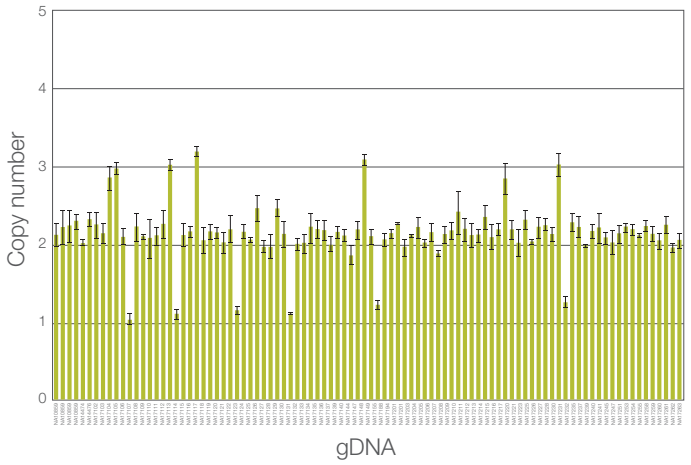


Figure 4. TaqMan Genotyping Master Mix is used for amplification in TaqMan Copy Number Assays. TaqMan Genotyping Master Mix is used with a TaqMan Copy Number Assay designed to target the *CYP2D6* gene, determining the copy number for this target in 92 gDNA samples. The RNase P reference gene is present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

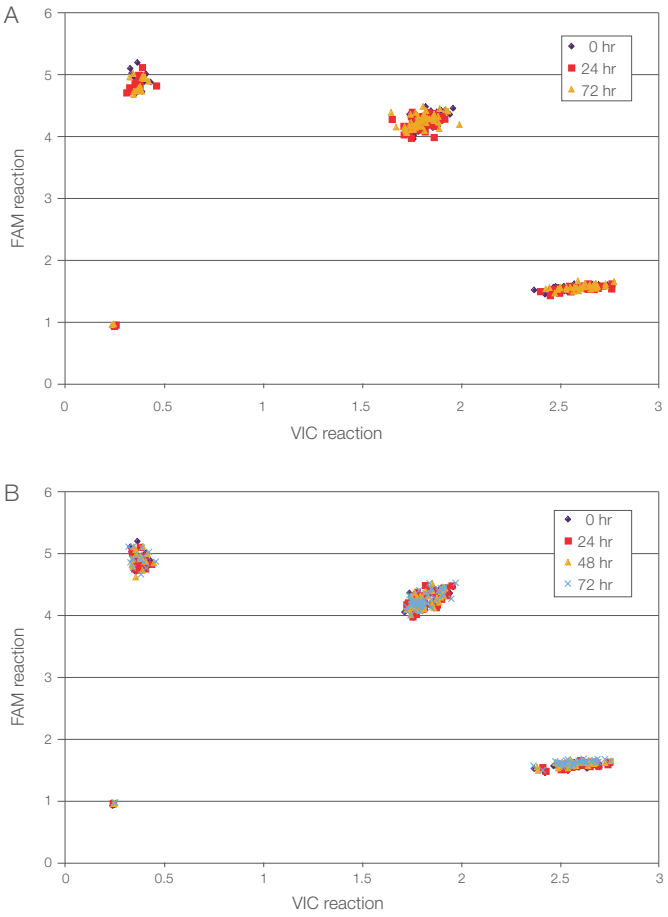


Figure 5. TaqMan Genotyping Master Mix provides pre- and post-PCR stability for up to 3 days. Genotyping reactions were set up using 94 gDNA samples and two no-template controls with TaqMan Genotyping Master Mix and TaqMan SNP Assay C___2188620_10. Reactions were left on the bench either **(A)** before 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

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Company code 122351387, VAT code LT223513811

CERTIFICATE OF ANALYSIS

4444557 **TaqMan™ Fast Advanced Master Mix, 5 mL**

Packaging Lot: 2691130
Manufacturing Date: 08.03.2023 (DD.MM.YYYY)
Expiration Date: 31.03.2025 (DD.MM.YYYY)

QUALITY CONTROL

References

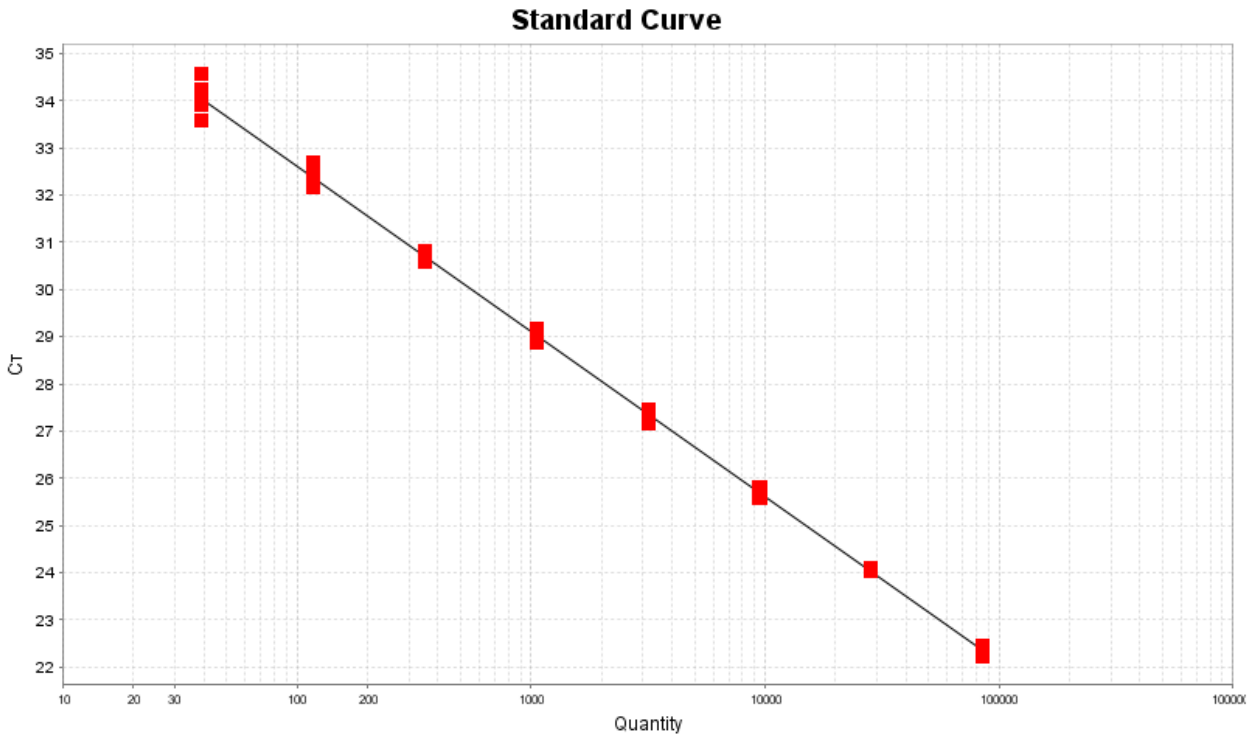
TEST	RESULT
ANALYTICAL TEST	
dATP Concentration by HPLC	Pass
dCTP Concentration by HPLC	Pass
dGTP Concentration by HPLC	Pass
dUTP Concentration by HPLC	Pass
Magnesium ion (Mg2+) concentration (measured by ion chromatography)	Pass
Bacterial DNA carryover	Pass
pH at 25°C	Pass
RNase level	Pass
DNase level	Pass
FUNCTIONAL TEST	
TaqMan™ Fast Advanced Master Mix is tested for Gene Expression Assay performance using a TaqMan™ RNaseP Assay coupled with the TaqMan™ Exogenous Internal Positive Control on ABI 7500 Fast qPCR System.	
PCR Efficiency	Pass
R2 value ≥ 98%	Pass
Fold Discrimination	Pass
Duplex Ct	Pass

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

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

Image for product number 4444557 lot number 2691130



Target: FAM-TAMRA Slope: -3,507 Y-Inter: 39,637 R^2: 0,998 Eff%: 92,82

TaqMan Fast Advanced Master Mix

Performance superior to standard master mixes in less than half the time

Features and benefits

- **Best-in-class performance**—superior sensitivity, accuracy, dynamic range, and specificity compared to standard mixes in standard mode
- **Engineered for enhanced benchtop stability**—stable at room temperature for up to 72 hours in preassembled reactions
- **Optimized for multiplexing**—validated for duplexing with exogenous and endogenous internal positive control assays
- **Reduced run times**—optimized on fast instruments and for fast cycling conditions on standard instruments
- **Seamless transition through the workflow**—validated with Applied Biosystems™ TaqMan® Assays for gene expression and microRNAs and Applied Biosystems™ TaqMan® Array Microfluidic Cards

Applied Biosystems™ TaqMan® Fast Advanced Master Mix has been designed to perform better than standard master mixes (Figure 1), requiring shorter run times (<40 minutes) and delivering superior results.

Our best-in-class gene expression master mix employs Applied Biosystems™ AmpliTaq™ Fast DNA Polymerase, which has been engineered for enhanced stability, allowing your preassembled reactions to be left at room temperature for up to 72 hours without impacting performance. The formulation has been optimized for duplex PCR with both endogenous and exogenous control assays, enabling you to run a control in every well to further increase confidence in your results.

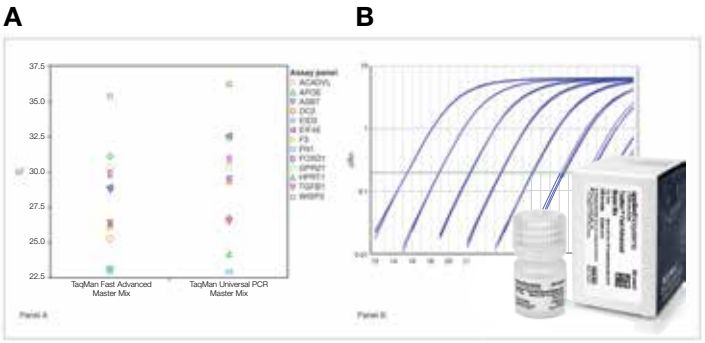


Figure 1. Performance of TaqMan Fast Advanced Master Mix vs. Applied Biosystems™ TaqMan® Universal PCR Master Mix. (A) Comparison of C_t values across a panel of 13 TaqMan Gene Expression Assays. **(B)** Representative amplification plot from real-time PCR of a dilution series of human cDNA amplified in 4 replicate reactions using the Applied Biosystems™ 7500 Fast Real-Time PCR System and the *FN1* TaqMan Gene Expression Assay.

For maximum flexibility, TaqMan Fast Advanced Master Mix has also been optimized for use on both fast instruments and fast PCR cycling conditions on standard instruments. TaqMan Fast Advanced Master Mix has been rigorously tested and optimized to help ensure success with all TaqMan gene expression and microRNA assays, enabling a seamless transition through your workflow.

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Table 1. Dynamic range comparison between TaqMan Fast Advanced Master Mix and products from other leading suppliers. Comparison of detection range (in number of logarithmic units) across a panel of various TaqMan Gene Expression Assays. The range of detection must have PCR efficiency between 85% and 115% and R^2 values ≥0.98. Each master mix was tested using cDNA template and run according to the manufacturers' respective recommended protocols. Reactions (6 replicates) were run on the Applied Biosystems™ 7900HT Fast Real-Time PCR System.

Assay	Assay type	TaqMan Fast Advanced Master Mix	Roche FastStart Reagent	Qiagen QuantiTect Reagent	Qiagen QuantiFast Reagent	Bio-Rad iTaq Supermix	Bio-Rad iTaq Fast Supermix
APOA1	Good Fast	7	5	5	5	5	5
APOA1 (FAM)/ *GAPDH* (VIC)	Good Fast	7	4	4	5	5	5
APOA1 (FAM)/ *GAPDH* (VIC)	Housekeeping	7	7	7	7	7	7
UBC	Housekeeping	6	4	4	5	5	5
HIST1H3F	LenAmpLong	5	3	3	3	3	3
TXNDC	GCAmpLow, PrimerLong	5	2	2	3	3	3
FOXD1	GCAmpHigh	4	2	2	2	2	2
GPR34	GCProbeLow, Low dRn	3	1	2	2	2	2
WISP	HighProbeTm	2	0	0	1	1	1

Orders of magnitude	Final (ng/µL)
7	0.00001
6	0.0001
5	0.001
4	0.01
3	0.1
2	1
1	10

Best-in-class performance

TaqMan Fast Advanced Master Mix was designed to perform better than current standard master mixes. Our master mix was benchmarked against the leading suppliers' standard and fast master mixes to demonstrate our superior sensitivity, accuracy, dynamic range, and specificity.

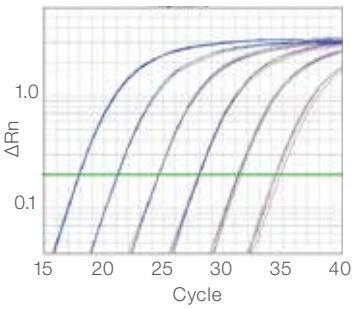
The unparalleled dynamic range of TaqMan Fast Advanced Master Mix is shown in Table 1. These results demonstrate the ability of the master mix to provide dependable target quantitation over a wider dynamic range compared to leading suppliers' standard and fast master mixes. For a variety of assays, TaqMan Fast Advanced Master Mix was capable of detection across 2 additional orders of magnitude when run under identical conditions.

Benchtop stability for high-throughput handling and convenience

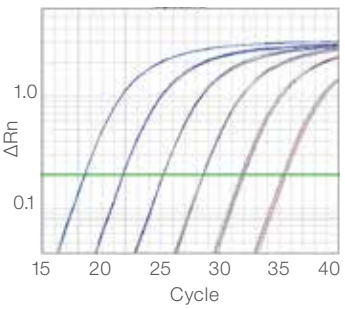
TaqMan Fast Advanced Master Mix has been engineered to retain its high level of performance in preassembled reactions for up to 72 hours. If you use high-throughput liquid handling systems, the stability of this mix helps to ensure that the results on the first plate will mimic those of the last plate. For less extreme throughput needs, the enhanced stability of this master mix provides overall added convenience to your workflow, as you are no longer constrained to immediately running your plates upon assembly.

Figure 2 shows an assay that was run upon assembly (time 0) and after 72 hours of incubation at 30°C, simulating the most extreme room temperature scenario. The results after 72 hours show excellent PCR efficiency and R^2 values, almost identical to those at time 0, as well as a ΔC_t between time 0 and 72 hours of less than 1.

A



B

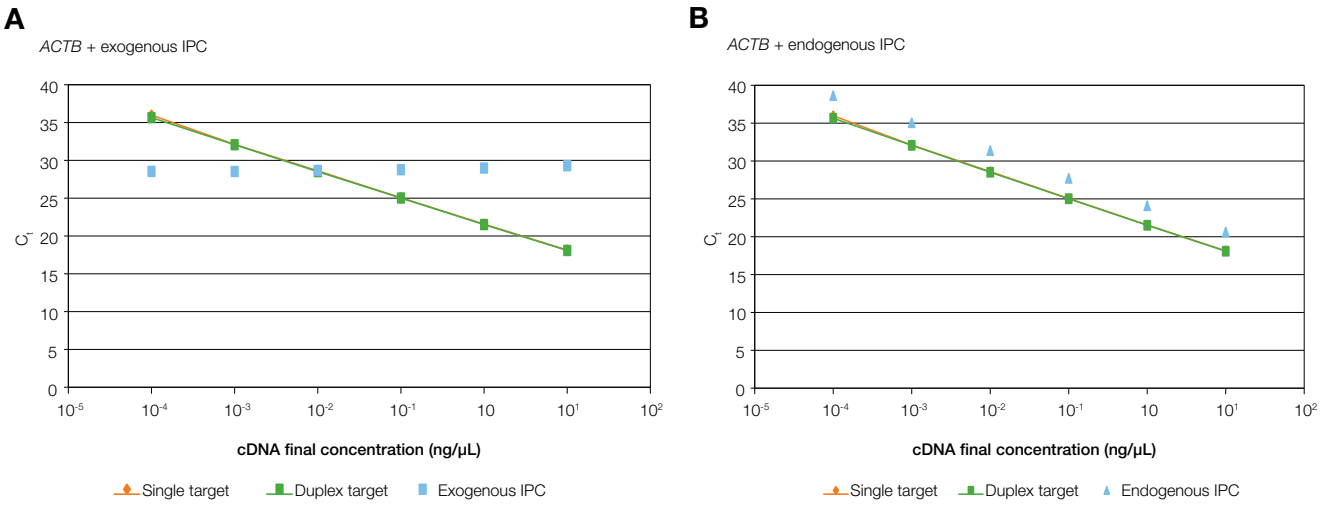


C

PCR efficiency		R^2	
0 hr	72 hr	0 hr	72 hr
99.7%	100.0%	0.9998	0.9997

Final concentration (ng/µL)	C_t	
cDNA	0 hr	72 hr
10	17.81	18.34
1	20.99	21.59
0.1	24.32	24.95
0.01	27.65	28.24
0.001	31.18	31.80
0.0001	34.34	34.80

Figure 2. Benchtop stability of TaqMan Fast Advanced Master Mix. This *B2M* TaqMan Gene Expression Assay was run **(A)** upon assembly (time 0) and **(B)** after 72 hr of incubation at 30°C. **(C)** The results after 72 hr show excellent PCR efficiency, R^2 values, and C_t values when compared to time 0.



Exogenous duplex

	PCR efficiency	R^2
Single target	92.9%	0.999
Duplex target	95.1%	1.000

Endogenous duplex

	PCR efficiency	R^2
Single target	92.9%	0.999
Duplex target	92.6%	1.000

Figure 3. TaqMan Fast Advanced Master Mix is optimized for multiplexing with exogenous or endogenous control assays. Results are shown for *ACTB* (β-actin gene), which was serially diluted and amplified in single-target reactions and duplex reactions. The duplex reactions included the single target *ACTB* and either **(A)** a constant quantity of exogenous target or **(B)** a relative quantity of endogenous target.

Optimized for multiplexing

We realize that confidence is paramount when it comes to your results. For added confidence in your results, TaqMan Fast Advanced Master Mix was designed to help deliver accurate results for duplex reactions using an internal positive control (IPC). Figure 3 shows results for the experimental target gene *ACTB* (β-actin), which was serially diluted and amplified in both single-target reactions and duplex reactions. The duplex reactions included the single target *ACTB* and either a constant quantity of exogenous target (Figure 3A) or a relative quantity of endogenous target (Figure 3B). TaqMan Fast Advanced Master Mix succeeded in providing nearly identical PCR efficiency, R^2, and C_t values for *ACTB* in both simplex and duplex environments.

Validated for microRNA assays

TaqMan Fast Advanced Master Mix has been validated for multiple real-time PCR applications, including microRNA assays. The formulation provides high specificity and a large dynamic range, the two most critical performance attributes that define successful results when working with microRNAs. The data in Figure 4 demonstrate excellent PCR linearity over a range of inputs, covering 6 orders of magnitude.

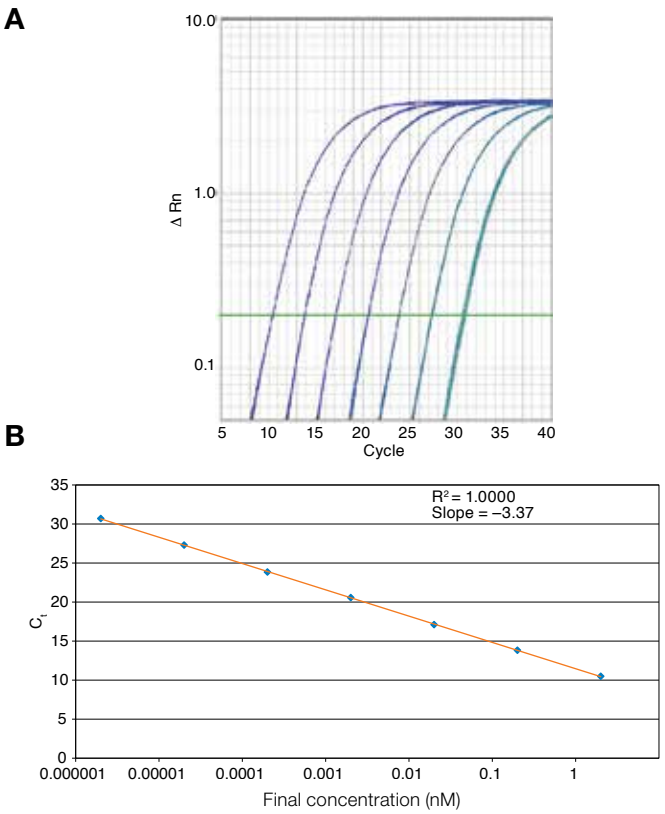


Figure 4. miRNA linear target amplification over a dynamic range of 6 orders of magnitude of input. (A) Amplification plot and **(B)** standard curve from real-time PCR of a dilution series of a synthetic target amplified in 4 replicate reactions using the 7900HT Fast Real-Time PCR System and the Let-7c TaqMan MicroRNA Assay.

applied
biosystems

Reduced run times on standard instrumentation

TaqMan Fast Advanced Master Mix has been optimized for use with both fast and standard instrumentation, enabling researchers who currently own standard instruments to realize the performance benefits and time savings this mix provides. Figure 5 showcases the impressive results achieved when using TaqMan Fast Advanced Master Mix under fast thermal cycling conditions on the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System. The mix has been tested with all Applied Biosystems™ standard real-time PCR instrumentation, including the QuantStudio, 7900HT, 7500, and 7300 systems, to enable success whether or not you own a fast-enabled instrument.

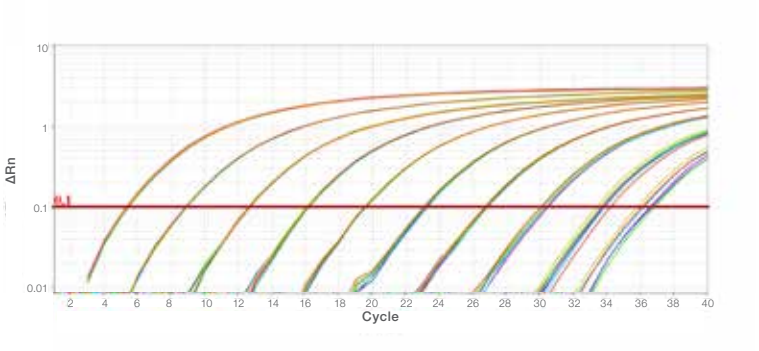


Figure 5. Results on the QuantStudio 12K Flex Real-Time PCR System using TaqMan Fast Advanced Master Mix. Amplification plot from real-time PCR of a dilution series of human cDNA amplified in 8 replicate reactions using the Eukaryotic 18S rRNA TaqMan Gene Expression Assay and the QuantStudio 12K Flex Real-Time PCR System.

Ordering information

Size	Quantity	No. of 20 µL rxns	Cat. No.
TaqMan Fast Advanced Master Mix			
Mini Pack	1 x 1 mL	100	4444556
1 Pack	1 x 5 mL	500	4444557
2 Pack	2 x 5 mL	1,000	4444963
5 Pack	5 x 5 mL	2,500	4444964
10 Pack	10 x 5 mL	5,000	4444965
Bulk Pack	1 x 50 mL	5,000	4444558

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

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