thermo scientific

PRODUCT INFORMATION

Thermo Scientific Phusion Flash High-Fidelity PCR Master Mix

Pub. No. MAN0012774
Rev. Date 27 June 2018 (Rev. B.00)

#__

Lot __ Expiry Date __

Store at -20°C

Ordering information

Component	#F-548S 100 rxns	#F-548L 500 rxns
2X Phusion Flash Master Mix	1 mL	5 ×1 mL

www.thermofisher.com

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

1. Introduction

Thermo Scientific™ Phusion™ Flash High-Fidelity PCR Master Mix is a 2X master mix based on modified Phusion Hot Start II DNA Polymerase. The unique composition of Phusion Flash High-Fidelity PCR Master Mix enables the use of extremely short PCR protocols (15 s/1 kb) with both low and high complexity DNA templates. Phusion Flash PCR Master Mix contains all the reagents required for PCR except for the DNA template and primers.

Phusion Flash II DNA Polymerase is a proofreading polymerase that contains a unique processivity-enhancing domain, making this polymerase accurate and rapid. Phusion Flash II DNA Polymerase is a hot start polymerase utilizing a reversibly binding Affibody® protein.[1,2] This protein inhibits DNA polymerase activity at ambient temperatures, thus preventing amplification of non-specific products. In addition, the Affibody protein blocks the 3'→5' exonuclease activity of the polymerase, preventing degradation of primers and template DNA during reaction setup. At polymerization temperatures, the Affibody protein dissociates from the polymerase rendering the enzyme fully active.

Phusion Flash II DNA Polymerase possesses 5'→3' DNA polymerase activity and 3'→5' exonuclease activity. The error rate using Phusion Flash PCR Master Mix is 9.5×10^{-7} when determined with a modified lacI-based method.[3] The error rate is approximately 25-fold lower than that of *Thermus aquaticus* DNA polymerase and 3-fold lower than that of *Pyrococcus furiosus* DNA polymerase. Phusion Flash DNA Polymerase is free of contaminating endo- and exonucleases.The polymerase is capable of amplifying long amplicons such as the 7.5 kb genomic and 20 kb lambda DNA used in Thermo Scientific's quality control assays.

2. Source

Thermostable Phusion DNA Polymerases are purified from recombinant *E.coli* strains. The Affibody ligand is purified from an *E.coli* strain carrying a plasmid encoding Affibody protein.

3. Important Notes

- Use 98 °C for denaturation (see 6.1 & 6.2).
- The annealing rules are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read Sections 5.2 and 6.3 carefully.
- Use 15 s/kb for extension (see 6.4).
- **Note:** Phusion Flash II DNA Polymerase produces blunt end DNA products.

4. Guidelines for using Phusion Flash PCR Master Mix

Carefully mix and spin down the Phusion Flash PCR Master Mix tube before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature.

Due to the nature of Phusion Flash II DNA Polymerase, optimal reaction conditions may differ from other amplification protocols. Please pay special attention to the conditions listed below when running your reactions. Following the guidelines will ensure optimal enzyme performance.

Table 1. Pipetting instructions (add items in this order)

Component	20 µL rxn	50 µL rxn	Final conc.
H_2O	Add to 20 µL	add to 50 µL	
2X Phusion Flash PCR Master Mix	10 µL	25 µL	1X
Primer A (see 5.2)	X µL	X µL	0.5 µM
Primer B (see 5.2)	X µL	X µL	0.5 µM
Template DNA (see 5.3)	X µL	X µL	

Table 2.Cycling instructions

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98 °C	10 s	98 °C	10 s	1
Denaturation (see 6.2)	98 °C	0 or 1 s	98 °C	0 or 1 s	30
Annealing (see 6.3)	-	-	X °C	5 s	
Extension (see 6.4)	72 °C	15 s/1 kb	72 °C	15 s/1 kb	
Final Extension	72 °C 4 °C	1 min hold	72 °C 4 °C	1 min hold	1

5. Notes about reaction components

5.1 Phusion Flash High-Fidelity PCR Master Mix

Phusion Flash PCR Master Mix contains all the necessary reaction components for PCR except for template DNA and primers. The composition of the Phusion Flash PCR Master Mix is designed to give optimal results.

When cloning fragments amplified with Phusion Flash II DNA Polymerase, blunt end cloning is recommended. If TA cloning is required, it can be performed by adding A overhangs to the blunt PCR product with *Taq* DNA Polymerase, for example. However, before adding the overhangs it is very important to remove all Phusion Flash II DNA Polymerase by purifying the PCR product carefully. Any remaining Phusion Flash II DNA Polymerase will degrade the A overhangs, creating blunt ends again.

5.2 Primers

The recommendation for final primer concentration is 0.5 µM. If required, the primer concentration may be optimized between 0.2-1.0 µM. To shorten the time required for a PCR protocol, it is advisable to design primers suitable for a two-step PCR protocol, if possible. In a two-step PCR protocol, primer annealing and extension occur at 72 °C and a separate annealing step can be omitted. However, Phusion Flash PCR Master Mix can also be used when performing a PCR protocol with a separate annealing step (see section 6.3). The results from primer Tm calculations can vary significantly depending on the method used. **Always use the Tm calculator and instructions on website: www.thermofisher.com/tmcalculator to determine the Tm values of primers and optimal annealing temperature.**

5.3 Template

General guidelines for low complexity DNA (e.g. plasmid, lambda or BAC DNA) are: 1 pg–10 ng per 20 µL reaction volume, or 2.5 pg–25 ng per 50 µL reaction volume. For high complexity genomic DNA, the amount of DNA template should be 10–100 ng per 20 µL reaction volume, or 25–250 ng per 50 µL reaction volume. If cDNA synthesis reaction mixture is used directly as a source for the template, the volume used should not exceed 10% of the final PCR reaction volume.

(Continued on reverse page)

6. Notes about cycling conditions

6.1 Initial denaturation

Denaturation should be performed at 98 °C. Due to the high thermostability of Phusion Flash II DNA Polymerase, even higher temperatures may be used. Initial denaturation of 10 seconds is recommended for all templates when using Phusion Flash PCR Master Mix.

6.2 Denaturation

A very short denaturation step is recommended. For this step, it is usually sufficient that the reaction mixture reaches the required 98 °C. If the PCR instrument used does not accept 0 seconds as a value, then a 1-second value can be programmed.

6.3 Primer annealing

For minimizing the total PCR cycling time, a two-step PCR protocol is recommended. It is applicable with primers whose Tm values are, when calculated with our Tm calculator (www.thermofisher.com/tmcalculator), at least 69 °C or 72 °C (primers >20 nt or ≤20 nt, respectively).

If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination. The annealing gradient should extend up to the extension temperature (two-step PCR).

6.4 Extension

The extension should be performed at 72 °C. Extension time of 15 seconds per 1 kb is suitable for most templates. Some amplicons can be successfully amplified with even shorter extension times, e.g. 1-5 seconds per 1 kb.

7. Troubleshooting

No product at all or low yield	
• Repeat and make sure that there are no pipetting errors. • Make sure that the cycling protocol was performed as recommended. • Optimize annealing temperature. • Titrate template amount. • Template DNA may be damaged. Use carefully purified template.	• Increase the number of cycles. • Check the purity and concentration of the primers.Check primer design. • Check primer design. • Increase extension time. • Increase denaturation time up to 5 seconds.
Non-specific products - High molecular weight smears	
• Make sure that the extension time used was not too long. (Recommended extension time is 15 s/kb). • Increase annealing temperature or perform a temperature gradient PCR.	• Titrate template amount. • Reduce the total number of cycles. • Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands	
• Increase annealing temperature. • Titrate template amount. • Shorten extension time.	• Perform a temperature gradient PCR. • Decrease primer concentration. • Design new primers.

TECHNICAL SUPPORT

EMEA: ts.molbio.eu@thermofisher.com

Americas & APAC: ts.molbio@thermofisher.com

8. References

1. Nord K. *et al.* (1997) *Nature Biotechnol.* 15: 772–777.
2. Wikman M. *et al.* (2004) *Protein Eng. Des.* Sel. 17: 455–462.
3. Frey M. & Suppmann B. (1995) *Biochemica* 2: 34–35.

CERTIFICATE OF ANALYSIS

DNA amplification assay

Performance in PCR is tested by the amplification of a 7.5 kb fragment of genomic DNA and a 20 kb fragment of lambda DNA.

Quality authorized by:  Jurgita Zilinskiene

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.

Phusion High-Fidelity DNA Polymerases

Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerases offer very high fidelity, speed, and yield for all PCR applications.

General instructions

- Due to the unique nature of Phusion DNA polymerases, always use the T_m calculator on our website to determine optimal annealing temperature (**thermofisher.com/tmcalculator**).
- Use 98°C for denaturation.
- Use 15–30 sec/kb for extension. Do not exceed 1 min/kb.
- Use Phusion DNA polymerases at 0.5–1.0 U per 50 µL reaction volume. Do not exceed 2 U per 50 µL reaction volume.
- Use 200 µM of each dNTP.
- If uracil is present in the dNTP mix or DNA template, use Thermo Scientific™ Phusion™ U Hot Start DNA Polymerase.

Note: Phusion DNA polymerases produce blunt-end DNA products.

Choosing the right Phusion product

		Phusion High-Fidelity DNA Polymerase (Cat. No. F530S)	Phusion Hot Start II High-Fidelity DNA Polymerase (Cat. No. F549S)	Phusion Flash High-Fidelity DNA Polymerase (Cat. No. F548S)	Phusion U Hot Start DNA Polymerase (Cat. No. F555S)	Phusion U Multiplex PCR Master Mix (Cat. No. F562S)
Characteristics	Blunt or 3′-A end	Blunt	Blunt	Blunt	Blunt	Blunt
	Target length, genomic/phage DNA	≤16/20 kb	≤16/20 kb	≤16/20 kb	≤7.5/20 kb	≤2.5/2.5 kb
	Hot start	No	Yes	Yes	Yes	Yes
	Recommended extension time	15–30 sec/kb	15–30 sec/kb	15 sec/kb	15–30 sec/kb	15–30 sec/kb
	Fidelity vs. *Taq*	52x	52x	25x	25x	NA
	dUTP tolerance	No	No	No	Yes	Yes
Formats	Enzyme*	✓	✓		✓	
	Green buffer**	✓	✓			
	Master mix†	✓	✓	✓	✓	✓
	Complete kit‡	✓				

* DNA polymerase, buffer, DMSO, and $MgCl_2$.
** DNA polymerase supplied with Phusion Green Buffer, which includes a density reagent and two tracking dyes for direct loading on gel.
† 2X master mix format.
‡ All the necessary PCR components, including control template and primers.

thermo scientific

Reaction setup

Component	50 µL reaction	20 µL reaction	Final concentration
5X Phusion buffer*	10 µL	4 µL	1X
10 mM dNTPs*	1 µL	0.4 µL	200 µM each
Primer A	x µL	x µL	0.5 µM
Primer B	y µL	y µL	0.5 µM
Template DNA	z µL	z µL	–
DMSO (optional)	(1.5 µL)	(0.6 µL)	(3%)
Phusion DNA polymerase	0.5 µL	0.2 µL	0.02 U/µL
Water	To 50 µL total	To 20 µL total	–

* If you are using any of the Phusion PCR master mix products, add 25 or 10 µL of the 2X master mix (depending on the final reaction volume). Do not add dNTPs.

Cycling instructions for Phusion and Phusion Hot Start II High-Fidelity DNA Polymerases

	2-step protocol		3-step protocol		
Cycle step	Temperature	Time	Temperature	Time	Cycles
Initial denaturation	98°C	30 sec	98°C	30 sec	1
Denaturation	98°C	5–10 sec	98°C	5–10 sec	25–35
Annealing*	–	–	X°C*	10–30 sec	
Extension	72°C	15–30 sec/kb	72°C	15–30 sec/kb	
Final extension	72°C	5–10 min	72°C	5–10 min	1
	4°C	Hold	4°C	Hold	

* Depends on the primer T_m values. Use the T_m calculator at **thermofisher.com/tmcalculator**

Cycling instructions for Phusion Flash High-Fidelity PCR Master Mix

	2-step protocol		3-step protocol		
Cycle step	Temperature	Time	Temperature	Time	Cycles
Initial denaturation	98°C	10 sec	98°C	10 sec	1
Denaturation*	98°C	0 or 1 sec	98°C	0 or 1 sec	30
Annealing**	–	–	50–72°C	5 sec	
Extension	72°C	15 sec/kb	72°C	15 sec/kb	
Final extension	72°C	1 min	72°C	1 min	1
	4°C	Hold	4°C	Hold	

* A very short denaturation step is recommended. If the PCR instrument used does not accept 0 sec as a value, then a 1 sec value can be programmed.
** Depends on the primer T_m values. Use the T_m calculator at **thermofisher.com/tmcalculator**

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

ThermoFisher SCIENTIFIC

ExoSAP-IT™ PCR Product Cleanup

Brief Protocol

Catalog Number 78200, 78201, 78202, 78205, and 78250

Doc. Part No. 78200b **Pub. No.** MAN0016836 **Rev.** A.0 (02/2017)

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

Product description

ExoSAP-IT™ reagent treats PCR products ranging in size from less than 100 bp to over 20 kb with absolutely no sample loss by removing unused primers and nucleotides. Add ExoSAP-IT™ reagent directly to the reaction products following PCR. ExoSAP-IT™ PCR Product Cleanup is active in commonly used PCR buffers, so no buffer exchange is required. After treatment, ExoSAP-IT™ reagent is inactivated by heating to 80°C for 15 minutes. The treated PCR products are now ready for subsequent analysis in applications that require DNA to be free of excess primers and nucleotides.

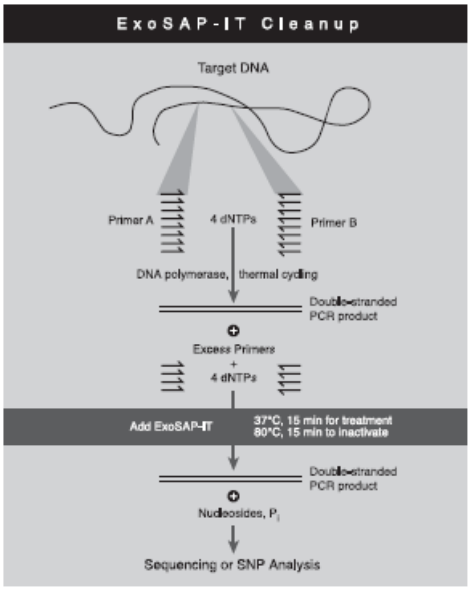
PCR cleanup protocol

Note: Store ExoSAP-IT™ reagent at –20°C in a non-frost-free freezer.

1. Remove ExoSAP-IT™ reagent from –20°C freezer and keep on ice throughout this procedure.
2. Mix 5 µL of a post-PCR reaction product with 2 µL of ExoSAP-IT™ reagent for a combined 7 µL reaction volume.

   When treating PCR product volumes greater than 5 µL, simply increase the amount of ExoSAP-IT™ reagent proportionally.
3. Incubate at 37°C for 15 minutes to degrade remaining primers and nucleotides.
4. Incubate at 80°C for 15 minutes to inactivate ExoSAP-IT™ reagent.
5. The PCR product is now ready for use in DNA sequencing, SNP analyses, or other primer-extension applications. Treated PCR products may be stored at –20°C until required.

ExoSAP-IT™ Cleanup product overview



Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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

Manufacturer's address: Affymetrix Inc. | 3450 Central Expressway | Santa Clara, CA 95051 | USA

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

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

Thermo Fisher Scientific Baltics UAB
V. A. Graiciuno 8, Vilnius, LT-02241 Lithuania
Phone +370 700 55131, Fax +370 700 55132
www.thermofisher.com
cofarequests@thermofisher.com
Company code 122351387, VAT code LT223513811

ThermoFisher SCIENTIFIC
The world leader in serving science

CERTIFICATE OF ANALYSIS

78202.4X.1.ML ExoSAP-IT™

Packaging Lot: **2585425**
Expiry Date: 30.09.2024 (DD.MM.YYYY)
Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
2580411	4 x 1 mL	ExoSAP-IT™

QUALITY CONTROL

Parameter	Method	Requirement	Result
Functional Assay	Test and Control ExoSAP-IT samples are used to eliminate unincorporated dNTP and unused primers from the PCR product. Exonuclease I/SAP-treated PCR product is sequenced. Quality of the sequence is evaluated visually.	There are no extra or missing bands, absence of stops in Test sequences. Test and Control sequences have uniform band intensity	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

Use of ExoSAP-IT PCR Product Cleanup Reagent in NGS

Cited in over 10,000 publications, Applied Biosystems™ ExoSAP-IT™ PCR Product Cleanup Reagent is widely used for enzymatic PCR cleanup. The one-tube, one-step ExoSAP-IT method has many advantages over using spin columns or magnetic beads for PCR cleanup. With its simple protocol and 100% recovery of both short and long amplicons, ExoSAP-IT reagent enables researchers to conserve limited samples and improve workflow efficiency. While Sanger sequencing–based methods remain popular for validation and long contiguous DNA sequence reads (>500 nucleotides), many genomic analysis applications are transitioning to next-generation sequencing (NGS) technology for its scalability and affordability for sequencing a large number of targets [1].

In addition to its routine use in Sanger sequencing, ExoSAP-IT reagent is proving beneficial in library preparation workflows across a broad range of NGS applications and platforms for Thermo Fisher Scientific, Illumina, and PacBio, among others (Table 1). This application note reviews NGS applications by platform and highlights the utility of ExoSAP-IT reagent and its benefits for each workflow.

Ion Torrent™ NGS instrument

The Ion Torrent™ Personal Genome Machine™ (PGM™) System uses semiconductor technology to sequence Ion Torrent™ DNA libraries like those prepared using the Ion Xpress™ Plus Fragment Library Kit. This technology works by detecting the positively charged hydrogen ion (H^+) that is released when a nucleotide is incorporated. By stepwise addition of one specific nucleotide after another, each position of a DNA template can be determined. Ion Torrent libraries comprise DNA fragments (~200 bp) ligated to blunt-ended adapters that enable sequencing on the Ion PGM platform.

Enzymatic cleanup applications

As shown in Table 1, ExoSAP-IT reagent for PCR cleanup is used in Ion Torrent workflows across a range of NGS applications, including species identification, genotyping, and targeted sequencing.

Table 1. Improved NGS workflows using ExoSAP-IT reagent across a broad range of applications and platforms.

Application	Platform	Workflow	ExoSAP-IT reagent benefits	Relevance	Ref.
Species identification	Ion PGM	Mitochondrial DNA PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Efficient PCR cleanup prior to library construction using Ion Xpress kit	Identify species of meat	2
	Ion PGM	16S PCR ⇨ ExoSAP-IT reagent ⇨ PCR2 (addition of adapters)	PCR2 efficiency	Identify bacterial population in water	3
	Ion PGM	16S PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Efficient PCR cleanup prior to library construction using Ion Xpress kit	Identify bacterial population in water	4, 5
Mutation analysis	Illumina MiSeq	Long range (LR) PCR ⇨ ExoSAP-IT reagent ⇨ Nextera library prep kit	Efficient PCR cleanup prior to library construction using Nextera kit	Detect markers for chronic fatigue syndrome	6
	Illumina MiSeq	LR-PCR ⇨ ExoSAP-IT reagent ⇨ column purification ⇨ TruSeq library prep	Improved purification efficiency	Validate indels from whole-genome sequencing (WGS)	7
	Illumina MiSeq	LR-PCR ⇨ ExoSAP-IT reagent ⇨ column purification ⇨ TruSeq library prep kit	Improved purification efficiency	Validate indels from WGS	8
	Illumina MiSeq	Multiplex PCR ⇨ ExoSAP-IT reagent ⇨ PCR2 (addition of adapters)	PCR2 efficiency	Tumor profiling for cancer mutations	9
Genotyping	Ion PGM	Human leukocyte antigen (HLA) PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Removal of ssDNA for improved enzymatic shearing by Ion Shear Plus Reagent	HLA genotyping research	10
	Illumina HiSeq	Shear gDNA ⇨ ExoSAP-IT reagent ⇨ PCR2 (addition of adapters)	Increase in percent on-target reads	Genotyping-in-thousands by sequencing (GT-Seq)	11
	Illumina Genome Analyzer$_{IIx}$	Add adapters with PCR ⇨ ExoSAP-IT reagent ⇨ direct sequencing	Removal of primers that would bind to the surface of the flow cell	Antenatal SNP genotyping	12
	NEBNext reagent set for 454 library prep	HLA PCR ⇨ ExoSAP-IT reagent ⇨ 454 library prep	Efficient PCR cleanup prior to library prep	HLA genotyping research	13
Targeted DNA/RNA sequencing	Ion PGM	PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Removal of ssDNA for improved enzymatic shearing by Ion Shear Plus Reagent	Identify growth-rate genes involved in development of pigs	14
	Ion PGM	RT-PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Efficient PCR cleanup prior to library construction using Ion Xpress kit	Rotavirus in South African children presenting diarrhea	15
	Illumina HiSeq	RT ⇨ ExoSAP-IT reagent ⇨ poly(A) tailing ⇨ PCR ⇨ TruSeq library prep	Poly(A) tailing efficiency	Identify circular (circ)RNAs involved in development	16
	Illumina HiSeq	IP ⇨ adapter ligation ⇨ RT ⇨ ExoSAP-IT reagent ⇨ RNase H ⇨ adapter ligation ⇨ PCR	Downstream ligation and PCR efficiency	ID RNA-binding protein binding sites	17
	Illumina MiSeq	PCR ⇨ ExoSAP-IT reagent ⇨ PCR2 (addition of adapters)	PCR2 efficiency	Detect nitrogenase gene diversity in trees	18
	Illumina MiSeq	PCR ⇨ ExoSAP-IT reagent ⇨ PCR2/3 (addition of indices/adapters)	PCR2/3 efficiency	Examine leaf mycobiome	19, 20
Epigenetics	PacBio SMRTbell	SMRTbell prep ⇨ exonuclease III, VII ⇨ direct single-molecule real-time (SMRT) sequencing	Removal of incomplete SMRTbell templates	Direct sequencing of intergenic modified sites	21

In a study by Bertolini et al. [2], mitochondrial DNA (mtDNA) extracted from meat samples was amplified by PCR to enrich the species-specific mtDNA loci. PCR products were then cleaned with ExoSAP-IT reagent prior to library prep using the Ion Xpress Plus kit and sequenced to identify the species of the meat samples. Similar research workflows were applied in studies using 16S sequencing to identify bacterial populations in water [3-5] and in HLA genotyping assays [10]. The utility of ExoSAP-IT PCR cleanup reagent has also been demonstrated in targeted sequencing workflows in studies of growth-rate genes in pigs [14] and RNA rotavirus in South African children presenting diarrhea [15]. Several of these research workflows benefited from the ability of ExoSAP-IT reagent to efficiently remove excess primers that can interfere with the enzymatic shearing by Ion Shear™ Plus Reagent, which is part of the Ion Xpress Plus Fragment Library Kit (Figure 1).

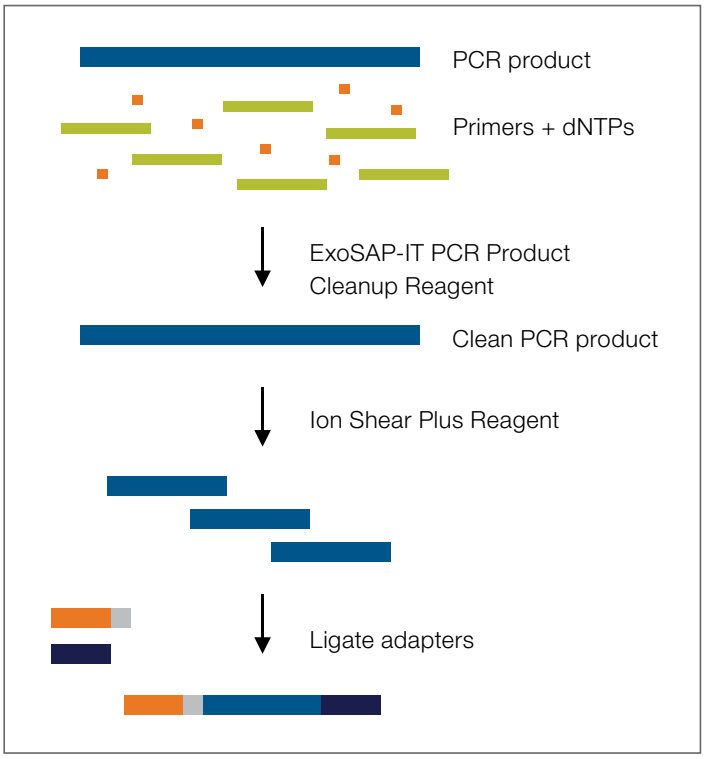


Figure 1. Ion Xpress Plus fragment library prep workflow. Example of Ion Xpress Plus Fragment Library Kit workflow starting with PCR-amplified DNA. ExoSAP-IT PCR reagent removes ssDNA and dNTPs, enabling more efficient fragmentation by Ion Shear Plus Reagent.

Illumina instruments

Illumina offers a range of instruments that vary in read length, sequencing depth, and throughput capacity. Illumina NGS technology is based on sequencing-by-synthesis (SBS) chemistry, which incorporates fluorescent nucleotides stepwise for base calling at each position of a DNA template. There are several methods available for preparing Illumina libraries, including a fragmentation approach using an Illumina Nextera™ kit, ligating T-tailed adapters with an Illumina TruSeq™ kit, and amplicon-based methods to add sequencing adapters.

Enzymatic cleanup applications

As shown in Table 1, ExoSAP-IT reagent for PCR cleanup is being used in Illumina workflows across a range of NGS applications, including mutation analysis, genotyping, and targeted sequencing.

In a study by Billing et al. [6], LR-PCR was used to amplify mtDNA to assess markers associated with chronic fatigue syndrome. LR-PCR products were cleaned with ExoSAP-IT reagent prior to Nextera library prep and sequenced for mutation analysis. This study demonstrated an efficient workflow for quantifying cleaned PCR products using ExoSAP-IT reagent with Invitrogen™ Quant-iT™ PicoGreen™ assay prior to pooling at equimolar ratios for Nextera library prep. ExoSAP-IT reagent has been cited in several publications involving amplification steps prior to TruSeq library prep. Studies by Fang et al. [7] and Narzisi et al. [8] describe a similar workflow where LR-PCR products were pooled, cleaned with ExoSAP-IT reagent, and then passed through a purification column prior to TruSeq library prep and NGS confirmation of indels. In this workflow, ExoSAP-IT reagent removes the large population of primers and nucleotides in the PCR pool, which is important for spin column efficiency and yield. In a study by Fan et al. [16], RNA from single cells was reverse-transcribed and cleaned with ExoSAP-IT reagent to enable poly(A) tailing in a sample prep workflow upstream of TruSeq library prep. Targeted NGS was then performed to sequence circular (circ)RNAs to investigate their regulatory function during development. An article by Van Nostrand et al. [17] describes another example of using ExoSAP-IT reagent to clean up after reverse transcription (RT) in a targeted NGS workflow for identifying binding sites for RNA-binding proteins with enhanced UV crosslinking and immunoprecipitation (eCLIP).

Based on recent publications, ExoSAP-IT reagent is proving to be very useful in workflows that add sequencing adapters using amplicon-based methods (Figure 2). Examples include a study by Doty et al. [18] that describes an amplicon-based library prep workflow incorporating ExoSAP-IT reagent in experiments investigating the plant microbiome, and similar studies by Siddique et al. [19] and Unterseher et al. [20] investigating the mycobiome. ExoSAP-IT reagent was also used to clean up multiplex PCR products in tumor profiling assays [9] and sheared DNA in GT-Seq [11] prior to amplicon-based library prep. In a study by Rieneck et al. [12], adapter sequences were included in PCR primers to amplify a single-nucleotide polymorphism (SNP) position, enabling direct sequencing of the SNP-containing PCR product in an antenatal genotyping assay. In this workflow, ExoSAP-IT PCR Product Cleanup Reagent effectively removed unincorporated primers that would otherwise bind to the Illumina flow cell and decrease NGS efficiency.

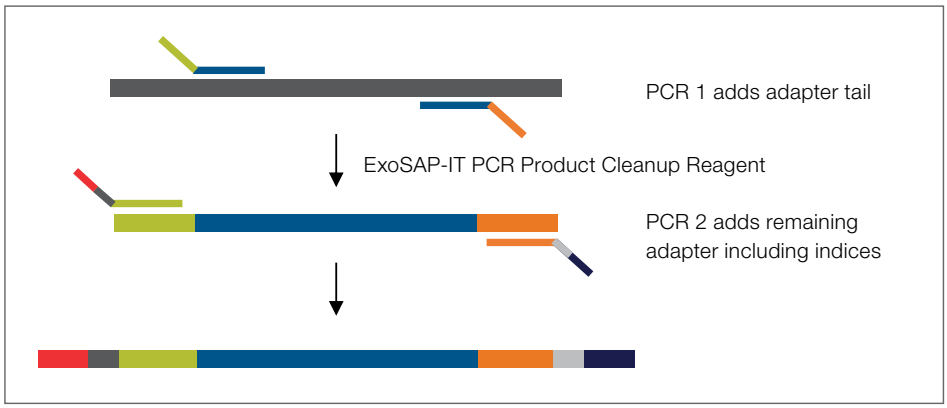


Figure 2. Amplicon-based library prep. Amplicon-based methods use PCR to target specific genes and add sequencing adapters. ExoSAP-IT PCR Product Cleanup Reagent can be used in between PCR steps to increase workflow efficiency.

PacBio platform

The Ion Torrent and Illumina platforms are similar in that the sequencing workflows include a clonal amplification step to augment the library to be sequenced. PacBio offers a third-generation sequencing platform that uses the novel SMRT sequencing technology to directly sequence single molecules in real-time (SMRT) using uniquely colored nucleotides. The SMRTbell™ Template Prep Kit is used to construct libraries of large DNA fragments (500 bp to >20 kb) flanked by SMRTbell hairpin adapters. During this process, exonuclease treatment is necessary to remove incomplete SMRTbell templates that cause inefficiencies in the direct sequencing reaction.

Enzymatic cleanup applications

Although only exonuclease III is used in this workflow, the PacBio technology is another example of how enzymatic cleanup is being applied in NGS. In a study by Seib et al. [21] that characterizes intergenic modified sites involved in gene regulation, the PacBio workflow includes use of USB™ Exonuclease VII for SMRTbell template purification.

Summary

The prevalence of ExoSAP-IT reagent usage in Sanger sequencing–based methods is reflective of its ease of use and ability to increase the efficiency and quality of traditional DNA sequencing results. As genomic research moves toward NGS applications run on platforms developed by Thermo Fisher Scientific, Illumina, and PacBio, the utility of ExoSAP-IT reagent in NGS library preparation protocols is becoming increasingly evident. Recent additions to the ExoSAP-IT reagent family (Table 2), including the fastest cleanup reagent on the market, Invitrogen™ ExoSAP-IT™ *Express* PCR Product Cleanup Reagent, are making it easier than ever to incorporate ExoSAP-IT PCR cleanup steps in NGS workflows for improved efficiency and more consistent results.

applied
biosystems

Table 2. Selection guide for ExoSAP-IT reagents.

	ExoSAP-IT *Express* reagent	ExoSAP-IT reagent (original formulation)	HT ExoSAP-IT *Fast* High-Throughput reagent (for automated liquid handlers)
Protocol time	5 min	30 min	14 min
Format	Single tube 8-tube strips	Single tube	Single tube 8-tube strips 96-well plate
Throughput level	Low to high; recommended for processing any sample size	Low to medium; recommended for processing 1–96 samples at a time	High; recommended for processing ≥96 samples at a time
Platform	Single- or multichannel pipette, automated liquid handling platforms	Single-channel pipette	Automated liquid handling platforms
Freezes at –20°C	No	No	Yes
Stability	At –20°C for up to 2 years	At –20°C for up to 2 years	At –20°C for up to 2 years; once thawed, stable at 4°C for 1 month and room temperature for 2 days

References

1. Goodwin S et al. (2016) *Nat Rev* 17:333-351.
2. Bertolini F et al. (2015) *PLoS One* 10:e0121701.
3. Jackson CR et al. (2014) *Appl Environ Microbiol* 80:7186-7195.
4. Saha M et al. (2014) *J Aquac Res Dev* 5(6). doi:10.4172/2155-9546.1000270.
5. Saha M et al. (2013) *J Bioremed Biodeg* 5(1). doi:10.4172/2155-6199.1000211.
6. Billing-Ross P et al. (2016) *J Transl Med* 14:19. doi: 10.1186/s12967-016-0771-6.
7. Fang H et al. (2014) *Genome Med* 6(10):89. doi: 10.1186/s13073-014-0089-z.
8. Narzisi G et al. (2014) *Nat Methods* 11(10):1033-1036.
9. Bourgon R et al. (2014) *Clin Cancer Res* 20(8). doi: 10.1158/1078-0432.CCR-13-3114.
10. Duke JL et al. (2015) *Int J Immunogenet* 42(5):346-358.
11. Campbell NR et al. (2014) *Mol Ecol Resour* 15(4):855-867.
12. Rieneck K et al. (2013) *Transfusion* 53:2892-2898.
13. Lind C et al. (2013) *Hum Immunol* 74(3):325-329.
14. Fontanesi L et al. (2015) *Anim Biotechnol* 262:92-97.
15. Magagula N et al. (2015) *J Med Virol* 87(1):79-101.
16. Fan X et al. (2015) *Genome Biol* 16:148. doi:10.1186/s13059-015-0706-1.
17. Van Nostrand EL et al. (2016) *Nat Methods* 13(6): 508-514.
18. Doty SL et al. (2016) *PLoS One* 11(5):e0155979.
19. Siddique AB et al. (2016) *Fungal Ecol* 20:175-185.
20. Unterseher M et al. (2016) *PLoS One* 11(4):e0152878.
21. Seib KL et al. (2015) *Nucleic Acids Res* 43(8): 4150-4162.

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

ThermoFisher SCIENTIFIC

GeneScan™ 500 LIZ™ Size Standard

SeqStudio™ Flex, SeqStudio™, 3500, 3730, and 3130 series instruments

Catalog Numbers 4322682

Pub. No. 4363115 Rev. D

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

Product description

The GeneScan™ 500 LIZ™ Dye Size Standard is an internal size standard for use with Applied Biosystems™ fluorescence-based DNA electrophoresis systems. An internal size standard enables automated data analysis during electrophoresis and precise DNA fragment size comparisons between electrophoresis runs. The GeneScan™ 500 LIZ™ Dye Size Standard sizes DNA fragments in the 35–500-bp range and provides 16 single-stranded, dye-labeled fragments of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500 bases. Each DNA fragment is labeled with the LIZ™ fluorophore, which results in a single peak when run under denaturing conditions.

This size standard is compatible with Dye Sets E5, G5, J6, and J6-T.

Contents and storage

Contents	Amount	Storage
GeneScan™ 500 LIZ™ Dye Size Standard	2 × 200 µL (400 reactions/tube; 800 reactions total)[1]	Store at 2–8°C, protected from light. Do not freeze.[2]

[1] The total number of reactions may vary depending on the specific application. This number is based on the volumes specified in this document.

[2] The product is stable for 1 year upon receipt.

Procedural guidelines

To optimize the analysis on capillary electrophoresis instruments, note the following:

- Use the size standard within 2 hours of preparation.
- The 250-bp peak is sensitive to small temperature variations. Do not use the 250-bp fragment when defining the size standard in the GeneMapper™ Software.
- The 340-bp peak is subject to large temperature variations.
- Fragment analysis primer peaks can often interfere with the detection of the 35-bp peak. If this occurs, copy the size standard definition and save it as a custom standard, then delete the 35-bp peak. Similarly, if the largest fragments are not collected with the run module that you are using, you can delete the largest fragments in a custom size standard definition.

Prepare the sample

1. Thoroughly mix the contents of the tube, then briefly centrifuge.
2. Combine the following components for the number of reactions required.

Component	Volume				
	SeqStudio™ Flex	SeqStudio™	3500 series	3730 series	3130 series
DNA sample	0.5 µL	0.5 µL	0.5 µL	0.5 µL	0.5 µL
Size standard	0.25 µL	0.25 µL	0.25 µL	0.5 µL	0.25 µL
Hi-Di™ Formamide (Cat. No. 4311320)	9.25 µL	9.25 µL	9.25 µL	9.0 µL	9.25 µL
Total volume per well	**10.0 µL**	**10.0 µL**	**10.0 µL**	**10.0 µL**	**10.0 µL**

Note: We recommend using the above ratios of DNA sample (PCR product) and size standard only as a starting point. Optimize these ratios as needed, based on your experimental results.

3. To denature the DNA fragments, incubate for 3 minutes at 95°C. Immediately place the mixture on ice for ≥2 minutes.

For information on setting up the run, see the instrument user guide.

Note: Discard any unused reagent that has been diluted in Hi-Di™ Formamide.

Limited product warranty

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

Life Technologies Ltd | 7 Kingsland Grange | Woolston, Warrington WA1 4SR | United Kingdom
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. 4363115

Revision	Date	Description
D	2 February 2022	Added the SeqStudio™ Flex Series Genetic Analyzer and SeqStudio™ Genetic Analyzer. Removed the 3100 and 310 series instruments. Added dye set compatibility. Added the manufacturing address. Made format, style, and legal updates.
C	13 December 2012	Baseline for this revision history

Certificate of Analysis

GeneScan™-500 LIZ™ Size Standard

Product No.	**4322682**
Lot No.	**2209474**
Date of Manufacture	**05SEP2022**

KIT CONFIGURATION

Part No.	Component Description	Lot No.
4322679	Tube, GS500 Liz Size Standard	2206188

TEST	SPECIFICATION	RESULT
Physical Inspection		
Tubes are inspected for correct label and lot number. Caps are tight with no signs of leakage.	PASS	PASS
Use Test		
Each lot of GeneScan™-500 LIZ™ Size Standard is tested for correct fragment pattern and peak height on an ABI Prism™ 3100 or 3130 Genetic Analyzer.	PASS	PASS

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

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

Quality Assurance
Issued 16SEP2022

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

TaqMan SNP Genotyping Assays

Applied Biosystems™ TaqMan™ SNP Genotyping Assays from Thermo Fisher Scientific provide a highly flexible technology for detection of polymorphisms within any genome. Applied Biosystems™ TaqMan™ Assays have the simplest workflow available and are the quickest way to generate genotyping data. Based on powerful Applied Biosystems™ TaqMan™ probe and primer chemistry and 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 screening, association, candidate region, candidate gene, and fine-mapping studies.

Content-rich marker selection tools simplify study design and help you select from a library of human and mouse assays. This library includes over 7 million genome-wide human assays (of which 3.5 million are HapMap SNP-based assays, 160,000 are validated assays, and over 950,000 are coding region assays) and 10,000 mouse assays. We also offer 2,700 inventoried drug metabolism genotyping assays. Additionally, with Applied Biosystems™ Custom TaqMan™ SNP Genotyping Assays you can confidentially submit target SNP sequences for any genome to create your own assays. Let TaqMan SNP Genotyping Assays accelerate the pace of your discovery by eliminating time-consuming experimental design and optimization.

Powerful, proven chemistry

Whether your genotyping studies require targeted detection of essential SNPs, or the flexibility to choose SNPs for mapping, TaqMan SNP Genotyping Assays are the technology of choice. Proven TaqMan

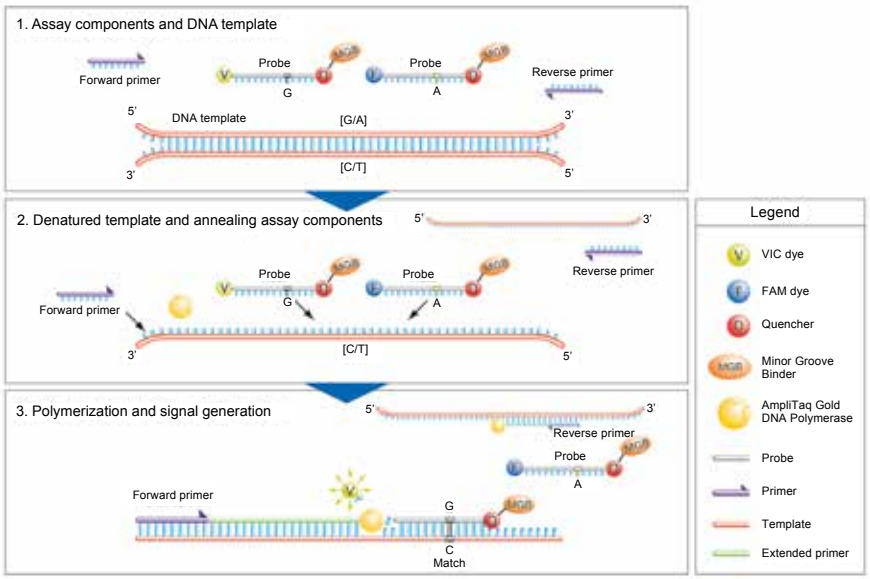


Figure 1. Allelic discrimination is achieved by the selective annealing of TaqMan MGB probes.

probes, which incorporate minor groove binder (MGB) technology at the 3′ end, deliver superior allelic discrimination. The MGB molecule binds to the minor groove of the DNA helix, improving hybridization-based assays by stabilizing the MGB probe–template complex. This increased binding stability permits the use of probes as short as 13 bases for improved mismatch discrimination and greater flexibility when designing assays for difficult or variable sequences. In addition to SNP detection, TaqMan probes can be designed to detect multiple nucleotide polymorphisms (MNPs) and insertion/deletions (indels).

Detection is achieved with proven 5′ nuclease chemistry by means of exonuclease cleavage of an allele-specific 5′ dye label, which generates the permanent assay signal (Figure 1). All MGB probes include a nonfluorescent quencher (NFQ) that virtually eliminates the background fluorescence associated with traditional quenchers, and provides a greater signal-to-noise ratio for superior assay sensitivity.

TaqMan SNP Genotyping Assays collection

TaqMan SNP Genotyping Assays are the world's largest collection of single-tube, ready-to-use SNP assays available. The TaqMan SNP Genotyping Assays library consists of two collections of human assays and one of mouse assays, and can be supplemented with assays designed using our Custom TaqMan SNP Genotyping Assays Service.

ThermoFisher SCIENTIFIC

Over 7 million human SNP genotyping assays

This assay group contains over 7 million genome-wide SNPs, providing unprecedented marker coverage. Included in this collection are 160,000 validated assays that have approximately 10 kb spacing across gene regions. These assays were subjected to an extensive minor allele frequency test in 2–4 ethnic populations (45 individual samples per ethnic group) and as a result, offer the highest success rate. Also included are over 600,000 assays for the detection of nonsynonymous SNPs in coding regions, including many putative functional SNPs. Visit **thermofisher.com/taqmansnp** for more information.

Over 10,000 mouse SNP genotyping assays

The Applied Biosystems™ Mouse TaqMan™ Predesigned SNP Genotyping Assays collection consists of over 10,000 assays, and can be supplemented with assays designed using our Custom TaqMan SNP Genotyping Assays Service.

TaqMan Drug Metabolism Genotyping Assays

The collection of Applied Biosystems™ TaqMan™ Drug Metabolism Genotyping Assays includes 2,700 assays that target high-value polymorphisms in 221 drug metabolism genes. These assays have proven performance in four different ethnic population samples, consisting of 45 individuals each. To enable easy identification, these assays have been mapped to the common public allele nomenclature websites where possible. Visit **thermofisher.com/taqmandme** for more information.

All TaqMan SNP Genotyping Assays are generated using next-generation algorithms from the Thermo Fisher Scientific bioinformatics pipeline. For all predesigned assays, bioinformatics evaluation of target SNP sequences includes the masking of adjacent SNPs and ambiguous bases so that assay design and subsequent performance is not affected by the poor quality of the underlying sequence. Lastly, the assay designs are aligned to the human genome using BLAST to ensure that each assay binds uniquely to the intended polymorphism. As the Custom TaqMan SNP Genotyping Assay Service is confidential and secure, you simply perform your own bioinformatics analysis prior to submitting your sequence for assay design.

Custom assay service for any possible SNP

Custom TaqMan SNP Genotyping Assays can be developed for any SNP in any organism. This service can generate assays for the detection of SNPs, MNPs, indels of up to 6 bases, or QSY™-labeled probes for multiplexing SNP assays.

Custom TaqMan SNP Genotyping Assays provide you with a complete service that includes secure and confidential ordering, assay design and manufacturing, and quality-control testing for synthesis accuracy and formulation completeness. Additionally, custom human assays are subjected to a functional test on 20 unique DNA samples.

Use the free Applied Biosystems™ Custom TaqMan™ Assay Design Tool to input and submit your sequence for assay design. This easy-to-use online resource lets you quickly submit your sequence information and start the ordering process securely and confidentially. Access the Custom TaqMan Assay Design Tool at **thermofisher.com/snpcadt**

Quality design and manufacturing

Probes and primers used in TaqMan SNP Genotyping Assays are designed using our rigorous bioinformatics pipeline. This proprietary group of algorithms has generated millions of TaqMan Assay designs by utilizing heuristic design rules deduced from both manufacturing and assay performance data. All assays are designed to perform under universal reaction conditions, as calculated probe and primer melting temperatures are consistent and include contributions from associated probe conjugates (i.e., dyes and MGB).

After manufacturing, assay components undergo extensive laboratory testing at our state-of-the-art manufacturing facility. Quality-control testing includes mass spectrometry for sequence verification and formulation assessments of probe and primer concentrations. Additionally, all human SNP genotyping assays are functionally tested to ensure allelic discrimination.

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 at ambient temperature in a convenient, single-tube format. 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 (Figure 2). There is no need to optimize probe, primer, salt concentrations, or temperature because all assays use universal reagent concentrations and thermal cycling conditions.

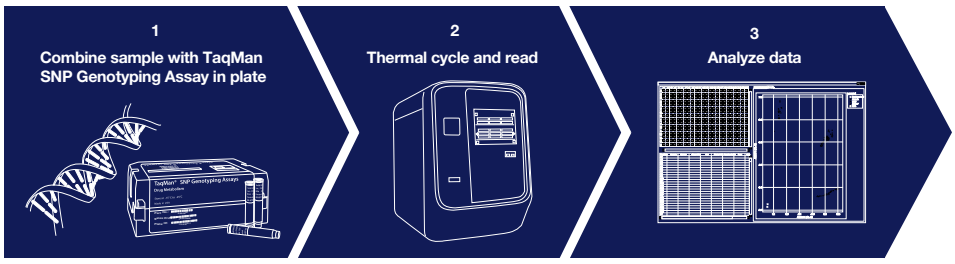


Figure 2. A simple workflow and reliable instruments combine to generate fast, high-confidence results.

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 reduces the chance of contamination, sample mix-up, 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 intervention.

Reliable real-time PCR platforms

A suite of superior Applied Biosystems instrument platforms is available for processing and analyzing TaqMan SNP Genotyping Assays (Table 1). These instruments, which meet all throughput needs and budgets, include the 7500, 7500 Fast, 7900HT Fast, ViiA™ 7, StepOne™, and StepOnePlus™ Real-Time PCR Systems, and the QuantStudio™ 3, 5, 6, 7, and 12K Flex Real-Time PCR Systems (Figure 3). Following PCR amplification, an endpoint read can be performed on any Applied Biosystems real-time PCR system. All of these dependable instruments offer the advanced multicolor detection capabilities required for highly accurate and reproducible allelic discrimination assays.

Data analysis software

The sophisticated SDS software package provided with all Applied Biosystems real-time PCR systems facilitates experimental setup, data collection, and assay performance analysis. The SDS software uses an advanced multicomponent algorithm to calculate the distinct signal contribution of each allele of a marker from the fluorescence measurements of each sample well during the assay plate read. The multicomponent data collected from the plate read are stored as SDS files, ready for genotype determination by the SDS software or optional Applied Biosystems™ TaqMan™ Genotyper Software (Figure 4).

Table 1. Applied Biosystems instrument capacities.

Instrument	Capacity
7500/7500 Fast Real-Time PCR System	96-well block (standard or Fast)
7900HT Fast Real-Time PCR System	96- and 384-well blocks (standard or Fast)
ViiA 7 Real-Time PCR System	96-well (standard or Fast), 384-well, and TaqMan Array Card blocks
StepOne Real-Time PCR System	48-well block (standard or Fast)
StepOnePlus Real-Time PCR System	96-well block (standard or Fast)
QuantStudio 3 Real-Time PCR System	96-well block (standard or Fast)
QuantStudio 5 Real-Time PCR System	96-well (standard or Fast) and 384-well blocks
QuantStudio 6 Real-Time PCR System	96-well (standard or Fast) and 384-well blocks
QuantStudio 7 Real-Time PCR System	96-well (standard or Fast), 384-well, and TaqMan Array Card blocks
QuantStudio 12K Flex Real-Time PCR System	96-well (standard or Fast), 384-well, TaqMan Array Card, and OpenArray plate blocks

Figure 3. The QuantStudio 5 Real-Time PCR System (left) and the QuantStudio 12K Flex Real-Time PCR System (right), which offers the highest throughput of all Applied Biosystems real-time PCR instruments.

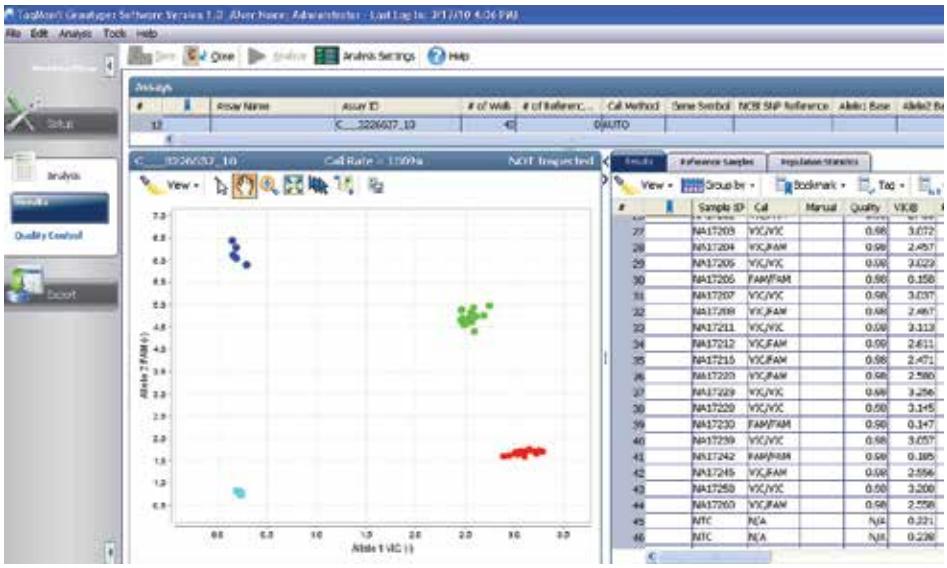


Figure 4. TaqMan Genotyper Software automatically determines sample genotypes and displays data.

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 on Applied Biosystems™ TaqMan™ OpenArray™ Plates. It has a state-of-the-art genotype-calling algorithm, an intuitive user interface, and enhanced study-based analysis features. The software enables multi-plate data analysis for high-throughput workflows and improved accuracy in genotype calling; versatile export features and comprehensive quality-control features facilitate streamlining of the entire workflow. TaqMan Genotyper Software can be downloaded at **thermofisher.com/taqmangenotyper**

Simple ordering

Selecting and ordering TaqMan SNP Genotyping Assays is as simple as "point and click." Use SNPbrowser Software to select the most informative SNPs for your genotyping studies. As you identify SNPs of interest, simply upload your selected TaqMan SNP Genotyping Assays to our online ordering tool.

The TaqMan Assay online ordering tool (Figure 5) enables you to search, select, and order from our catalog of over 7 million made-to-order predesigned TaqMan SNP Genotyping Assays. You can search for SNPs using any of several criteria: National Center for Biotechnology Information (NCBI) gene ID, NCBI SNP reference ID (rs#), or gene symbol. You can further refine your search by using SNP type (i.e., intragenic, 5′ or 3′ UTR, chromosome, etc.).

Our Custom TaqMan SNP Genotyping Assays supply you with SNPs that are not available from our predesigned assay collection, including those from any nonhuman organism. This service designs assays for all possible SNP, MNP, and indel targets but without the up-front bioinformatic preparation used for the predesigned made-to-order assays. Our complementary Custom TaqMan Assay Design Tool conveniently formats your target sequence for submission to our manufacturing facilities. To order custom assays, simply prepare your target sequence according to the Design and Ordering Guide, and upload your submission file at **thermofisher.com/snpcadt**

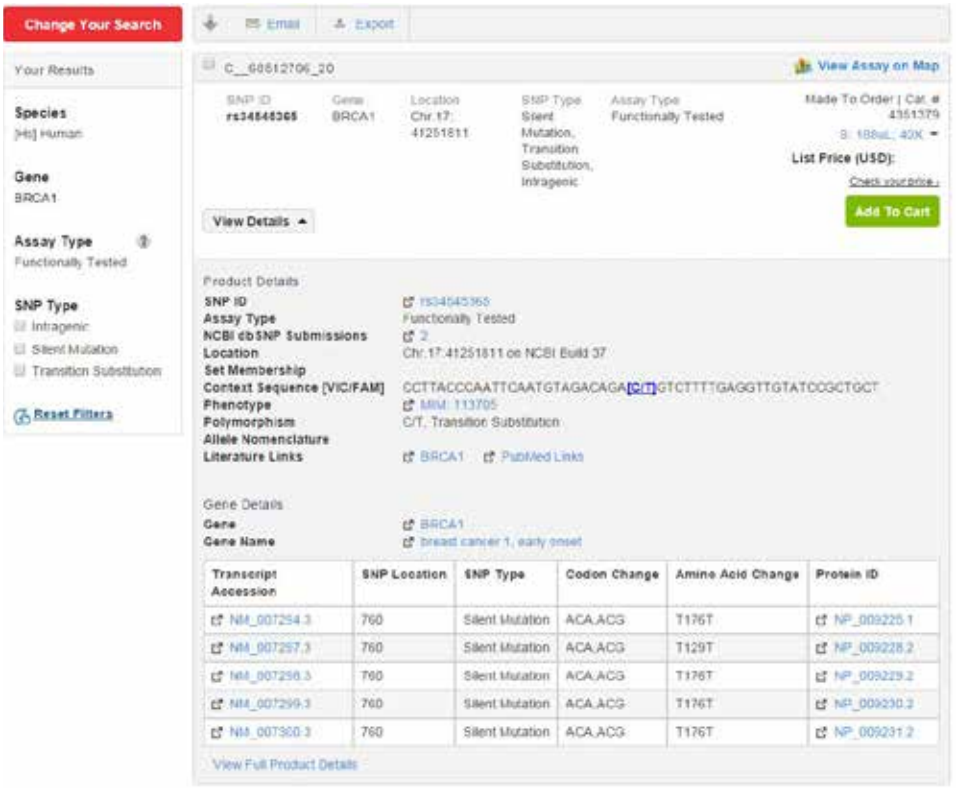


Figure 5. Our TaqMan Assay search and order tool makes online ordering easy. For convenient online ordering and multiple search options for all our genotyping assays, including keyword, batch, and location searches, visit **thermofisher.com/taqmansnp**

Ordering information

Size	Human assays (Cat. No.)	Nonhuman assays (Cat. No.)	Number of SNPs	No. of 5 µL reactions (384-well plate)	No. of 25 µL reactions (96-well plate)	Assay mix formulation	Assay type
Predesigned TaqMan SNP Genotyping Assays for Human and Mouse							
Small	4351379	4351384*	>4.5 million	1,500	300	40X	made-to-order
Medium	4351376	4351382*	>4.5 million	5,000	1,000	40X	made-to-order
Large	4351374	4351380*	>4.5 million	12,000	2,400	80X	made-to-order
Custom TaqMan SNP Genotyping Assays							
Small	4331349	4332077	∞	1,500	300	40X	made-to-order
Medium	4332072	4332075	∞	5,000	1,000	40X	made-to-order
Large	4332073	4332076	∞	12,000	2,400	80X	made-to-order
TaqMan Drug Metabolism Genotyping Assays							
Small	4362691	NA	2,700	750	150	20X	inventoried

All assays are quality-control tested using a mass spectrometer to verify sequence and yield. All assays have a VIC™ dye–labeled probe, a FAM™ dye–labeled probe, and two target-specific primers. All assays, excluding Custom TaqMan SNP Genotyping Assays, undergo bioinformatics evaluation of target SNP sequences.

Functional testing against 20 unique genomic DNA samples is performed on all custom and predesigned made-to-order human TaqMan SNP Genotyping Assays. Validation testing against four populations (45 samples/population) was performed on all 160,000 validated TaqMan SNP Genotyping Assays, and all TaqMan Drug Metabolism Genotyping Assays.

* Over 10,000 mouse assays available.

applied
biosystems

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

ThermoFisher
SCIENTIFIC

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.

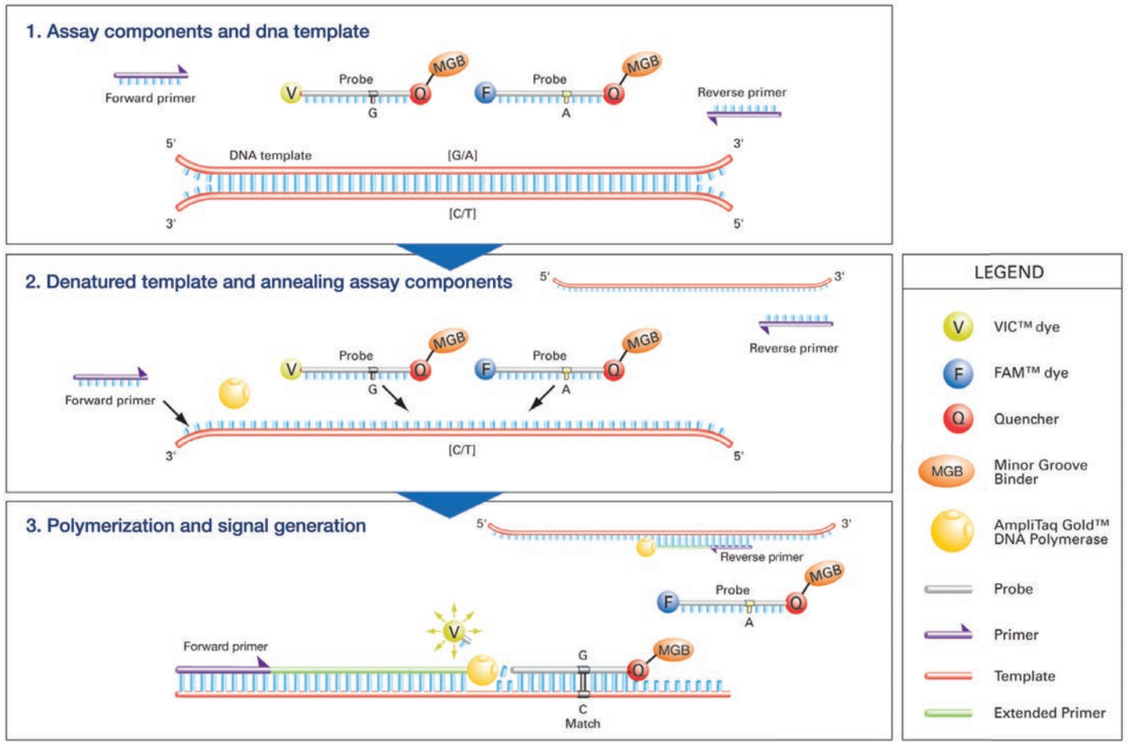


Figure 1. The TaqMan SNP Genotyping Assay. (1) The four TaqMan SNP Genotyping Assay components and the target DNA template with the SNP alleles (in brackets). **(2)** The denatured DNA target and annealing of the assay components. **(3)** Signal generation leading to specific allele detection.

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.

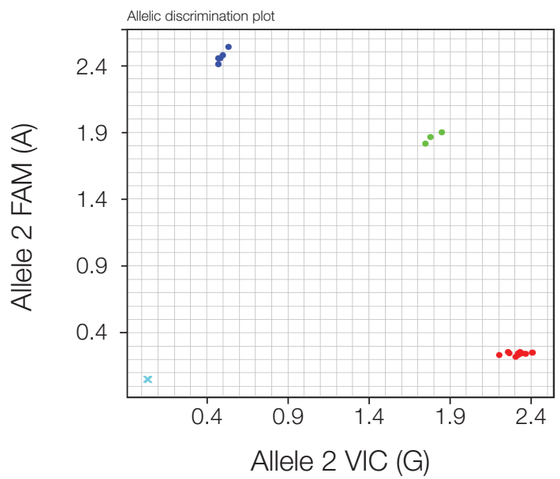


Figure 2. A three-cluster allelic discrimination plot generated with TaqMan SNP Genotyping Assay, C___1202883_20 (rs1801133) for *MTHFR* gene.

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**

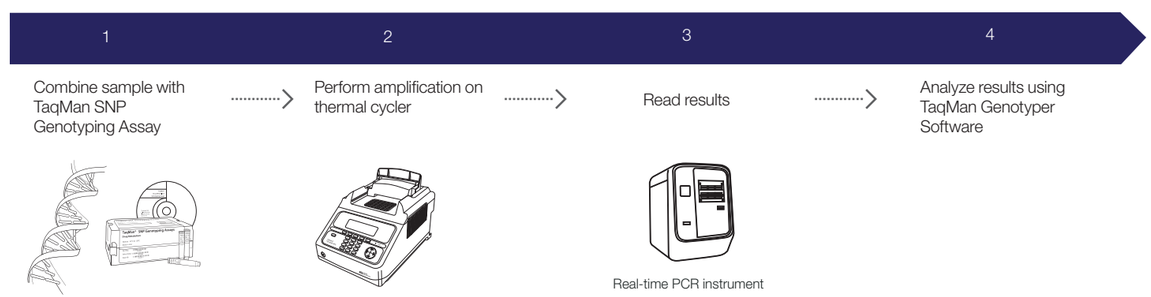


Figure 3. Workflow for TaqMan SNP Genotyping Assays.

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.

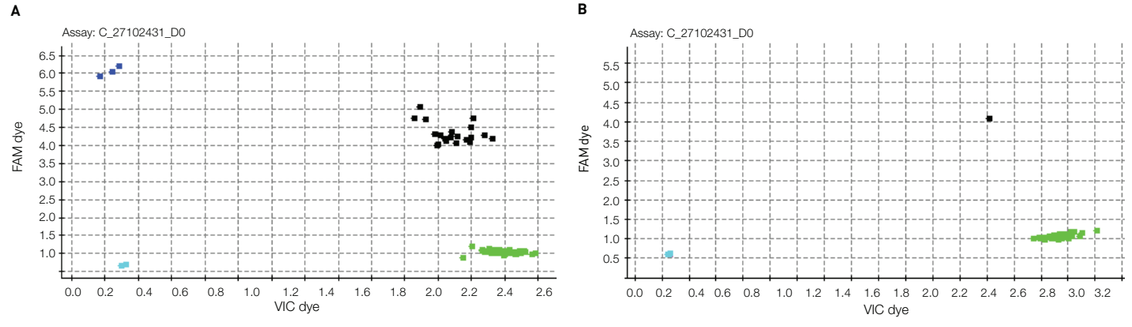


Figure 4. Allelic discrimination plots for the C__27102431_D0 assay run on (A) 45 each African-American and Caucasian, and (B) 45 each Chinese and Japanese gDNA samples. C__27102431_D0 targets the CYP2D6*4,g.1846G>A polymorphism, which encodes an mRNA splicing defect that results in a nonfunctional CYP2D6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

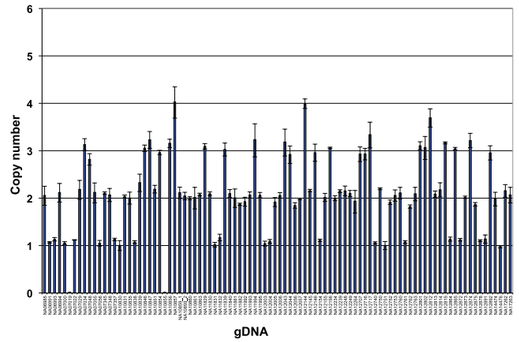
CNV, initially defined as variation in copy number of segments of DNA ≥1 kb in size, between individuals, is found in all humans as well as other animals and plants.

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

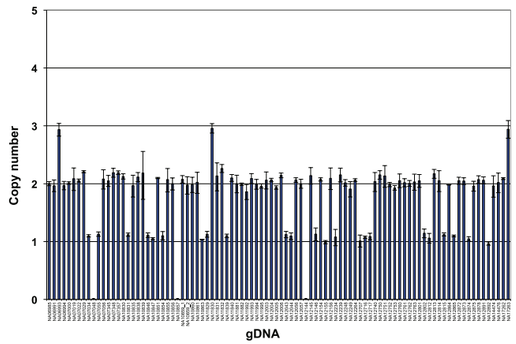


Figure 5. High specificity of TaqMan Copy Number Assays. C4A and C4B represent two isoforms of the C4 gene family. The sequences for these two genes differ in only 5 bases, but the encoded C4A and C4B proteins are functionally different. Differential detection of **(A)** C4A and **(B)** C4B is very challenging.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

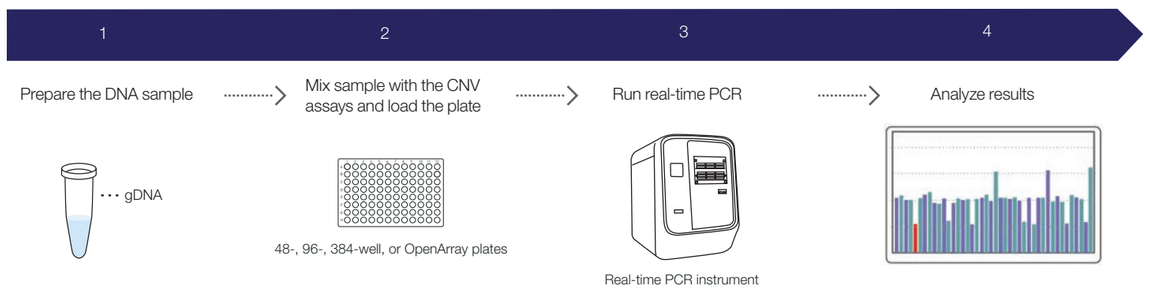


Figure 6. Workflow for TaqMan Copy Number Assays.

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.

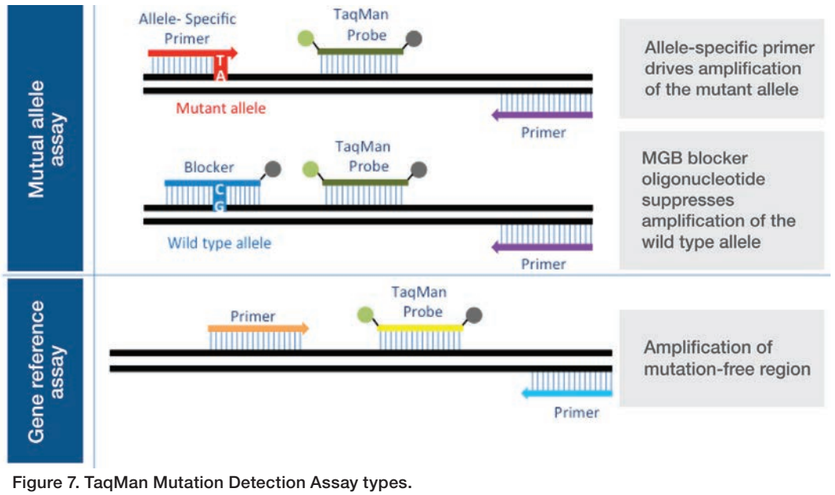


Figure 7. TaqMan Mutation Detection Assay types.

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.

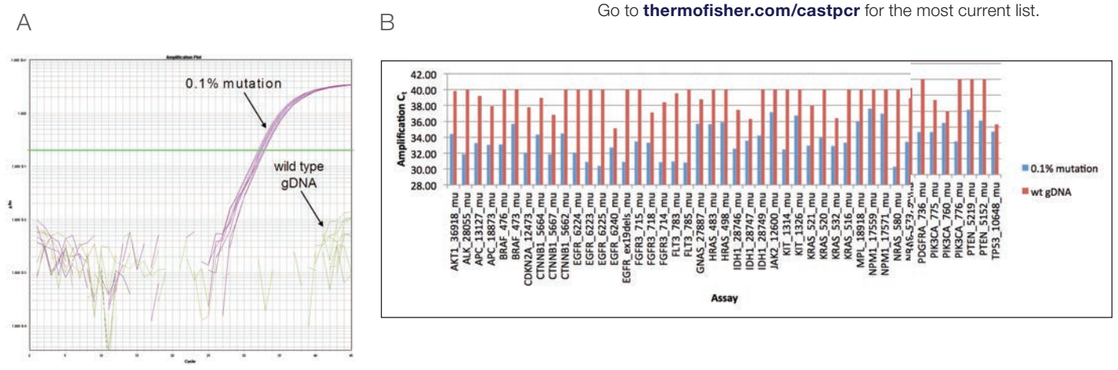


Figure 8. C_t differences between 0.1% mutation samples and wild type gDNA in TaqMan Mutation Detection Assays. For each mutant allele assay, 0.1% mutant allele samples were obtained by spiking cell line wild type gDNA (30 ng, ~10,000 copies) with 10 copies of mutant allele synthetic templates. **(A)**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

ThermoFisher SCIENTIFIC

applied biosystems™

Certificate of Analysis

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

Product No. **4351151**
Lot No. **2207524**

TEST	SPECIFICATION	RESULT

Material Test

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

Component	Part Number	Lot Number			
Cy3 Dye	4349412	2207234	562 ± 5 nm	**562 nm**	**Pass**
Cy5 Dye	4349413	2207244	659 ± 8 nm	**661 nm**	**Pass**
Texas Red® Dye	4349414	2207241	608 ± 5 nm	**608 nm**	**Pass**

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

ISO13485 REGISTERED

Singapore

Tshai Kai Yen
23 SEP 2022

Quality Assurance

Life Technologies Holdings Pte Ltd, Block 33, Marsil ng Industrial Estate Road 3, #07-06, Singapore 739256 Tel: (65) 6362 3300
To obtain a Certificate of Analysis on-line go to www.appliedbiosystems.com or email us at cofarequest@appliedbiosystems.com

Doc p/n: 100030349 Rev B

7500 Real-Time PCR Systems Spectral Calibration Kit II

Catalog Number 4351151

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

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

Contents and storage

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

Related documentation

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

Limited product warranty

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

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

1 December 2015

ThermoFisher SCIENTIFIC

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.

LIMITED USE LABEL LICENSE: Internal Research and Development Use Only.

The purchase of this product conveys to the buyer the limited, non-exclusive, non-transferable right (without the right to resell, repackage, or further sublicense) to use this product for internal research and development purposes. No other license is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of the product does not include or carry any right or license to use, develop, or otherwise exploit this product commercially and no rights are conveyed to the buyer to use the product or components of the product for purposes including but not limited to provision of services to a third party, generation of commercial databases or clinical diagnostics. This product is sold pursuant to authorization from Thermo Fisher Scientific and Thermo Fisher Scientific reserves all other rights. For information on purchasing a license for uses other than internal research and development purposes, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies Inc., 5781 Van Allen Way, Carlsbad, California 92008.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

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

Thermo Scientific molecular biology workflow solutions

High-quality essentials for everyday applications

ThermoFisher SCIENTIFIC

Supporting great science through innovation in molecular biology

For over two decades, the Thermo Scientific™ molecular biology portfolio has represented leading technology, enabling reliable performance for every step of the traditional molecular biology workflow. Our innovations include the first single-buffer restriction enzyme collection, the most widely used high-fidelity DNA polymerases, and the most comprehensive selection of PCR plastic consumables.

Today, the people behind our expanding portfolio remain committed to developing tools that deliver the best value for your research, with the performance and affordability that make it easy for you to do more great science.

To learn more, go to
thermofisher.com/thermoscientificmolbio

Contents

Nucleic acid isolation kits

High yields and exceptional value

Thermo Scientific™ GeneJET™ DNA and RNA purification kits are designed for rapid, efficient, and convenient purification of DNA and RNA from a wide range of samples. The kits utilize a proprietary 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. Purified DNA or RNA is ready to use in all common molecular biology procedures.

- Efficient nucleic acid extraction and high yields
- High purity of isolated DNA or RNA
- Simple and fast isolation procedure
- Convenient silica-based spin column format

Category	Description	Size	Cat. No.
Plasmid DNA purification	GeneJET Plasmid Miniprep Kit	50 preps/250 preps	K0502/K0503
	GeneJET Plasmid Midiprep Kit	25 preps/100 preps	K0481/K0482
	GeneJET Plasmid Maxiprep Kit	10 preps/25 preps	K0491/K0492
	GeneJET Endo-Free Plasmid Maxiprep Kit	10 preps	K0861
DNA and RNA fragment purification	GeneJET Gel Extraction Kit	50 preps/250 preps	K0691/K0692
	GeneJET PCR Purification Kit	50 preps/250 preps	K0701/K0702
	GeneJET RNA Cleanup and Concentration Micro Kit	50 preps/250 preps	K0841/K0842
	GeneJET Gel Extraction and DNA Cleanup Micro Kit	50 preps/250 preps	K0831/K0832
Genomic DNA purification	GeneJET Genomic DNA Purification Kit	50 preps/250 preps	K0721/K0722
	GeneJET Plant Genomic DNA Purification Mini Kit	50 preps/250 preps	K0791/K0792
	GeneJET Whole Blood Genomic DNA Purification Mini Kit	50 preps/250 preps	K0781/K0782
	GeneJET FFPE DNA Purification Kit	50 preps/250 preps	K0881/K0882
	GeneJET RNA Purification Kit	50 preps/250 preps	K0731/K0732
Total RNA purification	GeneJET Plant RNA Purification Mini Kit	50 preps/250 preps	K0801/K0802
	GeneJET Whole Blood RNA Purification Mini Kit	50 preps	K0761
	GeneJET Stabilized and Fresh Whole Blood RNA Kit	50 preps	K0871

To learn more, go to **thermofisher.com/genejet**

Reverse transcriptases

For optimal cDNA synthesis performance

Thermo Scientific™ Maxima™ reverse transcriptases (RTs) were developed through molecular evolution, which enabled the introduction and selection of multiple favorable mutations in traditional M-MuLV reverse transcriptase, boosting performance in cDNA synthesis. Maxima RTs are available in multiple formulations supporting a variety of molecular biology applications.

- Superior yields of full-length cDNA
- High reaction temperatures for improved transcription
- High transcription efficiency on long RNA templates
- Formats with integrated gDNA removal step for simplified workflows

Format	Description	Size	Cat. No.
Reverse transcriptases	Maxima Reverse Transcriptase	2,000 U/10,000 U	EP0741/EP0742
	Maxima H Minus Reverse Transcriptase	2,000 U/10,000 U	EP0751/EP0752
cDNA synthesis kits	Maxima First Strand cDNA Synthesis Kit for RT-qPCR	50 rxns/200 rxns	K1641/K1642
	Maxima First Strand cDNA Synthesis Kit for RT-qPCR, with dsDNase	50 rxns/200 rxns	K1671/K1672
	Maxima H Minus First Strand cDNA Synthesis Kit	20 rxns/100 rxns	K1651/K1652
	Maxima H Minus First Strand cDNA Synthesis Kit, with dsDNase	20 rxns/100 rxns	K1681/K1682
dsDNA synthesis kits	Maxima H Minus Double-Stranded cDNA Synthesis Kit	10 rxns	K2561

To learn more, go to **thermofisher.com/maxima**

For routine cDNA synthesis performance

Thermo Scientific™ RevertAid™ reverse transcriptases are based on M-MuLV enzymes and offer routine cDNA synthesis performance in molecular biology applications.

Format	Description	Size	Cat. No.
Reverse transcriptases	RevertAid Reverse Transcriptase	10,000 U/50,000 U	EP0441/EP0442
	RevertAid H Minus Reverse Transcriptase	10,000 U/50,000 U	EP0451/EP0452
cDNA synthesis kits	RevertAid First Strand cDNA Synthesis Kit	20 rxns/100 rxns	K1621/K1622
	RevertAid H Minus First Strand cDNA Synthesis Kit	20 rxns/100 rxns	K1631/K1632

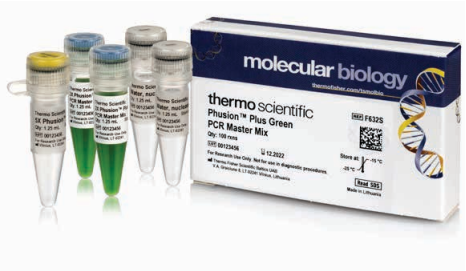
To learn more, go to **thermofisher.com/thermoscientificrt**

For reliable RNA protection

Thermo Scientific™ RiboLock™ RNase Inhibitor is an engineered thermostable enzyme that inhibits the activity of RNases A, B, and C. The enzyme is active under a wide range of reaction conditions and protects RNA at temperatures up to 55°C, helping to ensure successful reverse transcription in RT-PCR and RT-qPCR applications.

DNA polymerases

Trusted performance for high-fidelity PCR



Thermo Scientific™ Phusion™ high-fidelity DNA polymerases are designed to amplify DNA fragments with exceptional robustness and fidelity. Among the available Phusion formats, Thermo Scientific™ Phusion™ Plus DNA Polymerase allows you to skip calculation of annealing temperatures due to its universal annealing feature.

- High fidelity—Phusion Plus DNA Polymerase is >100x more accurate than *Taq* DNA polymerase
- Convenient—simplified PCR preparation and cycling with Phusion Plus DNA Polymerase due to a universal annealing temperature of 60°C

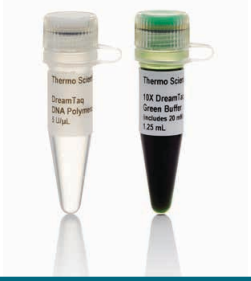
Green formats for Phusion and DreamTaq polymerases enable direct loading of PCR products on gels.

Format	Description	Size	Cat. No.
Standard	Phusion High-Fidelity DNA Polymerase	100 U/500 U	F530S/F530L
	Phusion High-Fidelity PCR Master Mix with HF Buffer	100 x 50 µL rxns/500 x 50 µL rxns	F531S/F531L
Hot-start	Phusion Hot Start II High-Fidelity DNA Polymerase	100 U/500 U	F549S/F549L
	Phusion Hot Start II High-Fidelity PCR Master Mix	100 x 50 µL rxns/500 x 50 µL rxns	F565S/F565L
	Phusion Plus DNA Polymerase	100 rxn/500 rxn	F630S/F630L
	Phusion Plus PCR Master Mix	100 rxn/500 rxn	F631S/F631L
	Phusion Plus Green PCR Master Mix	100 rxn/500 rxn	F632S/F632L
Uracil-tolerant	Phusion U Hot Start DNA Polymerase	100 U/500 U	F555S/F555L
	Phusion U Hot Start PCR Master Mix	100 x 50 µL rxns/500 x 50 µL rxns	F533S/F533L
Multiplex PCR	Phusion U Multiplex PCR Master Mix	100 x 50 µL rxns/500 x 50 µL rxns	F562S/F562L

To learn more, go to **thermofisher.com/phusion**

Enhanced *Taq* DNA polymerases for routine PCR

Thermo Scientific™ DreamTaq™ DNA polymerases offer a great balance between performance and value. Available in standard and hot-start formats, they deliver enhanced PCR performance that no conventional *Taq* enzyme can match.



- Featuring increased sensitivity and specificity; minimized optimization; and support of a wide range of amplicon lengths
- Multiple formats for maximum flexibility and reliability

Format	Description	Size	Cat. No.
Standard	DreamTaq DNA Polymerase	500 U/2,500 U	EP0702/EP0703
	DreamTaq Green DNA Polymerase	500 U/2,500 U	EP0712/EP0713
	DreamTaq PCR Master Mix	200 x 50 µL rxns/1,000 x 50 µL rxns	K1071/K1072
	DreamTaq Green PCR Master Mix	200 x 50 µL rxns/1,000 x 50 µL rxns	K1081/K1082
Hot-start	DreamTaq Hot Start DNA Polymerase	200 U/500 U/2,500 U	EP1701/EP1702/EP1703
	DreamTaq Hot Start Green DNA Polymerase	200 U/500 U/2,500 U	EP1711/EP1712/EP1713
	DreamTaq Hot Start PCR Master Mix	200 rxns/1,000 rxns	K9011/K9012
	DreamTaq Hot Start Green PCR Master Mix	200 rxns/1,000 rxns	K9021/K9022

To learn more, go to **thermofisher.com/dreamtaq**

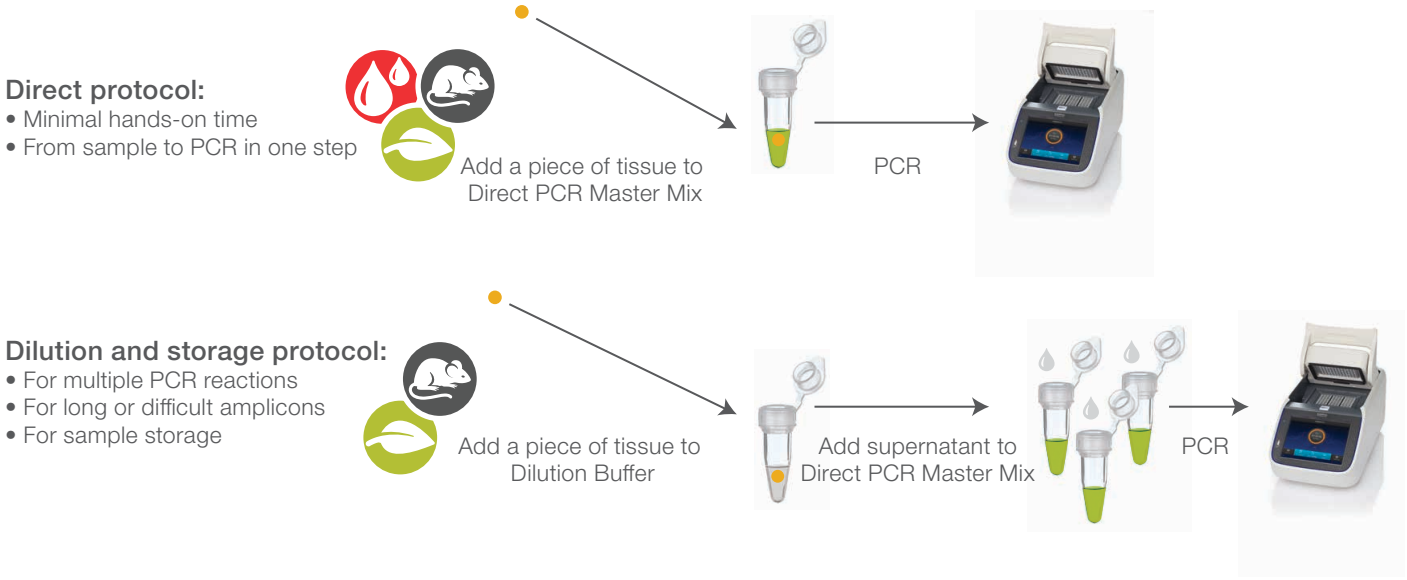
Solutions for direct PCR

Amplify without purification

Thermo Scientific™ Direct PCR master mixes offer outstanding convenience for DNA amplification by supporting PCR from unpurified samples. A tiny amount of source material is used in the PCR reaction without any purification steps, providing significant savings in both time and cost. Master mixes include a density reagent and two tracking dyes that allow for direct loading of PCR products on gels.

- PCR from crude samples—no DNA extraction or purification required
- Very short protocol times—from sample to results in 30 minutes
- Direct loading of PCR products on gels for simplified workflows
- Compatible with a variety of human, animal, and plant tissue samples

Two short protocols for different needs



Sample type	Description	Size		Cat. No.
		Direct protocol	Dilution and storage protocol	
Animal and human tissues	Phire Tissue Direct PCR Master Mix	100 rxns/500 rxns	250 rxns/1,250 rxns	F170S/F170L
Plant tissues, bacteria, yeast	Phire Plant Direct PCR Master Mix	100 rxns/500 rxns	250 rxns/1,250 rxns	F160S/F160L
Animal and human blood	Phusion Blood Direct PCR Master Mix	100 rxns/500 rxns	NA	F175S/F175L

To learn more, go to **thermofisher.com/directpcr**

PCR plastic consumables

Not all PCR plastics are created equal

For over 25 years, the Thermo Scientific™ PCR portfolio has been supplying high-quality PCR plastic consumables for molecular biology research. These products are designed to support maximum PCR performance and are manufactured with robust processes and extensive quality controls. The comprehensive portfolio of Thermo Scientific PCR plastic consumables includes individual tubes, tube strips, 96- and 384-well plates, and sealing options compatible with a broad range of PCR and qPCR instruments.

- Clean room production—certified free from DNA, RNases, and DNases
- Specialized solutions for low-, medium-, and high-throughput PCR and qPCR experiments
- Broad PCR and qPCR instrument compatibility including automated platforms
- Barcoded product options

Tubes
Individual PCR tubes with attached caps

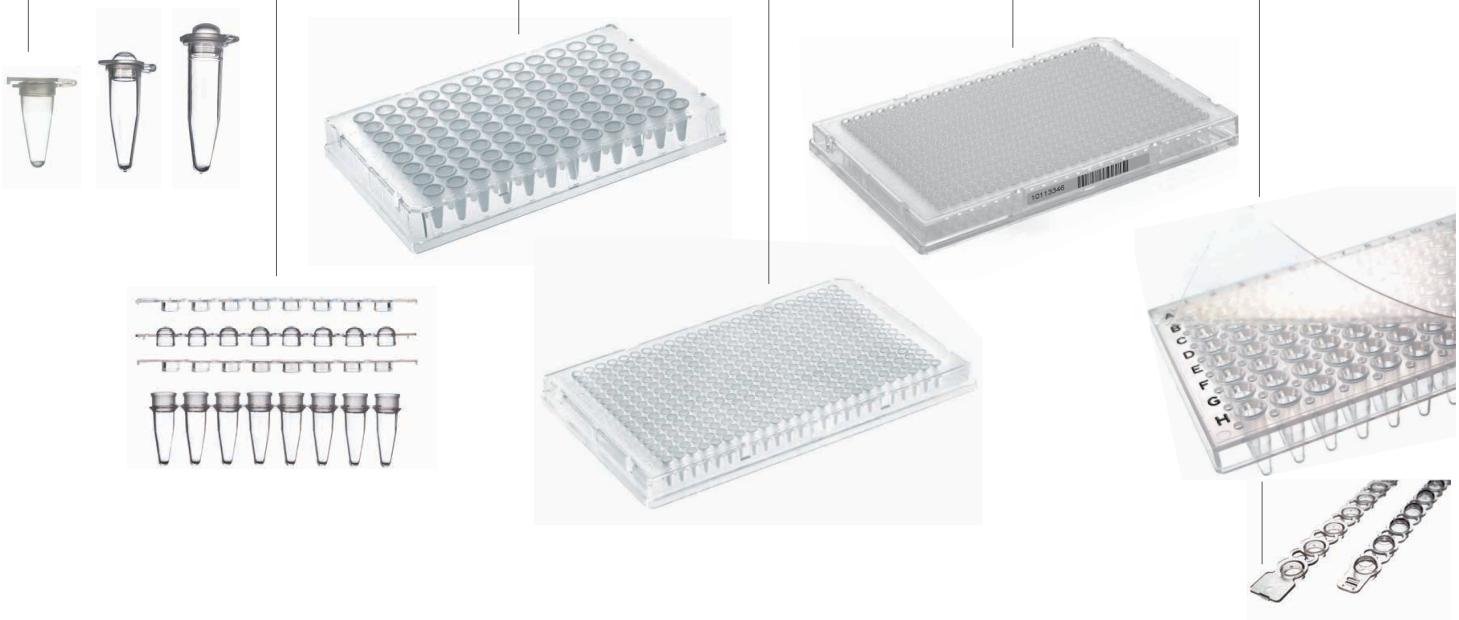
Tube strips
8-tube strips for PCR with separate or attached caps

96-well plates
Full-, semi-, or non-skirted 96-well PCR plates with different profiles; ultra-rigid plates for robotic applications

384-well plates
Full-skirted standard or extra-volume 384-well PCR plates; ultra-rigid plates for robotic applications

Barcoded plates
Barcoded 96- or 384-well PCR plates for reliable sample tracking

Sealing options
Cap strips, adhesive seals, and sealing mats compatible with PCR and qPCR applications



To learn more, go to **thermofisher.com/thermoscientificplastics**

Electrophoresis reagents

DNA ladders designed with accuracy in mind

Thermo Scientific™ GeneRuler™ DNA ladders are produced from chromatography-purified individual DNA fragments and are used for accurate analysis of DNA in agarose or polyacrylamide gels. They are ideal for sizing and in-gel DNA quantification. GeneRuler DNA ladders are available in conventional as well as ready-to-use formats (premixed with loading dye).

- Broad selection of DNA ladders that produce bright, sharp bands
- Environmentally friendly shipping

Range, bp*	Description	Size	Cat. No.
250–10,000	GeneRuler 1 kb DNA Ladder	5 x 50 µg/25 x 50 µg	SM0311/SM0312
	GeneRuler 1 kb DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0314/SM0313
75–20,000	GeneRuler 1 kb Plus DNA Ladder	5 x 50 µg/25 x 50 µg	SM1331/SM1332
	GeneRuler 1 kb Plus DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM1334/SM1333
100–1,000	GeneRuler 100 bp DNA Ladder	50 µg/5 x 50 µg	SM0241/SM0242
	GeneRuler 100 bp DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0243/SM0244
100–3,000	GeneRuler 100 bp Plus DNA Ladder	50 µg/5 x 50 µg	SM0321/SM0322
	GeneRuler 100 bp Plus DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0323/SM0324
50–1,000	GeneRuler 50 bp DNA Ladder	50 µg/5 x 50 µg	SM0371/SM0372
	GeneRuler 50 bp DNA Ladder, ready-to-use	50 µg	SM0373

* GeneRuler DNA ladders are also available in ultralow (10–300 bp), low (25–700 bp), and high (10,171–48,502 bp) ranges.

To learn more, go to **thermofisher.com/dnaladders**

RNA ladders for fragment sizing and in-gel quantification

Thermo Scientific™ RiboRuler™ RNA ladders are produced from chromatography-purified RNA transcripts and are free from degraded RNA or NTPs. They produce sharp bands of uniform intensity and have easy-to-remember band sizes and quantities allowing for RNA fragment sizing and approximate quantification. RiboRuler RNA ladders are available in conventional as well as ready-to-use formats (premixed with loading dye).

To learn more, go to **thermofisher.com/rnaladders**

High-quality agarose

Thermo Scientific™ TopVision™ Agarose is a highly purified DNase- and RNase-free agarose that comes in two melting point options (standard and low melting temperature) and two formats (powder and tablets).

- Suitable for DNA and RNA analysis
- Excellent gel transparency

Format	Description	Size	Cat. No.
Powder	TopVision Agarose	100 g/500 g	R0491/R0492
	TopVision Low Melting Point Agarose	25 g	R0801
Tablets	TopVision Agarose Tablets	200/1,000 tablets	R2801/R2802

To learn more, go to **thermofisher.com/topvision**

Restriction and modifying enzymes

Restriction digestion simplified

Thermo Scientific™ FastDigest™ enzymes are a line of restriction enzymes that are all 100% active in a single buffer. The universal Thermo Scientific™ FastDigest™ and FastDigest™ Green Buffers allow single, double, or multiple DNA digestion within 5–15 minutes, eliminating any need for buffer changes or subsequent DNA cleanup steps. Thermo Scientific™ DNA-modifying enzymes have 100% activity in this buffer as well. The FastDigest Green Buffer includes a density reagent and two tracking dyes that allow for direct loading of digestion reaction products on gels.

- 100% activity of all FastDigest enzymes in one buffer
- Complete DNA digestion in 5–15 minutes
- 100% buffer compatibility with downstream applications

FastDigest Value Pack

The Thermo Scientific™ FastDigest™ Value Pack (Cat. No. K1991) is a collection of 13 popular FastDigest enzymes supplied with FastDigest and FastDigest Green Buffers. Each enzyme is supplied in an amount sufficient for 20 standard restriction digestion reactions. The FastDigest enzymes included in the pack are: BamHI, BglII, EcoRI, EcoRV (Eco321), HindIII, KpnI, NdeI, NotI, PstI, SalI, SmaI, XbaI, and XhoI.

Find all 176 enzymes at **thermofisher.com/fastdigest**

DNA- and RNA-modifying enzymes

Thermo Scientific™ modifying enzymes are of high quality and purity, and support common modifications of RNA and DNA molecules. These enzymes include phosphatases, kinases, DNA and RNA polymerases, ligases, and other nucleases.

Enzyme type	Description	Size	Cat. No.
Phosphatases and kinases	FastAP Thermosensitive Alkaline Phosphatase (1 U/µL)	1,000 U/5 x 1,000 U/300 U	EF0651/EF0652/ EF0654
	T4 Polynucleotide Kinase (10 U/µL)	500 U/2,500 U	EK0031/EK0032
DNA polymerases	T4 DNA Polymerase (5 U/µL)	100 U/500 U	EP0061/EP0062
	T7 DNA Polymerase (10 U/µL)	300 U	EP0081
	Klenow Fragment (10 U/µL)	300 U/1,500 U	EP0051/EP0052
Deoxyribonucleases (DNases)	Exonuclease I (20 U/µL)	4,000 U/20,000 U	EN0581/EN0582
	DNase I, RNase-free (1 U/µL)	1,000 U	EN0521
Ligases	T4 DNA Ligase (5 U/µL)	200 U/1,000 U	EL0014/EL0011
RNA polymerases	T7 RNA Polymerase, HC (200 U/µL)	25,000 U	EP0113
Ribonucleases (RNases)	RNase A, DNase- and protease-free (10 mg/mL)	10 mg	EN0531
	RNase H (5 U/µL)	100 U/500 U	EN0201/EN0202

Find all modifying enzymes at **thermofisher.com/tsmodifyingenzymes**

Cloning kits

Universal cloning kit for any type of DNA fragment

The Thermo Scientific™ CloneJET™ PCR Cloning Kit utilizes positive selection for fast and simple cloning. This kit supports highly efficient cloning of PCR products generated with any thermostable DNA polymerase and allows both blunt- or sticky-end phosphorylated or non-phosphorylated DNA fragments to be cloned.

- Fast—ligation in only 5–10 minutes
- High efficiency—more than 99% positive clones
- No cloning background with the positive selection vector
- Eliminates the need for blue/white screening

To learn more, go to **thermofisher.com/clonejet**

Ligation-independent cloning kits

Streamline and facilitate the process of cloning an insert into an expression vector with the Thermo Scientific™ aLICator™ LIC Cloning and Expression System. The included pLATE bacterial expression vectors are designed for high levels of target protein expression as well as minimized basal (uninduced) expression.

- No need to cut and ligate DNA with traditional methods
- Tight control for protein production
- One-step on-column His-tag removal

To learn more, go to **thermofisher.com/alicator**

Kits for DNA ligation and end repair

The Thermo Scientific™ Rapid DNA Ligation Kit enables fast sticky-end or blunt-end DNA ligation in only 5 minutes at room temperature. The fast ligation efficiency is equal to that obtained with T4 DNA ligase in a standard 1-hour ligation. The reaction mixture can be used directly for bacterial transformation.

The Thermo Scientific™ Fast DNA End Repair Kit is used for blunting and phosphorylation of DNA ends in just 5 minutes for subsequent use in blunt-end ligation.

Cloning kit	Description	Size	Cat. No.
Universal cloning kit	CloneJET PCR Cloning Kit	20 rxns/40 rxns	K1231/K1232
Ligation-independent cloning kits	aLICator LIC Cloning and Expression Kits	20 rxns	K1241, K1251, K1261, K1281
	aLICator LIC Cloning and Expression Systems	30 rxns	K1271, K1291
Kit for DNA ligation	Rapid DNA Ligation Kit	50 rxns/150 rxns	K1422/K1423
Kit for DNA end repair	Fast DNA End Repair Kit	50 rxns	K0771

To learn more, go to **thermofisher.com/cloningtools**

Visit our molecular biology resource library for webinars, videos, and articles at **thermofisher.com/mbresources**

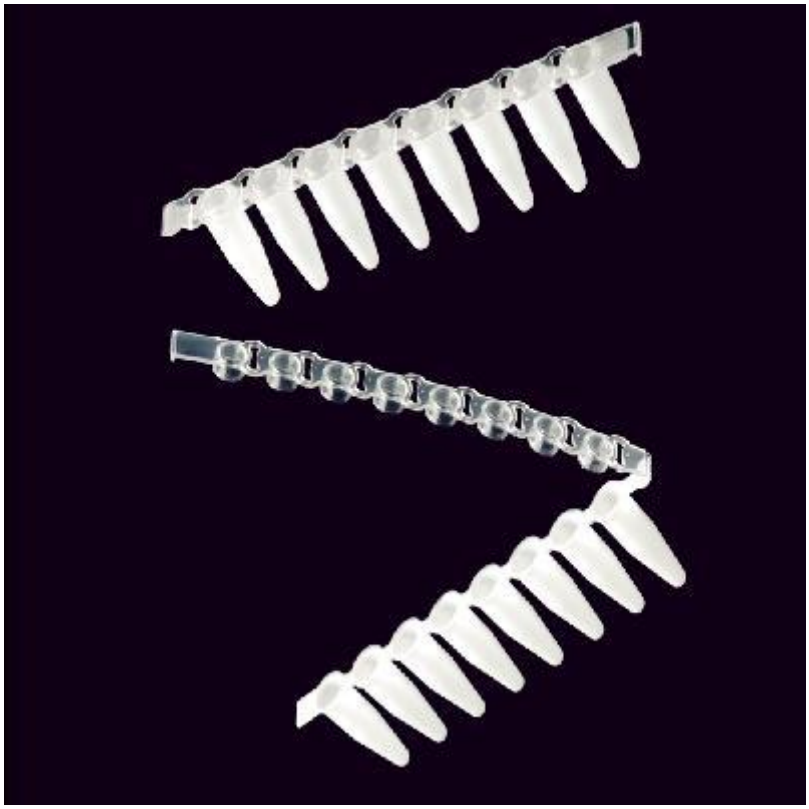
/ThermoSciBio @ThermoSciBio

To learn more, go to
thermofisher.com/thermoscientificmolbio

ThermoFisher SCIENTIFIC

0.2 ml PCR Real Time tubes in strips

Tubes made of polypropylene. Strip of 8 tubes, featuring an attached strip of 8 flat caps. Strips are manufactured by biomaterial molding, so the tubes are made of opaque white PP, while caps are compounded of transparent, optically clear PP. Opaque white tubes perform the highest performance in Real Time PCR, as they avoid crosstalking between wells. Specially conceived for Real Time PCR.
Caps are easily pierceable.
Certified RNAse, DNAse and pyrogen free.



Code/description/case qty.case/ weight (Kg)case vol. (m3)

4095.1BP/strip of 8 white/ 0.2 ml/ QPCR tubes and 8 caps 1250/2300/0040

This is a translation of the certificate ES16/20725.01

DELTALAB, S.L.

Pol.Ind. La Llana, Plaza de la Verneda, 1, 08191 Rubí, Barcelona

Has been assessed under the management system of the certified organisation defined in the main certificate ES16/20725 as meeting the requirements of

ISO 9001:2015

For the following activities

Design, manufacture and sale of laboratory material for the collection, transport and conservation of samples for microbiological, molecular biology, hematology, biochemistry, histology, microscopy and colorimetric analysis.
Commercialization of equipment for the storage of prepared samples, cryogenic stored samples, general labware and industrial packages.
Commercialization of equipment and instrumentation for the laboratory, diagnostic kits, healthcare products and cosmetics.

This certificate is valid from 11 October 2022 until 11 October 2025 and remains valid subject to satisfactory surveillance audits.

Issue 2.
The validity of this certificate depends on the validity of the main certificate.

SGS International Certification Services Iberica, S.A.U.
C/Trespaderne, 29. 28042 Madrid. España
t +34 91 313 8115 - www.sgs.com

This is a translation of the certificate ES19/86440.01

DELTALAB, S.L.

Pol.Ind. La Llana, Plaza de la Verneda, 1, 08191 Rubí, Barcelona

Has been assessed under the management system of the certified organisation defined in the main certificate ES19/86440 as meeting the requirements of

ISO 14001:2015

For the following activities

Design, manufacture and sale of laboratory material for the collection, transport and conservation of samples for microbiological, molecular biology, hematology, biochemistry, histology, microscopy and colorimetric analysis.
Commercialization of equipment for the storage of prepared samples, cryogenic stored samples, general labware and industrial packages.
Commercialization of equipment and instrumentation for the laboratory, diagnostic kits, healthcare products and cosmetics.

This certificate is valid from 31 August 2022 until 29 August 2025 and remains valid subject to satisfactory surveillance audits.

Issue 2.
The validity of this certificate depends on the validity of the main certificate.

SGS International Certification Services Iberica, S.A.U.
C/Trespaderne, 29. 28042 Madrid. España
t +34 91 313 8115 - www.sgs.com

Deltalab, S.L. defines and makes public its commitment with the Standard ISO 9001:2015 Quality Management Systems, ISO 14001:2015 Environmental Management Systems and ISO 13485:2016 Medical devices – Quality Management Systems, with the aim to create value and satisfy all its interested parties:

- Shareholders
- Members of the organisation
- Customers and suppliers
- All members of the surrounding community

The development of this Integrated Management System Policy is carried out with the philosophy of Continuous Improvement and with the support of all the processes described in our Integrated Management System, in order to achieve the following objectives:

1. Become leaders in the design and manufacture of single use products for the laboratory.
2. Bring solutions to cover the current and future customer needs, related to:

    - Design, manufacture and sale of laboratory material for the collection, transport and conservation of samples for microbiology, molecular biology, haematology, biochemistry, histology, microscopy and colorimetric analysis.
    - Design, manufacture and sale of sterile and non-sterile medical devices for the collection, transport and conservation of biological samples for clinical and IVD analysis.
    - Commercialization of diagnosis reagents, equipment and instrumentation for laboratory and equipment for the storage of prepared samples, cryogenic stored samples, general labware and industrial packages.
    - Commercialization of personal care, cosmetics and dietetic products

3. Maintain a constant growth, both in local and international markets, by means of mergers, acquisitions and by launching new products.
4. Achieve the full satisfaction of our customers, by means of a strict compliance to the agreements and expectations agreed with them, as well as the excellence in the service.
5. Reach a high level of innovation of our products and processes, in cooperation with universities, research centers, key opinion leaders and experts, both local and international.
6. Fulfil the legislation and regulatory requirements applicable to the activities carried out by the company, including those applicable to the quality of products and the environmental management.
7. Commit ourselves with the environmental protection, including the prevention of pollution.
8. Achieve and keep a high motivation and involvement of all members of the organisation, suppliers, distributors and customers, by fulfilling the highest Quality and environmental protection standards.
9. Improve the working conditions of all employees and ensure the technical capacity of the personnel by giving them the adequate training with the aim to achieve the required competence.
10. Establish a close relationship with the suppliers and guarantee the maximum quality of materials supplied by means of quality agreements.

The Integrated Management System is periodically reviewed to define the required actions to ensure that:

- ✓ The System is efficient, so that it is a tool for the routine of all the members of the organisation.
- ✓ The customer needs and requirements are duly identified, and their expectations are always met.
- ✓ All members of the organisation are familiar with and know the objectives and policy of the Integrated Management System, and that adequate training plans are defined to achieve them.
- ✓ Encourage the Continuous Improvement Philosophy, both related to Quality and Environmental Management.

This Policy is made available for the public and all interested parties.

JOSEP SAEZ
Managing Director
January 2019

Certificate ES10/81671

The management system of

DELTALAB, S.L.

Polígono Industrial La Llana, Plaza de la Verneda 1, 08191 Rubi, Barcelona, Spain

has been assessed and certified as meeting the requirements of

ISO 13485:2016
EN ISO 13485:2016

For the following activities

Design, manufacture and sale of sterile and non-sterile medical devices for the collection, transport and conservation of biological samples for clinical and IVD analysis.
Distribution of non-active medical devices and in vitro diagnostic medical devices.

Diseño, fabricación y comercialización de productos sanitarios estériles y no estériles para la toma, transporte y conservación de muestras biológicas para análisis clínicos y de IVD.
Distribución de productos sanitarios no activos y productos sanitarios para diagnóstico in vitro.

Disseny, fabricació i comercialització de productes sanitaris estèrils i no estèrils per a la presa, transport i conservació de mostres biològiques per a anàlisis clíniques i de IVD.
Distribució de productes sanitaris no actius i productes sanitaris per a diagnòstic in vitro.

This certificate is valid from 12 October 2022 until 11 October 2025 and remains valid subject to satisfactory surveillance audits.

Issue 10. Certified since 12 October 2010.

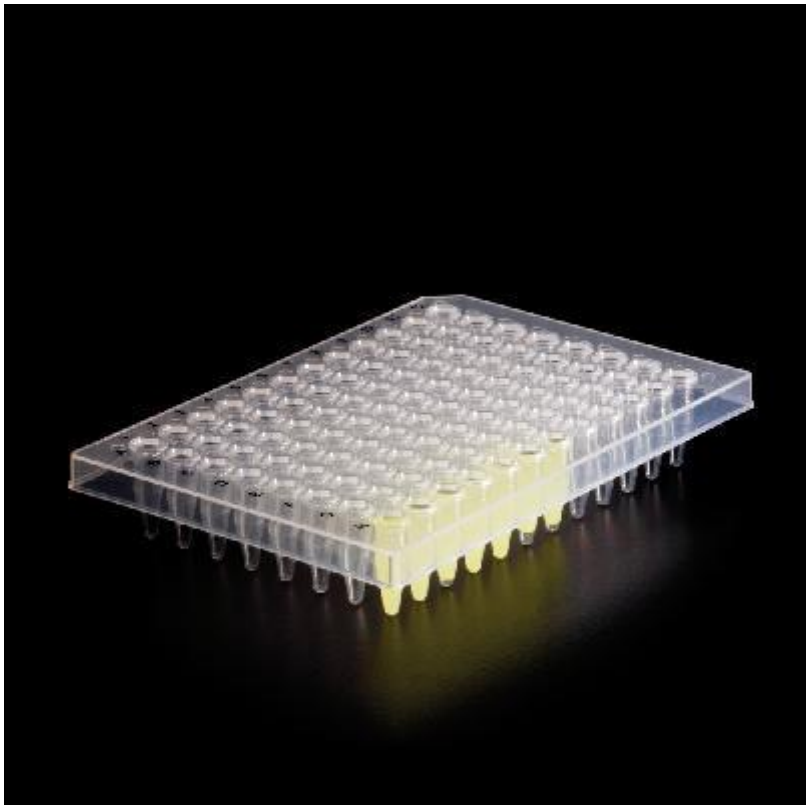
Jonathan Hall
Global Head - Certification Services

SGS United Kingdom Ltd
Rossmore Business Park, Ellesmere Port, Cheshire, CH65 3EN, UK
t +44 (0)151 350-6666 - www.sgs.com

UKAS MANAGEMENT SYSTEMS 0005

96 well semi-skirt plate

Made of transparent polypropylene.
This plate has a semi skirt ±7.5 mm high.
Each well has a capacity of 200 µl and embodies a low rim around its top which prevents accidental cross-contamination and it makes easy the sealing with foils.
Orientation cut is at position A 12 (upper right).
Alphanumeric identification printed in black.
DNAse, RNAse, DNA and PCR inhibitors free.
UV transparent.
Dimensions according to the SBS standard.



Code/description/case qty.case/ weight (Kg)/case vol. (m3)

900122 /semi-skirt/ PCR plate/100/3200/0030

MicroAmp™ plastic consumables compatibility chart for Applied Biosystems™ endpoint PCR systems and genetic analyzers

		3 x 32-well	96-well 0.2 mL			96-well 0.1 mL	384-well		Genetic analyzers	
Product	**Cat. No.**	ProFlex™	ProFlex, SimpliAmp™, VeritiPro™, Veriti™, MiniAmp™ Plus, MiniAmp	2720	9700	Veriti	ProFlex, Veriti , VeritiPro™	9700	310	3130, 3130xl, 3500, 3500xL, 3730, 3730xl
96-well 0.2 mL reaction plates										
Optical 96-Well Plate	N8010560, 4316813		•	•	•					•
Optical 96-Well Plate with Barcode	4306737, 4326659		•	•	•					•
96-Well Plate with Barcode & Optical Caps	403012		•	•	•					
Optical 96-Well Plate with Barcode & Optical Adhesive Films	4314320		•	•	•					
EnduraPlate Optical 96-Well Clear Plate	A36924		•	•	•					•
EnduraPlate Optical 96-Well Clear Plate with Barcode*	4483354, 4483352		•	•	•					•
TriFlex 3 x 32-Well Reaction Plate	A32810, A32811	•	•	•	•					
96-well 0.1 mL reaction plates										
Fast Optical 96-Well Plate, 0.1 mL	4346907					•			•	•
Fast Optical 96-Well Plate with Barcode, 0.1 mL	4346906, 4366932					•			•	•
EnduraPlate Optical 96-Well Fast Plate	A36930					•			•	•
EnduraPlate Optical 96-Well Fast Clear Plate with Barcode*	4483485, 4483494					•			•	•
384-well reaction plates										
Optical 384-Well Plate	4343370						•	•		•
Optical 384-Well Plate with Barcode	4309849, 4326270, 4343814						•	•		•
EnduraPlate Optical 384-Well Plate	A36931						•	•		•
EnduraPlate Optical 384-Well Clear Plate with Barcode*	4483285, 4483273						•	•		•
Strip tubes and caps										
Fast 8-Tube Strip, 0.1 mL	4358293					•				
Optical 8-Tube Strip with Attached Optical Caps, 0.2 mL	A30588	•	•	•	•					
8-Tube Strip with Attached Domed Caps, 0.2 mL	A30589	•	•	•	•					
8-Tube Strip, 0.2 mL*	N8010580	•	•	•	•				•	
Optical 8-Tube Strip, 0.2 mL	4316567	•	•	•	•					
8-Cap Strip*	N8010535, N8011535	•	•	•	•	•				
Optical 8-Cap Strip	4323032	•	•	•	•	•				
12-Cap Strip*	N8010534, N8011534		•	•	•	•				
Single tubes										
Fast Reaction Tube with Cap, 0.1 mL	4358297, 4358293					•				
Reaction Tube with Cap, 0.2 mL*	N8010540, N8010612, N8011540	•	•	•	•					
Reaction Tube without Cap, 0.2 mL*	N8010533, N8011533	•	•	•	•					
Optical Tube without Cap, 0.2 mL	N8010933	•	•	•	•					
Seals and covers										
Clear Adhesive Film	4306311		•		•	•	•	•		
Optical Adhesive Film	4360954, 4311971		•		•	•	•	•		
96-Well Full Plate Cover	N8010550			•	•					
32-Well Clear Adhesive Film	A32812	•	•	•	•					
Accessories										
Splash-Free 96-Well Base	4312063		•	•	•	•				
96-Well Support Base	4379590		•	•	•	•				•
96-Well Base	N8010531		•	•	•					
96-Well Reaction Tube/Tray/Retainer Set, 0.2 mL	403083, 403086			•	•					

* Multiple colors are available.

Note: Experiments using one or two 8-tube strips with attached caps require blank tube strips to balance lid pressure on the block or the use of the Applied Biosystems™ MicroAmp™ 96-Well Tray/Retainer Set (Cat. No. 4381850)—bottom part of the tray *only*. For use with the 96-well block of the ProFlex, SimpliAmp, Veriti, VeritiPro, MiniAmp Plus, and MiniAmp thermal cyclers.

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

ThermoFisher SCIENTIFIC

applied biosystems

MicroAmp™ plastic consumables compatibility chart for Applied Biosystems™ real-time PCR systems

		48-well	96-well 0.2 mL			96-well 0.1 mL			384-well
Product	Cat. No.	StepOne™	7000	7300, 7500	QuantStudio™ 3, 5, 6, 6 Pro, 7, 7 Pro, 12K; ViiA™ 7; 7900HT	StepOnePlus™	7500	QuantStudio 3, 5, 6, 6 Pro, 7, 7 Pro, 12K; ViiA 7; 7900HT**	QuantStudio 5, 6, 6 Pro, 7, 7 Pro, 12K; ViiA 7; 7900HT
96-well 0.2 mL reaction plates									
Optical 96-Well Plate	N8010560, 4316813		•	•	•				
Optical 96-Well Plate with Barcode	4306737, 4326659		•	•	•				
Optical 96-Well Plate with Barcode & Optical Caps	403012		•	•	•				
Optical 96-Well Plate with Barcode & Optical Adhesive Films	4314320		•	•	•				
EnduraPlate Optical 96-Well Clear Plate	A36924			•	•				
EnduraPlate Optical 96-Well Clear Plate with Barcode*	4483354, 4483352			•	•				
96-well 0.1 mL reaction plates									
Fast Optical 96-Well Plate, 0.1 mL	4346907					•	•	•	
Fast Optical 96-Well Plate with Barcode, 0.1 mL	4346906, 4366932					•	•	•	
EnduraPlate Optical 96-Well Fast Plate	A36930					•	•	•	
EnduraPlate Optical 96-Well Fast Clear Plate with Barcode*	4483485, 4483494					•	•	•	
384-well reaction plates									
Optical 384-Well Plate	4343370								•
Optical 384-Well Plate with Barcode	4309849, 4326270, 4343814								•
EnduraPlate Optical 384-Well Plate	A36931								•
EnduraPlate Optical 384-Well Clear Plate with Barcode*	4483285, 4483273								•
48-well reaction plates									
Fast Optical 48-Well Plate	4375816	•							
Strip tubes and caps									
Fast 8-Tube Strip, 0.1 mL	4358293	•				•	•	•	
Optical 8-Tube Strip with Attached Optical Caps, 0.2 mL	A30588		•	•	•				
Optical 8-Tube Strip, 0.2 mL	4316567		•	•	•				
Optical 8-Cap Strip	4323032	•	•	•	•	•	•	•	
Single tubes and caps									
Fast Reaction Tube with Cap, 0.1 mL	4358297	•				•		•	
Optical Tube without Cap, 0.2 mL	N8010933		•	•					
Seals and covers									
Optical Adhesive Film	4360954, 4311971		•	•	•	•	•	•	•
48-Well Optical Adhesive Film	4375323	•							
Reaction trays									
96-Well Tray/Retainer Set	403081		•						
Fast 48-Well Tray	4375282	•							
96-Well Tray for VeriFlex Blocks	4379983					•			
Accessories									
Splash-Free 96-Well Base	4312063		•	•	•	•	•	•	
96-Well Support Base	4379590		•	•	•	•	•	•	
96-Well Base	N8010531		•	•	•	•	•	•	

* Multiple colors are available.

** 7900HT (0.2 mL and 0.1 mL) instruments are not recommended for use with individual tubes or tube strips.

Note: Experiments using one or two 8-tube strips with attached caps require blank tube strips to balance lid pressure on the block or the use of the MicroAmp 96-Well Tray/Retainer Set (Cat. No. 4381850)—bottom part of the tray *only*. For use with the 96-well block of the 7000, 7300, 7500, and ViiA 7 systems, and the QuantStudio 3, 5, 6, 6 Pro, 7, 7 Pro, and 12K instruments.

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

ThermoFisher SCIENTIFIC

How to Use MicroAmp™ Reaction Plates, Tube Strips, and Tubes

For use with: Applied Biosystems™ thermal cyclers and real-time PCR systems

Publication Number 100033471 Revision B

How to use MicroAmp™ plates

MicroAmp™ plates and accessories

This table is representative of available plastics and other consumables. To find a complete list, or to search using our online PCR/qPCR plastic selection guide, visit **thermofisher.com/plastics**.

Item	Cat. No. (Quantity)
MicroAmp™ EnduraPlate™ Optical 96-Well Reaction Plates with Barcode	• 4483354 (20 plates; clear) • 4483343 (20 plates; blue) • 4483349 (20 plates; green) • 4483350 (20 plates; red) • 4483395 (20 plates; yellow) • 4483355 (5 plates; assorted colors) • 4483352 (500 plates; clear) • 4483356 (500 plates; assorted colors)
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	• 4306737 (20 plates) • 4326659 (500 plates)
MicroAmp™ Optical 96-Well Reaction Plate with Barcode and Optical Adhesive Films	4314320 (100 plates)
MicroAmp™ Optical 96-Well Reaction Plate	• 4316813 (500 plates) • N8010560 (10 plates)
MicroAmp™ Optical 8-Cap Strips	4323032 (300 strips)

Item	Cat. No. (Quantity)
MicroAmp™ 12-Cap Strip	• N8010534 (200 strips) • N8011534 (1,000 strips)
MicroAmp™ 8-Cap Strip, clear	N8010535 (300 strips)
MicroAmp™ 8-Cap Strip, assorted colors	N8010835 (300 strips of assorted colors)
MicroAmp™ 12-Cap Strip, assorted colors	N8010834 (200 strips of assorted colors)
MicroAmp™ Clear Adhesive Film	4306311 (100 films)
MicroAmp™ Optical Adhesive Film	• 4311971 (100 films) • 4360954 (25 films)
MicroAmp™ Splash Free 96-Well Base	4312063 (10 bases)
MicroAmp™ Adhesive Film Applicator	4333183 (5 applicators)
MicroAmp™ Cap Installing Tool (Handle)	4330015 (1 tool)

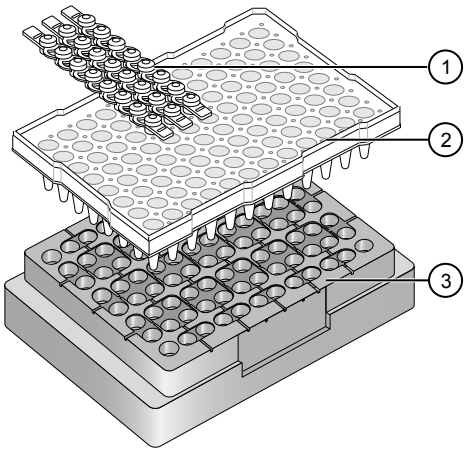
Fill, seal, and load reaction plates

The following procedure describes how to properly load and seal MicroAmp™ reaction plates using cap strips or adhesive seals. For a visual demonstration, watch the video "How to seal a PCR plate" by visiting **PCR/ qPCR Plastics and Seals Education**.

1. Place the reaction plate on a splash-free 96-well base.
2. Pipette the samples into the sample wells.
3. Seal the plates using one of the following:
   - MicroAmp™ Cap Strips.
   - MicroAmp™ Adhesive Film.
4. Place the sealed reaction plate into the instrument without the splash-free base.

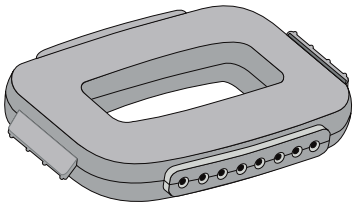
Seal plates with cap strips

1. Align and place the MicroAmp™ Cap Strip on the appropriate wells on the MicroAmp™ Optical 96-Well Reaction Plate.



① MicroAmp™ 8-Cap Strip
② MicroAmp™ Optical 96-Well Reaction Plate 0.2-mL
③ MicroAmp™ Splash Free 96-Well Base

2. Seal the cap strips using the rocking capping tool:



   a. Slip your fingers through the handle with the holes in the tool facing down for domed caps and with the holes facing up for optical caps.

   b. Align the tool over the first eight caps in a row.

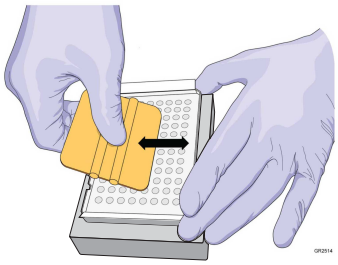
   c. Rock the tool back and forth a few times to seal the caps.

   d. Repeat for all remaining rows.

Seal plates with adhesive covers

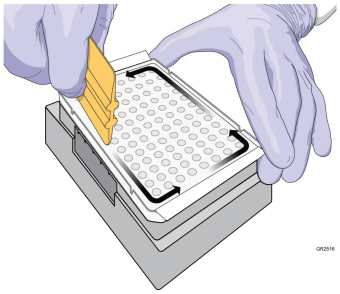
IMPORTANT! Apply significant downward pressure on the applicator in all steps to form a complete seal on top of the wells. Pressure is required to activate the adhesive on the optical cover.

1. Remove the backing of the adhesive film.
2. Align the adhesive film so as to cover all wells while placing on the plate, then rub the flat edge of the applicator back and forth along the long edge of the plate.



3. Rub the flat edge of the applicator back and forth along the short edge (width) of the plate.

4. Rub the end of the applicator horizontally and vertically between all wells.
5. Rub the end of the applicator around all outside edges of the plate using small back and forth motions to form a complete seal around the outside wells.



How to use MicroAmp™ tube strips and cap strips

MicroAmp™ tube strips and accessories

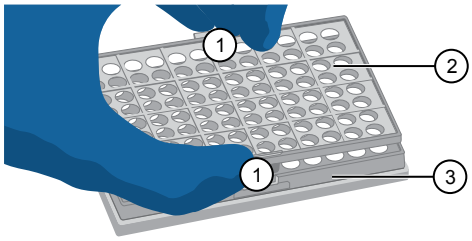
This table is representative of available plastics and other consumables. To find a complete list, or to search using our online PCR/qPCR plastic selection guide, visit **thermofisher.com/plastics**.

Consumables	Cat. No. (Quantity)
MicroAmp™ 8-Tube Strip (0.2 mL)	• N8010580 (125 tube strips) • N8010838 (120 tube strips; assorted colors)
MicroAmp™ Fast 8-Tube Strip (0.1 mL)	4358293 (125 strips)
MicroAmp™ 96-Well Tray/ Retainer Set - Blue color	4381850 (10 tray/retainer sets)
MicroAmp™ 8-Cap Strip Natural color, dome cap.	N8010535 (300 strips)
MicroAmp™ 8-Cap Strip, assorted colors Assorted color, dome cap.	N8010835 (300 strips)
MicroAmp™ Optical 8-Cap Strip	4323032 (300 strips)
MicroAmp™ Splash Free 96-Well Base	4312063 (10 bases)
MicroAmp™ Cap Installing Tool (Handle)	4330015 (1 tool)

Prepare samples using MicroAmp™ tubes/tube strips with separate cap strips

The following procedure describes how to properly load and seal MicroAmp tube strips using cap strips and the MicroAmp 96-well tray/retainer set. For a visual demonstration, watch the video "How to use adapter tray retainers" by visiting **PCR/qPCR Plastics and Seals Education**.

1. Separate the **blue** tray and retainer by squeezing the release catch as indicated in the graphic.

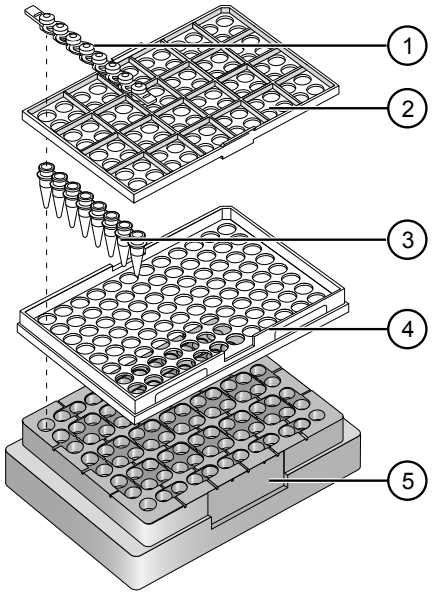


① Release catch
② MicroAmp™ 96-Well Retainer
③ MicroAmp™ 96-Well Tray

2. Place the **blue** tray on the 96-well base.

3. Load the tube strips on the tray.

4. Pipette the reaction mixture into the tubes.
5. Place the **blue** retainer over the tubes and snap the retainer into the tray.
6. Seal the tube strip using a MicroAmp™ cap strip. See “Seal tube strips with cap strips” on page 6 for instructions.
7. Remove the **blue** tray/retainer assembly containing the sealed tube strips from the 96-well base and place the assembly into the instrument.

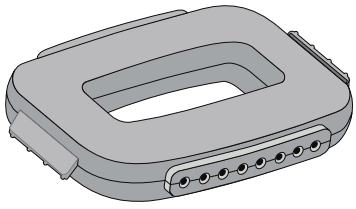


① MicroAmp™ 8-Cap strip
② MicroAmp™ 96-Well Retainer
③ MicroAmp™ 8-Tube Strip (0.2-mL) or MicroAmp™ Reaction Tube without Cap (0.2-mL)
④ MicroAmp™ 96-Well Tray
⑤ MicroAmp™ Splash Free 96-Well Base

Seal tube strips with cap strips

IMPORTANT! Apply significant downward pressure on the sealing tool in all steps to form a complete seal on top of the tubes.

1. Align and place the cap strips on the tubes.
2. Seal the cap strips using the rocking capping tool:



   a. Slip your fingers through the handle with the holes in the tool facing down for domed caps and with holes facing up for optical caps.

b. Align the tool over the first eight caps in a row.

c. Rock the tool back and forth a few times to seal the caps.

d. Repeat for all remaining rows.

How to use MicroAmp™ tube strips with attached caps

MicroAmp™ tubes and accessories

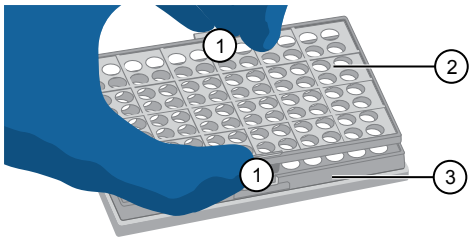
This table is representative of available plastics and other consumables. To find a complete list, or to search using our online PCR/qPCR plastic selection guide, visit **thermofisher.com/plastics**.

Consumables	Cat. No. (Quantity)
MicroAmp™ 8-Tube Strip with Attached Domed Caps (0.2 mL)	A30589 (125 strips)
MicroAmp™ Optical 8-Tube Strip with Attached Optical Caps (0.2 mL)	A30588 (125 strips)
MicroAmp™ 96-Well Tray / Retainer Set- Blue color	4381850 (10 trays/retainer sets)
MicroAmp™ Splash Free 96-Well Base	4312063 (10 bases)
MicroAmp™ Cap Installing Tool (Handle)	4330015 (1 tool)

Prepare samples using MicroAmp™ tube strips with attached caps

The following procedure describes how to properly load and seal MicroAmp tube strips with attached caps using the MicroAmp 96-well tray/retainer set. For a visual demonstration, watch the video "How to use adapter tray retainers" by visiting **PCR/qPCR Plastics and Seals Education**.

1. Separate the **blue** tray from the retainer by squeezing the release catch as indicated in the graphic.



① Release catch
② MicroAmp™ 96-Well Retainer
③ MicroAmp™ 96-Well Tray

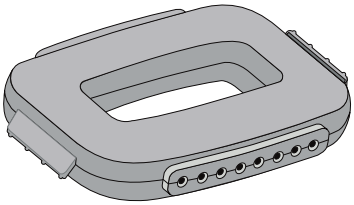
2. Place the **blue** tray on the splash-free 96-well base.

   Note: Only the bottom tray is used for tubes with attached caps.

3. Load the tube strips with attached caps into the tray.

4. Pipette the reaction mixture into the tubes.

5. Seal the cap strips using the rocking capping tool:



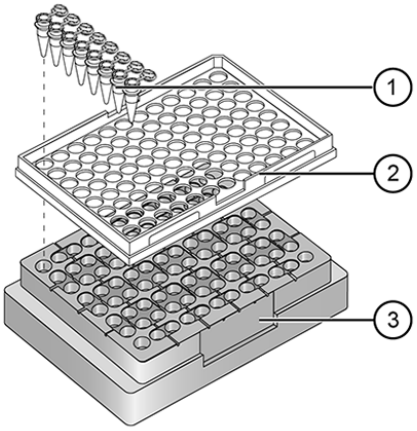
   a. Slip your fingers through the handle with the holes in the tool facing down for domed caps and with the holes facing up for optical caps.

   b. Align the tool over the first eight caps in a row.

   c. Rock the tool back and forth a few times to seal the caps.

   d. Repeat for all remaining rows.

6. Remove the **blue** tray containing the sealed tube strips from the 96-well base and place the tray and sealed tube strips into the instrument.



① MicroAmp™ 8-Tube Strip with Attached Caps (0.2-mL)
② MicroAmp™ 96-Well Tray
③ MicroAmp™ Splash Free 96-Well Base

How to use MicroAmp™ individual tubes

MicroAmp™ tubes and accessories

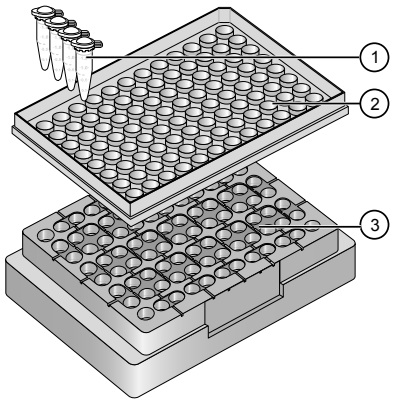
This table is representative of available plastics and other consumables. To find a complete list, or to search using our online PCR/qPCR plastic selection guide, visit **thermofisher.com/plastics**.

Consumables	Cat. No. (Quantity)
MicroAmp™ 96-Well Tray for VeriFlex™ Systems - black color	4379983 (10 trays)
MicroAmp™ Reaction Tubes with Cap (0.2 mL)	• N8010540 (1,000 tubes) • N8011540 (10,000 tubes) • N8010840 (1,000 tubes; assorted colors) • N8010612 (1,000 tubes; autoclaved)
MicroAmp™ Fast Reaction Tube with Cap (0.1 mL)	4358297 (1000 tubes)
MicroAmp™ Multi-Removal Tool	4313950 (1 tool)
MicroAmp™ Splash Free 96-Well Base	4312063 (10 bases)

Prepare samples using MicroAmp™ Reaction Tubes

The following procedure describes how to properly load and seal MicroAmp™ individual tubes with attached caps and the MicroAmp™ 96-well tray for VeriFlex systems. For a visual demonstration, watch the video "How to use adapter tray retainers" by visiting **PCR/qPCR Plastics and Seals Education**.

1. Set the **black** tray on a 96-well base.
2. Place the reaction tubes in the **black** tray.



① MicroAmp™ Reaction Tube with Cap (0.2-mL)
② MicroAmp™ 96-Well Tray for VeriFlex™ Blocks
③ MicroAmp™ Splash Free 96-Well Base

3. Pipette the reaction mixture into the reaction tubes.

4. Cap the tubes.

5. Remove the **black** tray with sealed reaction tubes from the 96-well base and place the tray and sealed tubes into the instrument.

Limited product warranty

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

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

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

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

TRADEMARKS: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

thermofisher.com/support | thermofisher.com/askaquestion

thermofisher.com

9 June 2020