Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific DreamTaq Hot Start DNA Polymerase

Pub. No. MAN0015972
Rev. Date 29 July 2016 (Rev. A.00)

Lot: __ Expiry Date: __

Ordering Information

Catalog No.	DreamTaq Hot Start DNA Polymerase, 5 U/µL	10X DreamTaq Buffer*
EP1701	200 U	1.25 mL
EP1702	500 U	2 × 1.25 mL
EP1703	2500 U	10 × 1.25 mL
EP1704	4 × 2500 U	40 × 1.25 mL

* includes 20 mM $MgCl_2$

Store at –20°C

www.thermofisher.com

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

DESCRIPTION

Thermo Scientific™ DreamTaq™ Hot Start DNA Polymerase is an enhanced hot start *Taq* DNA polymerase optimized for most PCR applications. It ensures higher sensitivity, specificity, and yields compared to conventional hot start *Taq* DNA polymerase. It is capable of amplifying long amplicons such as 6 kb genomic DNA and 20 kb λ DNA.

DreamTaq Hot Start DNA Polymerase combines *Taq* DNA polymerase and a specific antibody that inhibits the DNA polymerase activity at ambient temperatures, thus preventing the amplification of non-specific products. At polymerization temperatures, the antibody molecule is released, rendering the polymerase fully active.

DreamTaq Hot Start DNA Polymerase uses the same reaction set-up and cycling conditions as conventional *Taq* DNA polymerases, but the antibody-based hot start allows the reactions to be set up at room temperature. Because the enzyme is supplied with the optimized DreamTaq buffer, which includes 20 mM $MgCl_2$, extensive optimization of reaction conditions is not required.

DreamTaq Hot Start DNA Polymerase generates PCR products with 3'-dA overhangs. The enzyme tolerates dUTP and can incorporate modified nucleotides.

FEATURES

- High specificity due to antibody based hot start.
- Robust amplification with minimal optimization.
- High yields of PCR products.
- Higher sensitivity compared to conventional hot start *Taq* DNA polymerases.
- Amplification of long targets up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- Generates 3'-dA overhangs.
- Incorporates dUTP and modified nucleotides.

APPLICATIONS

- Routine PCR amplification of DNA fragments up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

CONCENTRATION

5 U/µL

DEFINITION OF ACTIVITY UNIT

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 minutes at 74°C.

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Location: Moldova

10X DREAMTAQ BUFFER

DreamTaq Buffer is a proprietary formulation, which contains KCl and $(NH_4)_2SO_4$ at a ratio optimized for robust performance of DreamTaq Hot Start DNA Polymerase in PCR. DreamTaq Buffer also includes $MgCl_2$ at a concentration of 20 mM.

INHIBITION AND INACTIVATION

- Inhibitors: Ionic detergents (deoxycholate, sarkosyl, and SDS) at concentrations higher than 0.06, 0.02, and 0.01%, respectively.
- Inactivated by phenol/chloroform extraction.

PROTOCOL

To set up parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers, and DreamTaq Hot Start DNA Polymerase. Prepare sufficient master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes, then add template DNA.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. For each 50-µL reaction, add the following components into a thin-walled PCR tube:

10X DreamTaq Buffer*	5 µL
dNTP Mix, 2 mM each (#R0241)	5 µL (0.2 mM of each)
Forward primer	0.1–1.0 µM
Reverse primer	0.1–1.0 µM
Template DNA	10 pg–1 µg
DreamTaq Hot Start DNA Polymerase	1.25 U
Water, nuclease-free (#R0581)	to 50 µL
Total volume	50 µL

*10X DreamTaq Buffer contains 20 mM $MgCl_2$, which is optimal for most applications. If further optimization is required, additional $MgCl_2$ can be added to the master mix. The volume of water should be reduced accordingly.

Volumes of 25 mM $MgCl_2$ (#R0971), required for specific final $MgCl_2$ concentration:

Final concentration of $MgCl_2$	2 mM	2.5 mM	3 mM	4 mM
Volume of 25 mM $MgCl_2$ to be added for 50-µL reaction	0 µL	1 µL	2 µL	4 µL

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

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

* The recommended extension step is 1 minute for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 minute/kb.

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

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

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Use PCR-certified reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Always perform "no template control" (NTC) reactions to check for contamination.

DreamTaq Hot Start DNA Polymerase incorporates dUTP; therefore, you can control carry-over contamination using Uracil-DNA Glycosylase (#EN0361).

GUIDELINES FOR PRIMER DESIGN

Use special design software or follow the general recommendations for PCR primer design as outlined below to design optimal primers:

- Use PCR primers that are 15–30 nucleotides long.
- Optimal GC content of the primer is 40 –60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3'-end.

(continued on reverse page)

- Avoid self-complementary primer regions, and complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementarity between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

ESTIMATION OF PRIMER MELTING TEMPERATURE

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

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

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

If the primer contains more than 25 nucleotides, we recommend using specialized computer programs to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amount of template DNA for a 50-µL reaction volume is 1 pg–1 ng for both plasmid and phage DNA, and 100 pg–1 µg for genomic DNA. Higher amounts of template increase the risk of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

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

$MgCl_2$ concentration

DreamTaq Hot Start DNA Polymerase is provided with an optimized 10X DreamTaq Buffer, which includes $MgCl_2$ at a concentration of 20 mM. A final $MgCl_2$ concentration of 2 mM is generally ideal for PCR. $MgCl_2$ concentration can be further increased up to 4 mM by the addition of 25 mM $MgCl_2$ (#R0971).

If the DNA samples contain EDTA or other metal chelators, Mg^{2+} ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds 1 Mg^{2+}).

dNTPs

The recommended final concentration of each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be necessary. It is essential to have equal concentrations of all four nucleotides (dATP, dCTP, dGTP and dTTP) in the reaction mixture.

To obtain a 0.2 mM concentration of each dNTP in the PCR mixture, refer to the table below.

Volume of PCR mixture	dNTP Mix, 2 mM each (#R0241)	dNTP Mix, 10 mM each (#R0191)	dNTP Mix, 25 mM each (#R1121)
50 µL	5 µL	1 µL	0.4 µL
25 µL	2.5 µL	0.5 µL	0.2 µL
20 µL	2 µL	0.4 µL	0.16 µL

Use 200 µM of each dNTP. dUTP or dITP can be added up to 200 µM. For longer amplicons, a lower dUTP concentration (20–100 µM) may be required for high yields.

Primers

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

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

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

DreamTaq Hot Start DNA polymerase is inactive at room temperature during the reaction set up and is activated during the 1–3 minute initial denaturation/enzyme activation step.

It is essential to completely denature the template DNA at the beginning of the PCR run to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 60% or less, an initial 1–3 minute denaturation at 95°C is sufficient. For GC-rich templates this step can be prolonged.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3–4 minutes. DNA denaturation can also be enhanced by the addition of 5–10% glycerol, 5% DMSO, 1% formamide, or 1–1.5 M betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature has to be adjusted accordingly.

Note that higher than 10% DMSO or 5% formamide in the reaction mix inhibit DNA polymerases. Therefore, it may be necessary to increase the amount of the enzyme in the reaction if these additives are used.

Primer annealing

The annealing temperature should be equal to the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments. When additives that change the melting temperature of the primer-template complex are used (glycerol, DMSO, formamide and betaine), the annealing temperature must also be adjusted.

Extension

The optimal extension temperature for DreamTaq Hot Start DNA Polymerase is 70–75°C. The recommended extension step is 1 minute at 72°C for PCR products up to 2 kb. For longer products, the extension time should be increased by 1 minute/kb. For amplification of templates >6 kb, we recommend reducing the extension temperature to 68°C.

Number of cycles

The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield.

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

Final extension

After the last cycle, we recommend incubating the PCR mixture at 72°C for an additional 5–15 minutes to fill in any possible incomplete reaction products. If the PCR product will be cloned into TA vectors such as the Thermo Scientific™ InsTAclone™ PCR Cloning Kit (#K1213), the final extension step may be prolonged to 15 minutes to ensure the complete 3'-dA tailing of the PCR product. If the PCR product will be used for cloning using Thermo Scientific™ CloneJET™ PCR Cloning Kit (#K1231), the final extension step can be omitted.

TROUBLESHOOTING

For troubleshooting, visit **www.thermofisher.com.**

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable conversion of supercoiled plasmid DNA to a nicked form was observed.

Residual Activity Assay

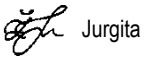
No detectable extension of labeled double stranded oligonucleotide with 5'-overhangs after incubation in the presence of dNTPs.

E. coli DNA Assay

No detectable E.coli DNA was observed.

Functional Assay

Performance in PCR is tested by the amplification of a 594 bp and 7.5 kb fragments of human genomic 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.

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

Refer to **www.thermofisher.com/support** for the Safety Data Sheets.

Safety Data Sheet

Safety Data Sheet according to Regulation (EC) No. 1907/2006 (REACH)
Classification according to Regulation (EC) No. 1272/2008 [CLP]

SECTION 1: Identification of the substance/mixture and of the company/undertaking

Product identifier

Product code	C4221
Product name	10X DreamTaq Buffer
Chemical Name	Not Applicable
REACH registration number	No registration number is given yet for this substance / substances in this mixture since the annual import quantity is less than one tonnage per annum or the transition period for its registration according to Article 23 of REACH has not yet expired.

Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses	For research use only. Not for use in diagnostic procedures
Use Description Code	SU22 - Professional uses: Public domain (administration, education, entertainment, services, craftsmen), PROC15 - Use as laboratory reagent, PC21 - Laboratory chemicals, SU24 - Scientific research and development
Uses advised against	Not for consumer use.

Details of the supplier of the safety data sheet

Manufacturer / Supplier

Thermo Fisher Scientific Baltics UAB
V.Graiciuno 8
LT-02241 Vilnius
Lithuania
Tel.: +370 5 2602131
Fax.: +370 5 2602142

LIFE TECHNOLOGIES EUROPE BV
KWARTSWEG 2
2665 NN BLEISWIJK
NETHERLANDS
31-(0)180 392 400
Email: MSDS@lifetech.com

Life Technologies Limited
3 Fountain Drive
Inchinnan Business Park
Paisley
PA4 9RF, UK
+44 (0)141 814 6100

24 hour Emergency Response for Hazardous Materials [or Dangerous Goods] Incident. Spill, Leak, Fire, Exposure, or Accident. Call CHEMTREC	Within the USA + Canada: 1-800-424-9300 and 1-703-527-3887 Outside the USA + Canada: 1-703-741-5970

Country Specific Emergency Number (if available):

CHEMTREC Ireland (Dublin)	+(353)-19014670 (Greeting Language: English and Irish)
CHEMTREC UK (London)	+(44)-870-8200418 (Greeting Language: English)

SECTION 2: Hazards identification

Classification of the substance or mixture

Classification according to Regulation (EC) No. 1272/2008 [CLP]

Physical hazards
Not Hazardous

Health hazards
Not Hazardous

Environmental hazards
Not Hazardous

Additional information
Not Applicable

Label elements

Labelling according to Regulation (EC) No 1272/2008 [CLP]

Hazard pictograms
None

Signal Word
None

Hazard Statements
Not Applicable

EU Specific Hazard Statements
Not Applicable

Precautionary Statements

Prevention
Not Applicable

Response
Not Applicable

Storage
Not Applicable

Disposal
Not Applicable

Other hazards
Contains a known or suspected endocrine disruptor

SECTION 3: Composition/information on ingredients

We recommend handling all chemicals with caution.

Component	CAS No	EINECS-No.	Weight-%	REACH registration number	Classification according to Regulation (EC) No. 1272/2008 [CLP]
Nonylphenol ethoxylates 9016-45-9 (<0.9)	9016-45-9	500-024-6	<0.9	-	Acute Tox. 4 - H302 Eye Dam. 1 - H318 Chronic Aquatic 2 - H411

SECTION 4: First aid measures

Description of first aid measures

Skin contact Rinse skin with water. Immediate medical attention is not required.

Eye contact Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Ingestion Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

Inhalation Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary.

Notes to Physician Treat symptomatically.

Most important symptoms and effects, both acute and delayed
Not Applicable

Indication of any immediate medical attention and special treatment needed
None.

SECTION 5: Firefighting measures

Extinguishing media

Suitable extinguishing media Water spray. Carbon dioxide (CO_2). Foam. Dry chemical.

Unsuitable extinguishing media No information available.

Special hazards arising from the substance or mixture
Not known

Protective equipment and precautions for firefighters
Standard procedure for chemical fires.

SECTION 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures
Ensure adequate ventilation
Always wear recommended Personal Protective Equipment
Use personal protection equipment
See section 8 for more information

Environmental precautions
Prevent product from entering drains. Do not allow material to contaminate ground water system.

Methods and material for containment and cleaning up
Soak up with inert absorbent material.

Reference to other sections
See section 8 for more information.

SECTION 7: Handling and storage

Precautions for safe handling
Use personal protective equipment as required. No special handling advices are necessary.

Conditions for safe storage, including any incompatibilities
Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers.

Specific end use(s)
For research use only. Not for use in diagnostic procedures.

SECTION 8: Exposure controls/personal protection

Control parameters

Chemical Name	EU OEL (TWA)	EU OEL (STEL)	EU Skin Notation
Nonylphenol ethoxylates 9016-45-9	None	None	None

Chemical Name	Austria	Belgium (TWA)	Czech Republic
Nonylphenol ethoxylates 9016-45-9	None	None	None

Chemical Name	Denmark (TWA)	Finland OEL (TWA)	France OEL (VME)
Nonylphenol ethoxylates 9016-45-9	None	None	None

Chemical Name	Germany OEL (TWA)	Ireland (TWA)	Italy OEL (TWA)
Nonylphenol ethoxylates 9016-45-9	None	None	None

Chemical Name	Lithuania OEL (TWA)	Netherlands OEL (MAC)	Norway
Nonylphenol ethoxylates 9016-45-9	None	None	None

Chemical Name	Poland	Portugal	Spain OEL (TWA)
Nonylphenol ethoxylates 9016-45-9	None	None	None

Chemical Name	Sweden - Occupational Exposure Limits - TLVs (LLVs)	Switzerland	United Kingdom
Nonylphenol ethoxylates 9016-45-9	None	None	None

Engineering Measures Ensure adequate ventilation, especially in confined areas.

Exposure controls

Personal protection equipment

Respiratory protection In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards.

Hand protection Wear suitable gloves Glove material: Compatible chemical-resistant gloves.

Eye protection Tight sealing safety goggles.

Skin and Body Protection Wear suitable protective clothing.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

Environmental exposure controls
Prevent product from entering drains. Do not allow material to contaminate ground water system.

SECTION 9: Physical and chemical properties

Information on basic physical and chemical properties

Appearance	liquid	
Odour	No data available	
Odour Threshold	No data	
Molecular Weight	No data	
pH	No data available	
Melting point / melting range	**°C** No data	**°F** No data
Boiling point / boiling range	**°C** No data	**°F** No data
Flash point	**°C** No data	**°F** No data
Autoignition Temperature	**°C** No data	**°F** No data
Decomposition temperature	**°C** No data	**°F** No data
Evaporation rate	No data	
Flammability (solid, gas)	No data available	
Upper explosion limit	No data	
Lower explosion limit	No data	
Vapour Pressure	No data	
Vapour density	No data	
Relative density	No data	
Specific gravity	No data	
Solubility	No data available	
Partition coefficient: n-octanol/water	No data available	
Viscosity	No data	
Explosive properties	No data	
Oxidising properties	No data	

Other information
No data available.

SECTION 10: Stability and reactivity

Reactivity	None known.
Chemical stability	Stable under normal conditions.
Possibility of hazardous reactions	Hazardous reaction has not been reported.
Conditions to avoid	No information available.
Incompatible materials	No dangerous reaction known under conditions of normal use.
Hazardous decomposition products	No data available.

SECTION 11: Toxicological information

Information on toxicological effects

Chemical Name	Oral LD50	Dermal LD50	Inhalation LC50
Nonylphenol ethoxylates	= 1310 mg/kg (Rat) = 2590 mg/kg (Rat) = 1300 mg/kg (Rat) = 1410 µL/kg (Rat)	No data available	No data available

Principal Routes of Exposure

Skin corrosion/irritation Data are conclusive but insufficient for classification

Serious eye damage/irritation Data are conclusive but insufficient for classification

Respiratory or skin sensitisation Data are conclusive but insufficient for classification

Specific target organ toxicity (STOT) – single exposure Data are conclusive but insufficient for classification

Specific target organ toxicity (STOT) – repeated exposure Data are conclusive but insufficient for classification

Carcinogenicity Data are conclusive but insufficient for classification

Germ cell mutagenicity Data are conclusive but insufficient for classification

Reproductive Toxicity Data are conclusive but insufficient for classification

Aspiration Hazard Data are conclusive but insufficient for classification

SECTION 12: Ecological information

Ecotoxicity
Hazardous to the Aquatic Environment.

Chemical Name	Toxicity to algae	Toxicity to daphnia and other aquatic invertebrates	Toxicity to fish	Microtox Data	log Pow
Nonylphenol ethoxylates	No data available	No data available	No data available	No data available	No data available

Persistence and degradability No information available.

Bioaccumulative potential No information available.

Results of PBT and vPvB assessment
No information available.

Other adverse effects
Contains a known or suspected endocrine disruptor.

Chemical Name	EU - Endocrine Disrupters Candidate List
Nonylphenol ethoxylates	Group III Chemical; Group III Chemical (9<EO<19); Group III Chemical (with EO>19); Group III Chemical (with EO<9) Group III Chemical

SECTION 13: Disposal considerations

Waste treatment methods
The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.
Do not empty into drains
Do not dispose of waste into sewer.

SECTION 14: Transport information

IATA / ADR / DOT-US / IMDG
Not regulated in the meaning of transport regulations

UN number	Not Applicable
UN proper shipping name	Not Applicable
Transport hazard class(es)	Not Applicable
Packing group	Not Applicable

Environmental hazards
Not Applicable

Special precautions for user
Not Applicable

Transport in bulk according to Annex II of MARPOL and the IBC Code
Not Applicable.

SECTION 15: Regulatory information

Safety, health and environmental regulations/legislation specific for the substance or mixture
None.

Substances of Very High Concern

Chemical Name	Weight-%	EU - REACH (1907/2006) - Article 59(1) - Candidate List of Substances for Eventual Inclusion in Annex XIV
Nonylphenol ethoxylates	<0.9	Reason for inclusion Endocrine disrupting properties, Article 57f - environment

Substance subject to authorisation per REACH Annex XIV
None

Chemical Name	Weight-%	Substance subject to authorisation per REACH Annex XIV
Nonylphenol ethoxylates	<0.9	Intrinsic properties: Endocrine disrupting properties (Article 57(f) - environment) Application date: July 4, 2019 Sunset date: January 4, 2021 Exempted uses: None

Restricted substances under EC 1907/2006, Annex XVII

None.

Chemical Name	Weight-%	EU - REACH (1907/2006) - Annex XVII - Restrictions on Certain Dangerous Substances
Nonylphenol ethoxylates	<0.9	Use restricted. See item 46[b]. Use restricted. See item 46a.

Substances listed under Annex I of Regulation (EC) No 689/2008
None.

Restricted substances under Annex V of Regulation (EC) No 689/2008
None.

Substances under Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC
None.

German Water hazard classes (Wassergefährdungsklassen)

Chemical Name	Weight-%	Water hazard class (WGK)
Nonylphenol ethoxylates	<0.9	hazard class 3 - highly hazardous to water hazard class 2 - obviously hazardous to water

Other International Inventories

Chemical Name	EINECS (European Union)	ELINCS (European List of Notified Chemical Substances)	ENCS (Japan)	PICCS (Philippines)
Nonylphenol ethoxylates	-	-	Listed	Listed

Chemical Name	AICS (Australia)	South Korea (KECL)	Canada (DSL)	NDSL
Nonylphenol ethoxylates	Listed	Listed	Listed	-

Chemical safety assessment
No Chemical safety assessment has been carried out.

SECTION 16: Other information

Reason for revision Update according to Commission Regulation (EU) No 830/2015
Revision number 2
Revision date 15-Mar-2019

References
• ECHA: http://echa.europa.eu/
• TOXNET: http://toxnet.nlm.nih.gov/
• eChemPortal: http://www.echemportal.org/
• LOLI database: https://www.chemadvisor.com/loli-database

Classification and procedure used to derive the classification for mixtures according to Regulation (EC) 1272/2008 [CLP]:
Not classified

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED,INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE"

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

EN0581 Exonuclease I (ExoI)

Packaging Lot: **01016122**
Expiry Date: 31.10.2024 (DD.MM.YYYY)
Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
00988399	4 ku	Exonuclease I (ExoI)
00977842	1 mL	10X React Buffer for Exo I

QUALITY CONTROL

Parameter	Method	Requirement	Result
Unit definition	One unit of the enzyme catalyzes the release of 10 nmol of acid soluble nucleotides in 30 min at 37 °C.	20 U/µL ± 10%	Conforms
Endodeoxyribonucleases (nicking activity)	Incubation of supercoiled plasmid DNA with enzyme.	Not detectable	Conforms
Single-stranded Endodeoxyribonucleases	Incubation of circular single-stranded DNA with enzyme.	Not detectable	Conforms
Double-stranded Exodeoxyribonucleases	Incubation of double-stranded DNA fragments with enzyme.	Not detectable	Conforms
Ribonucleases	Incubation of [3H]-RNA with enzyme.	Not detectable	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

Exonuclease I (Exo I)

Catalog Number EN0581, EN0582

Pub. No. MAN0012007 **Rev.** C.00

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

Contents and storage

Cat. No.	Contents	Amount	Storage
EN0581	Exonuclease I (Exo I)	4000 U, 20 U/µL	-25 °C to -15 °C
	10X Reaction Buffer	1 mL	
EN0582	Exonuclease I (Exo I)	20000 U, 20 U/µL	
	10X Reaction Buffer	5 x 1 mL	

Description

Exonuclease I (Exo I) degrades single-stranded DNA in a 3'→5' direction, releasing deoxyribonucleoside 5'-monophosphates in a stepwise manner and leaving 5'-terminal dinucleotides intact. It does not cleave DNA strands with terminal 3'-OH groups blocked by phosphoryl or acetyl groups (1).

Applications

- Primer removal from PCR mixtures:
  - prior to PCR product sequencing (2),
  - for one-tube "megaprimer" PCR mutagenesis (3).
- Removal of single-stranded DNA containing a 3'-hydroxyl terminus from nucleic acid mixtures.
- Assay for the presence of single-stranded DNA with a 3'-hydroxyl terminus (4).

Source

E.coli cells with a cloned *E.coli sbcB* gene.

Definition of Activity Unit

One unit of the enzyme catalyzes the release of 10 nmol of acid soluble nucleotides in 30 min at 37 °C.
Enzyme activity is assayed in the following mixture: 67 mM glycine-KOH (pH 9.5), 6.7 mM $MgCl_2$, 1 mM DTT and 0.17 mg/mL single-stranded [^{3}H]-DNA.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50 % (v/v) glycerol.

10X Reaction Buffer

670 mM glycine-KOH (pH 9.5 at 25 °C), 67 mM $MgCl_2$, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: 20 % (w/v) PEG 8000 (5).
- Inactivated by heating at 80 °C for 15 min.

Note

The enzyme is not suitable for removing 3'-overhangs of dsDNA.

Protocol for PCR product clean-up prior to sequencing

The clean-up reaction removes unincorporated primers and degrades unincorporated nucleotides. The resulting PCR product is ready to use for sequencing without additional purification, e.g., using column purification kits.

1. Prepare the following reaction mixture:

Components	Volume
PCR mixture (directly after completion of PCR)	5 µL
Exonuclease I	0.5 µL (10 U)
Thermo Scientific™ FastAP™ Thermosensitive Alkaline Phosphatase (#EF0651)	1 µL (1 U)

2. Mix well and incubate at 37 °C for 15 min.
3. Stop the reaction by heating the mixture at 85 °C for 15 min.

Note

- Up to 5 µL of purified PCR products can be used directly for DNA sequencing without further purification.
- For reliable sequencing results there should not be nonspecific PCR products.
- The protocol may be applied for clean-up of PCR products, generated by any thermophilic DNA polymerase or polymerase mix.
- The procedure is not recommended for downstream cloning applications.

Reference

1. Lehman, I.R., Nussbaum A.L., The deoxyribonucleases of *Escherichia coli*. V. On the specificity of exonuclease I (phosphodiesterase), J. Biol. Chem., 239, 2628-2636, 1964.
2. Werle, E., et al., Convenient single-step, one tube purification of PCR products for direct sequencing, Nucleic Acids Res., 22, 4354-4355, 1994.
3. Nabavi S., Nazar R.N., Simplified one tube “megaprimer” polymerase chain reaction mutagenesis, Anal Biochem., 2, 346-348, 2005.
4. Rosamond, J., et al., Modulation of the action of the recBC enzyme of *Escherichia coli* K-12 by Ca^{2+}, J. Biol. Chem., 254, 8646-8652, 1979.
5. Sasaki, Y., Miyoshi, D. and Sugimoto, N., Regulation of DN nucleases by molecular crowding., Nucleic Acids Res., 35, 4086-4093, 2007.

Limited product warranty

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

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

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

EF0651 FastAP Thermosensitive AP

Packaging Lot: **01017842**
Expiry Date: 31.08.2023 (DD.MM.YYYY)
Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
00991580	1 ku	FastAP Thermosensitive AP
01003773	2 x 1.5 mL	10X Buffer for fastAP

QUALITY CONTROL

Parameter	Method	Requirement	Result
Unit definition	One unit is the amount of the enzyme required to dephosphorylate 5'-termini of 1 µg of linearized pUC57 DNA in 10 min at 37 °C in FastAP buffer.	1.0 U/µL ± 15%	Conforms
Endodeoxyribonucleases (nicking activity)	Incubation of supercoiled plasmid DNA with enzyme.	Not detectable	Conforms
Ribonucleases	Incubation of [3H]-RNA with enzyme.	Not detectable	Conforms
Endo- and Exodeoxyribonucleases	Incubation of single stranded and double stranded radiolabeled oligonucleotides with enzyme.	Not detectable	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

Dephosphorylation of Proteins

Protocol

This protocol is for the Dephosphorylation of Proteins

Reaction Mixture:

1X Thermo Scientific™ FastAP™ reaction buffer,

0.1 - 0.2 mg/mL of phosphoprotein,

10 u of FastAP Thermosensitive Alkaline Phosphatase.

Incubate at 37 °C for 1 hour.

For example: if you are doing a 20 µL reaction setup you need 2 µL 10x FastAP buffer, 2-4 µg of protein (to be in the range of 0.1 - 0.2 mg/mL) and 10 U of FastAP Thermosensitive Alkaline Phosphatase (1 u/µL).

Note:

- The reaction can be stopped by addition of a final concentration of 50 mM EDTA (#R1021) or by addition of a final concentration of 10 mM sodium orthovanadate (Na3VO4).
- The optimal incubation time and the enzyme concentration must be determined experimentally for each substrate

Europe

Customer Service
cs.molbio.eu@thermofisher.com

Technical Support
ts.molbio.eu@thermofisher.com

Tel 00800 222 00 888
Fax 00800 222 00 889

United States

Customer Service
cs.molbio@thermofisher.com

Technical Support
ts.molbio@thermofisher.com

Tel 800 235 9880
Fax 800 292 6088

Canada

Customer Service
cs.molbio@thermofisher.com

Technical Support
ts.molbio@thermofisher.com

Tel 800 340 9026
Fax 800 472 8322

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SAFETY DATA SHEET

SECTION 1: Identification of the substance/mixture and of the company/undertaking

Identification of the substance or mixture

Product code 4482777
Product name TAQMAN QSY PROBE 6,000 PMOLES

Company/undertaking identification

Life Technologies
5791 Van Allen Way
PO Box 6482
Carlsbad, CA 92008
+1 760 603 7200

LIFE TECHNOLOGIES LIMITED
3 FOUNTAIN DRIVE
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
44-141 814-6100

24 hour Emergency Response:

866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

SECTION 2: Hazards identification

In accordance with local and national regulations
In accordance with local and national regulations Regulation (EC) No 1272/2008 Classification according to Directive 67/548/EEC or 1999/45/EC

GHS - Classification

Signal word
Not Hazardous

Health hazards
Not Hazardous

Physical hazards
Not Hazardous

European Union

EU Specific Hazard Statements

R-phrase(s)
None

S phrases
None

Principle Routes of Exposure/ Potential Health effects

eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	May be harmful by inhalation.
INGESTION	May be harmful if swallowed.

Specific effects

Carcinogenic effects	None
Mutagenic effects	None
Reproductive toxicity	None
Sensitisation	None
Target Organ Effects	No known effects under normal use conditions

SECTION 3: Composition/information on ingredients

The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

SECTION 4: First aid measures

Skin contact	Rinse cautiously with water for several minutes. If symptoms occur, obtain medical advice.
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. If symptoms persist, call a doctor.
INGESTION	Never give anything by mouth to an unconscious person. If symptoms persist, call a doctor. Do not induce vomiting without medical advice.
Inhalation	Remove to fresh air. If symptoms persist, call a doctor. If not breathing, give artificial respiration.
Notes to Physician	Treat symptomatically.

SECTION 5: Firefighting measures

Suitable extinguishing media	Water spray. Carbon dioxide (CO2). Foam. Dry chemical.
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit.

SECTION 6: Accidental release measures

Personal precautions	Use personal protection equipment.
Methods for cleaning up	Soak up with inert absorbent material.

Environmental precautions

Prevent further leakage or spillage if safe to do so.

See Section 12 for more information.

SECTION 7: Handling and storage

Handling	Always wear recommended Personal Protective Equipment. Wear personal protective equipment.
Storage	Keep in a dry, cool and well-ventilated place.

SECTION 8: Exposure controls/personal protection

Exposure Limits

We are not aware of any national exposure limit.

Engineering measures	Ensure adequate ventilation, especially in confined areas.

Personal protective equipment

Personal Protective Equipment requirements are dependent on the user institution's risk assessment and are specific to the risk assessment for each laboratory where this material may be used.

Respiratory protection	In case of insufficient ventilation, wear suitable respiratory equipment.
Hand protection	Impervious gloves.
Eye protection	Safety glasses with side-shields.
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice.
Environmental exposure controls	Prevent product from entering drains.

SECTION 9: Physical and chemical properties

General information

Form	liquid	
Appearance	No information available	
Odour	No information available	
Boiling point / boiling range	**°C** No data available	**°F** No data available

Melting point / melting range	**°C** No data available	**°F** No data available
flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidising properties	No information available	
Water solubility	soluble	

SECTION 10: Stability and reactivity

Stability	Stable under normal conditions.
Materials to avoid	No dangerous reaction known under conditions of normal use.
Hazardous decomposition products	None under normal use conditions.
polymerisation	Hazardous polymerisation does not occur.

SECTION 11: Toxicological information

Acute toxicity

Not Hazardous.

Principle Routes of Exposure/ Potential Health effects

eyes	May cause eye irritation with susceptible persons
Skin	May cause skin irritation in susceptible persons
Inhalation	May be harmful by inhalation
INGESTION	May be harmful if swallowed
Carcinogenic effects	None
Mutagenic effects	None
Reproductive toxicity	None.
Sensitisation	None
Target Organ Effects	No known effects under normal use conditions

SECTION 12: Ecological information

Ecotoxicity	No information available
Mobility	No information available
Biodegradation	Inherently biodegradable.
Bioaccumulation	Material does not bioaccumulate

SECTION 13: Disposal considerations

Dispose of contents/containers in accordance with local regulations.

SECTION 14: Transport information

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations.
Hazard Class	None
Subsidiary class	None
Packing group	None
UN-No	None

SECTION 15: Regulatory information

International Inventories

Complies

SECTION 16: Other information

Reason for revision SDS sections updated

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRENTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PUPOSE"

End of Safety Data Sheet

TaqMan multiplex real-time PCR

Get more data out of your sample

- A complete multiplex real-time PCR (qPCR) solution for gene expression and genotyping applications
- Applied Biosystems™ ABY™ and JUN™ dyes, QSY™ quencher, and a multiplex master mix for optimal amplification performance
- Up to 4-plex reactions—as sensitive as singleplex reactions, decreases the starting material required, and minimizes optimization processes

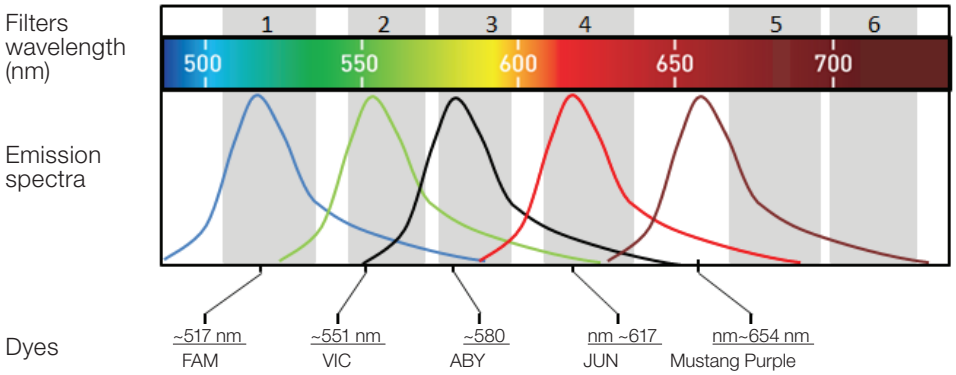

Figure 1. Fluorescence emission spectra of FAM, VIC, ABY, and JUN dyes used for multiplex real-time PCR. Grey zones represent the filters available on Applied Biosystems™ real-time PCR systems: 1 through 6 for the QuantStudio™ 7 or 12K Flex Real-Time PCR Systems; 1 through 5 for the QuantStudio™ 6 Flex Real-Time PCR System, ViiA™ 7 Real-Time PCR System, and 7500 or 7500 Fast Real-Time PCR System. MP = Mustang Purple™ dye.

Obtaining the maximum amount of genetic information from an important but small amount of sample can be challenging. This is particularly true with formalin-fixed, paraffin-embedded (FFPE) samples or tumor biopsies that are used for translational research studies. Singleplex qPCR is frequently used for these clinical research samples, but this typically has a higher cost per sample than running in multiplex format. The additional time and materials required to set up multiple single-assay reactions could also significantly increase the cost of a complex project.

Multiplex qPCR, a strategy where more than one target in a sample is amplified and quantified in a single tube, can decrease the quantity of sample material and reagents required. A complete solution for multiplex qPCR is presented here, with components designed to work together for better data quality and less time for optimization. The solution consists of the following:

- Applied Biosystems™ TaqMan® probes using QSY quencher, providing maximal PCR efficiency in a multiplex format. These probes can be ordered with Applied Biosystems™ FAM™ and VIC™ dyes and also with the ABY and JUN dyes, allowing amplification of up to 4 targets in a single reaction. These reporter dyes are optimized to work together with minimal spectral overlap for improved performance (Figure 1). In addition, the QSY quencher is fully compatible with probes that have minor-groove binder (MGB) quenchers.
- The Applied Biosystems™ TaqMan® Multiplex Master Mix was developed to allow amplification of 4 targets simultaneously, without competition between targets. This master mix contains the Applied Biosystems™ Mustang Purple™ dye, a passive reference used for normalization instead of the Applied Biosystems™ ROX™ dye, allowing for measurement of JUN dye in the channel previously used to measure ROX dye.

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- Off-the-shelf, predesigned assays—an RNase P assay using an ABY-QSY probe and a GAPDH assay using a JUN-QSY probe. Both assays are available in limited and nonlimited primer concentrations.
- Calibration plates for ABY, JUN, and Mustang Purple dyes, available in 96-well, 96-well Fast, and 384-well formats.
- Additional services provided through our custom services program—save time and let our Applied Biosystems™ TaqMan® Assay experts design your multiplex assays.

This multiplex solution is compatible with the Applied Biosystems™ QuantStudio™ 6, 7, and 12K Flex Real-Time PCR Systems, as well as the Applied Biosystems™ ViiA™ 7 Real-Time PCR System and the Applied Biosystems™ 7500 and 7500 Fast Real-Time PCR Systems.

Multiplexing without compromise

The multiplex format enables cost savings and preservation of limited sample, but it's important to obtain the same sensitivity as in the singleplex format. Figure 2 demonstrates comparable results between reactions performed in individual tubes or in 4-plex reactions for a gene quantification experiment.

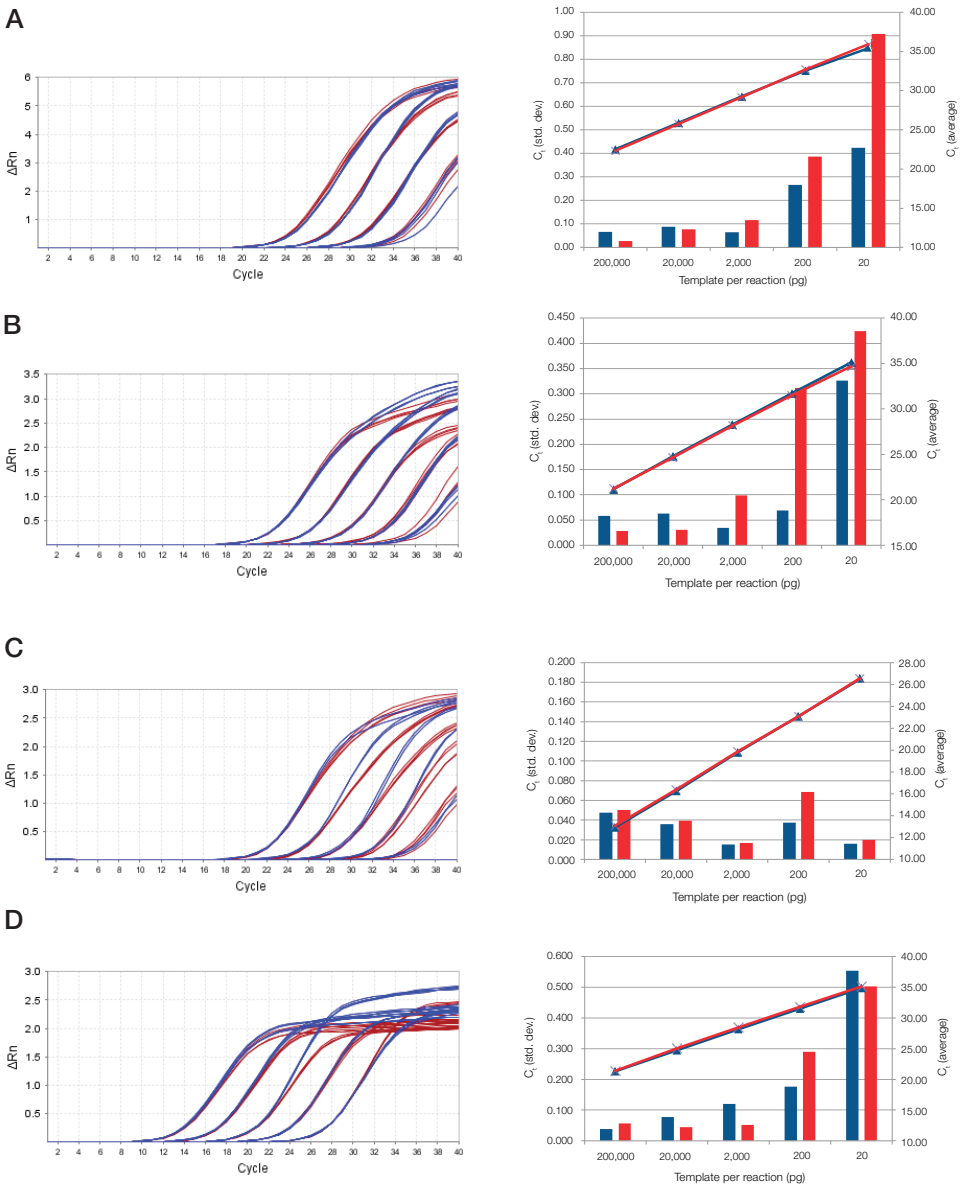

Figure 2. Comparison of singleplex and multiplex gene expression assays. (A) EGFR assay, FAM dye; **(B)** BRCA1 assay, VIC dye; **(C)** ESR1 assay, JUN dye; **(D)** RNase P assay, ABY dye. Amplification was performed on the QuantStudio 7 Real-Time PCR System using TaqMan Multiplex Master Mix. The figure shows amplification plots (left) and linear curves (right) for 4 assays amplified in singleplex (blue) and 4-plex reactions (red) in a dilution series from 20,000 pg to 2 pg of reference colon cDNA per 10 µL reaction. Average C_t value (lines) and average standard deviation (bars) for the dilution series are represented in their respective graphs and show the concordance between singleplex and 4-plex reactions. PCR efficiencies are: 96.09% for EGFR singleplex and 96.39% for EGFR 4-plex; 93.56% for BRCA1 singleplex and 94.93% for BRCA1 4-plex; 97.13% for ESR1 singleplex and 95.81% for ESR1 4-plex; 96.91% for RNase P singleplex and 98.1% for RNase P 4-plex.

Improved probe performance

Introduction of ABY and JUN reporter dyes and Mustang Purple passive reference dye allows for optimal 4-color multiplex assays when used with our FAM and VIC reporter dyes. Please note that ABY and JUN reporter dyes are available only with QSY quencher, while FAM and VIC dyes are available with either MGB or QSY quencher. A comparison with a set of dyes from another supplier shows that our combination of dyes provides an earlier C_t for the majority of assays (Figure 3).

Optimized multiplex master mix

In multiplex PCR, it's important to have a robust master mix that allows for amplification of each target in a highly competitive environment. Our new master mix composition was developed to provide optimal multiplex performance for each target in the reaction. A comparison of our master mix and a master mix from another supplier in a 4-plex reaction shows an earlier C_t for 3 of the targets amplified with our new master mix and a lower standard deviation for most of the dilution points, demonstrating the excellent performance of our solution (Figure 4).

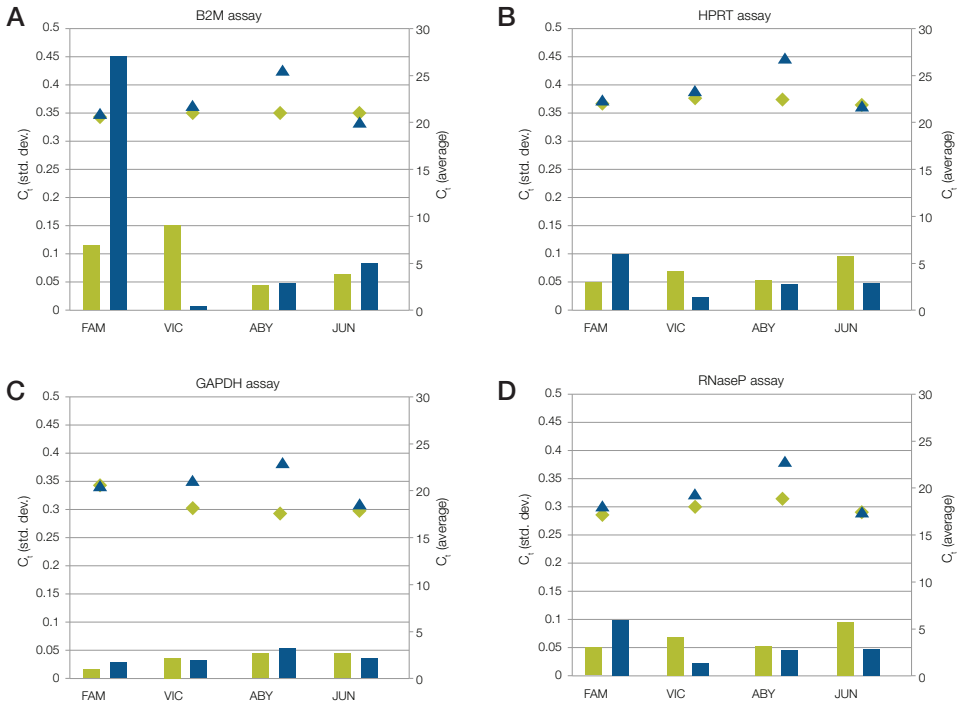

Figure 3. Comparison of our new dye combination with a dye combination from another supplier. Probes for **(A)** B2M, **(B)** HPRT, **(C)** GAPDH, and **(D)** RNase P gene expression assays were synthesized with FAM, VIC, ABY, and JUN dyes with QSY quencher (green bars and diamonds) and with another commercially available dye combination (blue bars and triangles). All possible gene-dye combinations were tested. Reactions were prepared with TaqMan Multiplex Master Mix using 900 nM of primer, 250 nM of probe, and 10 ng of cDNA. Amplification was performed on the QuantStudio 7 Real-Time PCR System using TaqMan Multiplex Master Mix. Bars represent average standard deviation. Triangles and diamonds represent average C_t values.

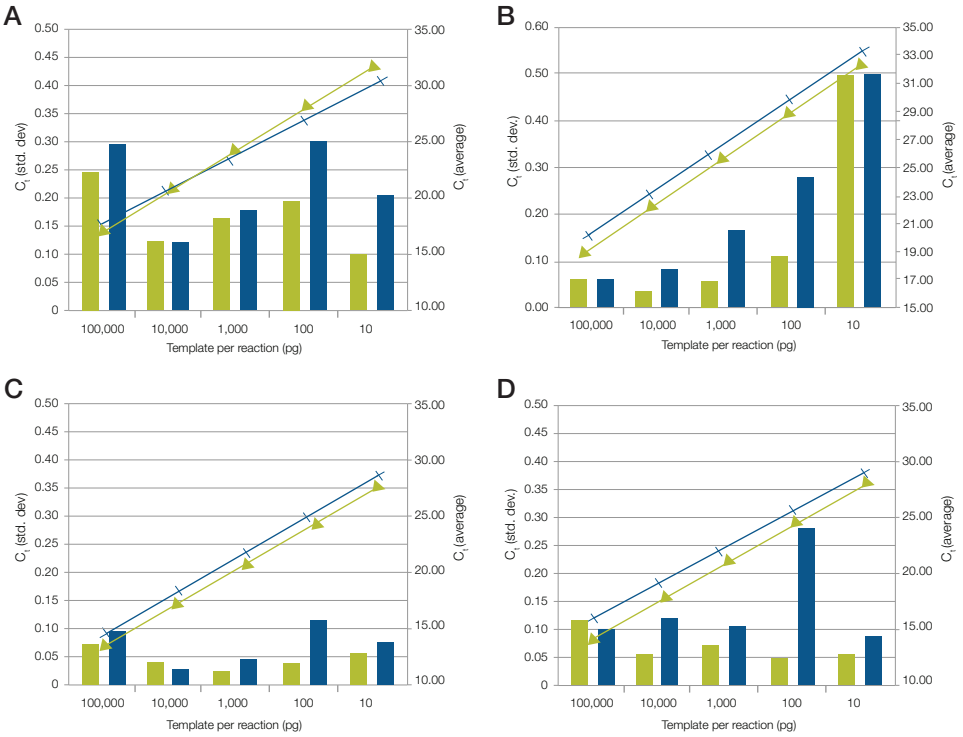

Figure 4. Comparison of TaqMan Multiplex Master Mix with another commercially available master mix. (A) B2M assay, FAM dye; **(B)** RNase P assay, VIC dye; **(C)** GAPDH assay, ABY dye; **(D)** HPRT assay, JUN dye. All assays used QSY quencher. The graph shows average standard deviation (bars) and average C_t values (cross and triangle) for 4-plex reactions using a dilution series from 100 ng to 10 pg of cDNA per 10 µL reaction. All amplifications were performed on the ViiA 7 Real-Time PCR System using the cycling conditions recommended for each master mix. Green represents TaqMan Multiplex Master Mix, and blue represents 4-plex reactions with another commercially available master mix.

applied biosystems

Optimized to minimize time-to-results

Developing a multiplex PCR assay requires time to correctly design the assay and optimize the reaction. Using our complete solution, for which all components were developed to work together, helps increase your chances of success and limits your development time. A new multiplex PCR user guide was developed to guide you through the development and optimization process [1], and our custom services will allow you to delegate assay design to our experienced team to minimize your efforts.

References

1. Multiplex PCR User Guide. Available at thermofisher.com/multiplexqpcr
2. TaqMan multiplex qPCR: Accurate, sensitive, and as efficient as traditional singleplex qPCR. Application note available at lifetechnologies.com/multiplexqpcr

Ordering information

Product	Cat. No.
TaqMan QSY probes	
TaqMan QSY Probe, 6,000 pmol	4482777
TaqMan QSY Probe, 20,000 pmol	4482778
TaqMan QSY Probe, 50,000 pmol	4482779
Control kits	
TaqMan GAPDH Assay, JUN-QSY 20X	4485712
TaqMan GAPDH Assay, JUN-QSY PL 20X	4485713
TaqMan RNaseP Assay, ABY-QSY 20X	4485714
TaqMan RNaseP Assay, ABY-QSY PL 20X	4485715
Multiplex master mixes	
TaqMan Multiplex Master Mix, 1 mL	4461881
TaqMan Multiplex Master Mix, 5 mL	4461882
TaqMan Multiplex Master Mix, 50 mL	4486295

Other formats are available at **lifetechnologies.com/multiplexqpcr**

Calibration plates	
96-Well Calibration Plate, Mustang Purple dye	4461599
96-Well Calibration Plate, JUN dye	A24737
96-Well Calibration Plate, ABY dye	A24738

Calibration plates are also available for 96-well Fast and 384-well plate formats.
Visit **thermofisher.com/multiplexqpcr** for more information.

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

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Custom Primers and TaqMan® Probes shipped at ambient temperature reduce environmental impact and retain their quality and stability

Abstract

To minimize the adverse environmental impact of packaging and shipping products on gel or dry ice, Thermo Fisher Scientific investigated the feasibility of shipping its Custom Primers and TaqMan® Probes at ambient temperatures. This report describes stability testing of dye-labeled primers and MGB, TAMRA™, and QSY® probes after subjecting them to simulated summer shipping conditions. Analytical and stability testing demonstrated that Custom Primers and TaqMan® Probes that underwent simulated summer ambient-temperature shipping conditions maintained the same integrity and functionality as primers and probes that were kept at the recommended storage condition. By shipping at ambient temperatures, the need for expanded polystyrene (EPS) coolers and added refrigerant is eliminated and the fuel consumption and greenhouse gas emissions from transporting the product are significantly reduced.

Introduction

The adverse environmental impact of shipping refrigerated or frozen products is tremendous. The annual carbon footprint to manufacture EPS and convert it into coolers for our Custom Primers and TaqMan® Probes is approximately 6 tons CO_2-equivalents (CO_2e) [1]. Factoring in the number of shipments, the average distance traveled per package, and the fact that most packages are shipped via air, the annual total carbon footprint for transporting Custom Primers and TaqMan® Probes is 32 tons CO_2e [2].

There are other facts to consider beyond the greenhouse gas emissions. When a cooler arrives at the laboratory, the researcher is often put in the untenable position of deciding whether to burn additional fossil fuels to transport the empty cooler across country for reuse/recycling or to dispose of the cooler in a landfill. The best way to address the total environmental impact of "cold-chain" transport is to follow the hierarchy of "reduce, reuse, recycle": 1) Design the product for stability to ensure it can withstand the rigors of ambient shipping conditions without added refrigerant or insulation; 2) Design the packaging to be reusable, without increasing source material consumption; and 3) Recycle locally. We have opted to reduce whenever possible, reuse when it is an environmentally preferable option, and to encourage our customers to recycle locally.

Thermo Fisher Scientific has been systematically evaluating novel ways to minimize the impact of shipping Life Technologies™ products on gel or dry ice, and the CO_2 footprint left by these products during distribution. Here we demonstrate that selected Custom Primers and TaqMan® Probes are stable at ambient temperatures during shipping. By avoiding the cooler and refrigerant, the product can be shipped in a smaller, corrugated cardboard box, which improves the carrier's freight density (less fuel and emissions per box) and reduces the amount of packaging materials requiring disposal or recycling. By eliminating the cooler and gel or dry ice for these products, Thermo Fisher Scientific is helping to divert an annual total of nearly 1,826 kg (5,062 cubic feet)

life technologies

of EPS from landfills and incinerators by replacing it with recyclable corrugated paper packaging, and to reduce the annual total carbon footprint by 38 tons CO_2e [1,2].

In 2009, we investigated the stability of five TaqMan® Assays: TaqMan® Gene Expression, Custom TaqMan® Gene Expression, TaqMan® MicroRNA, TaqMan® Drug Metabolism Genotyping, and TaqMan® SNP Genotyping Assays [3]. These assays comprise a preformulated set of unlabeled locus-specific oligonucleotide primers and minor grove binder–nonfluorescent quencher (MGB-NFQ) probes labeled with a fluorescent dye (VIC® or FAM™ dye), and are supplied in liquid form. A total of 42 different TaqMan® Assays were selected to represent the widest range of performance as well as chemical, sequence, and structural motifs. Assays were subjected to simulated summer ambient shipping conditions and subsequently analyzed for physical integrity and functional performance. Stressed samples were compared to controls in analytical HPLC and functional real-time PCR assays. In all cases, simulated ambient shipping of the assays had no effect on their quality, integrity, or functional performance. This study provided ample evidence for the stability of a wide range of structural motifs and oligonucleotide sequences under ambient shipping conditions and also demonstrated the stability of the VIC® and FAM™ dyes and the MGB moiety at the concentrations found in the assays.

For many years, Custom Primers and TaqMan® Probes have been shipped refrigerated on gel ice (with storage after shipping at +4°C or –20°C, depending on the product). Building on our 2009 study, this paper describes results from stability testing carried out after the Custom Primers and TaqMan® Probes were exposed to established summer shipping temperature profiles. These experiments demonstrate that by shipping selected Custom Primers and TaqMan® Probes under ambient conditions, not only can we supply researchers with the same superior-quality product they are used to receiving, but we can also reduce our environmental footprint in the process. This is a win for our customers (eliminating packaging waste and extra costs associated with refrigerated shipments), a win for our planet (reducing resource consumption and total carbon footprint), and a win for our company (eliminating the need to manage cold-chain transport).

Materials and methods

Products tested. Custom Primers are 5'-labeled oligos that come with a choice of six dyes: 6-FAM™, TET™, VIC®, HEX™, NED™, or PET™ dye. The Custom Primer Pairs also come with an unlabeled oligo in a separate tube. Primers and Primer Pairs may be HPLC purified and can be ordered in two or three different quantities, with the largest having the highest concentration. For this study, four different labeled primers at the highest concentration were selected to represent the variety of primer types and dyes available (Table 1). The FAM™ and VIC® dyes were not tested with the primers because the 2009 study demonstrated the stability of these dyes in the TaqMan® Assays under ambient shipping conditions. Additionally, the Sequence Detection Primers, which are unlabeled, were not tested because the 2009 study established that unlabeled oligos are not affected by simulated ambient shipping conditions. All primers tested were formulated in Tris-EDTA (TE) buffer and were not HPLC purified. HPLC purification has no impact on the stability of the oligo or dye. Formulations in water were not evaluated because the pH of Tris buffers is known to vary inversely with temperature [4,5], something that does not occur in water, making TE a higher-risk formulation for ambient shipping.

TaqMan® MGB Probes incorporate a 5' reporter dye (FAM™, VIC®, TET™, or NED™ dye) and a 3' nonfluorescent quencher, with the MGB moiety attached to the quencher molecule. The TAMRA™ probes incorporate a 5' reporter dye (FAM™, TET™, or VIC® dye) and a 3' TAMRA™ quencher dye. The TaqMan® QSY® Probes can be ordered with a 5' reporter dye (FAM™, VIC®, ABY®, or JUN® dye) and the QSY® quencher. All TaqMan® Probes are HPLC purified and supplied at a single concentration in TE buffer. The MGB, TAMRA™, and QSY® probes were each tested with their respective dyes, with the exception of the MGB probe. This probe was not tested with VIC® dye because our own unpublished studies have shown that FAM™ is more labile than VIC® at elevated temperatures; therefore, FAM™ was used to represent a "worst-case" scenario. Because the 2009 study showed that variation in sequence and length did not affect oligo stability, a single sequence was chosen for all primers and probes:

5' - TGGACAGCCACCGACGAGAGCCTGG - 3'

Table 1. Custom Primers and TaqMan® Probes represented in this study.

Product Description	Reporter dye	Cat. No.
Custom Primers		
Sequence Detection Primers, 10,000 pmol, 80,000 pmol, 130,000 pmol	None	4304970, 4304971, 4304972
Custom 5'-Labeled Primer Pair Di-Repeats, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, **NED™**, PET®, 6-FAM™, VIC®, TET™	4304976, 4304977, **4304978**
Custom 5'-Labeled Primer, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, NED™, **PET®,** 6-FAM™, VIC®, TET™	450007, 450006, **450017**
Custom 5'-Labeled Primer Pair, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, NED™, PET®, 6-FAM™, VIC®, **TET™**	450056, 450059, **450062**
Custom 5'-Labeled Primer Pair Di-Repeat + Tail, 10,000 pmol, 80,000 pmol, **300,000 pmol**	**HEX™**, NED™, PET®, 6-FAM™, VIC®, TET™	4304979, 4304981, **4304982**
TaqMan® Custom Probes		
TaqMan® MGB Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**6-FAM™**, VIC®, **NED™, TET™**	4316034, 4316033, **4316032**
TaqMan® TAMRA™ Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**VIC®, 6-FAM™, TET™**	450025, 450024, **450003**
TaqMan® QSY® Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**6-FAM™, VIC®, ABY®, JUN®**	4482777, 4482778, **4482779**

Products tested are in **bold**

Creating replicates. To help eliminate manufacturing lot variability when creating the replicates, individual tubes of the primers and probes were manufactured, pooled, and aliquoted into the same packaging tube at the same fill volume as specified for the manufactured product. A total of 10 lots for each primer and probe were used to create five replicate stress tubes and five replicate control tubes. The control tubes were kept at –20°C for the duration of the study.

Simulated shipping conditions. To simulate temperatures experienced during shipping, samples were placed in a cycling environmental chamber (Thermotron® S-16) programmed to reproduce a "worst-case" 288-hour (12-day) summer temperature profile (Figure 1). This profile is adopted from one developed and validated by Amgen to simulate global ambient shipping conditions and mimics product temperature extremes encountered during transit of over 2,500 shipments during summer months between the latitudes of 59.9° N and 37.8° S [6]. Testing of winter ambient conditions was not considered, due to the low risk of exposing the Custom Primers and TaqMan® Probes to cold conditions.

Stability and integrity testing. Structural integrity changes in stressed samples compared to controls were measured by reverse-phase HPLC (RP-HPLC) and MALDI mass spectrometry. RP-HPLC samples were analyzed using an Agilent® 1200 HPLC. The HPLC column used was a Phenomenex® Clarity® 3 µm Oligo-RP, 2.0 mm ID x 50 mm. Mobile phases used were 0.1 M TEAA (triethylamine acetate) in water and 0.1 M TEAA in

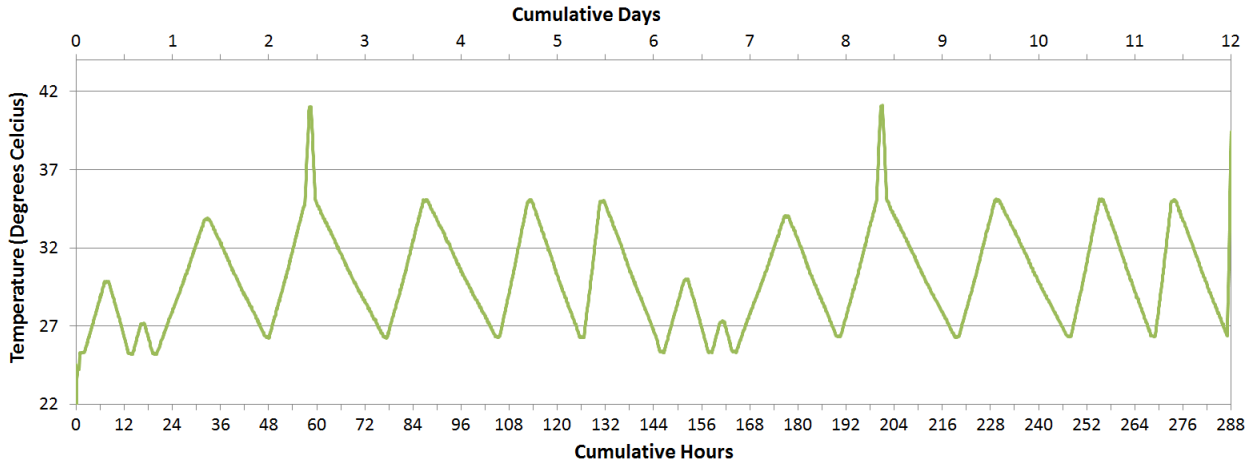

Figure 1. 288 hr summer temperature profile used to simulate shipping conditions. The summer temperature profile was used to mimic average high temperature extremes between the latitudes of 59.9° N and 37.8° S.

50% water/40% acetonitrile/10% methanol for the primers, MGB, and TAMRA™ probes, and 0.1 M TEAA in water and 2.0 M TEAA in 5% water/95% methanol for the QSY® probes. Absorbance was monitored at 260 nm for the oligonucleotide and at the maximum absorbance wavelength of the dye. Samples for MALDI mass spectrometry were analyzed on an AB Sciex® 4800 Plus MALDI TOF/TOF™ Analyzer.

Results

RP-HPLC. RP-HPLC was used to create peak profiles of the dye-labeled primers using UV/Vis absorbance detection. Matched test and control tubes from each assay were analyzed. An example of the data is shown in Figure 2. Test and control peak profiles were compared, and the purity (peak areas) were calculated (data not shown). For all samples analyzed, test samples were judged as identical to matched controls (no degradation), confirming that the simulated shipping stress did not affect product integrity.

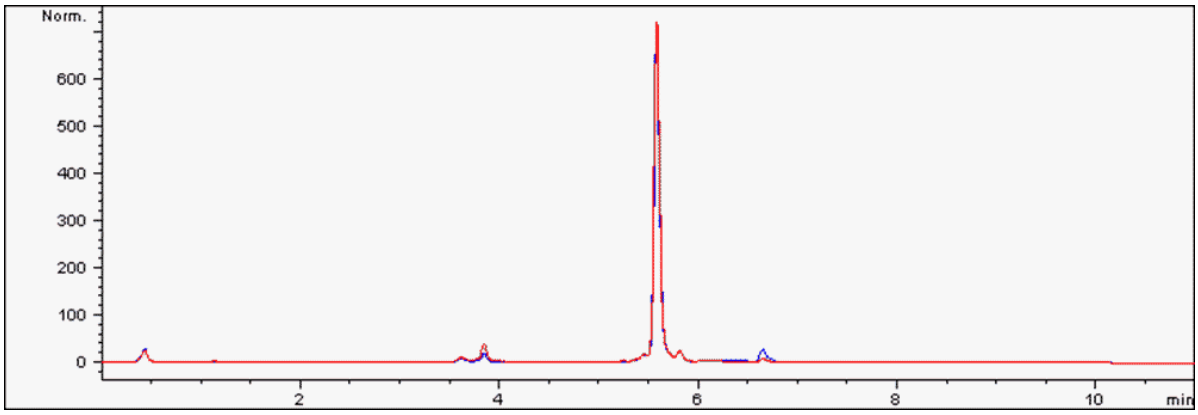

Figure 2. Simulated summer ambient shipping does not affect oligonucleotide stability—representative HPLC data. The effect of simulated summer ambient shipping on oligonucleotide integrity was measured by comparing RP-HPLC profiles of matched test and control samples. The HPLC chromatogram profiles of the test samples are comparable to the profiles of the control samples. There was no indication of probe or primer degradation in the simulated ambient-shipped 5'-Labeled Primer Pair Di-Repeats with the NED™ dye (red) compared to the matched control (blue).

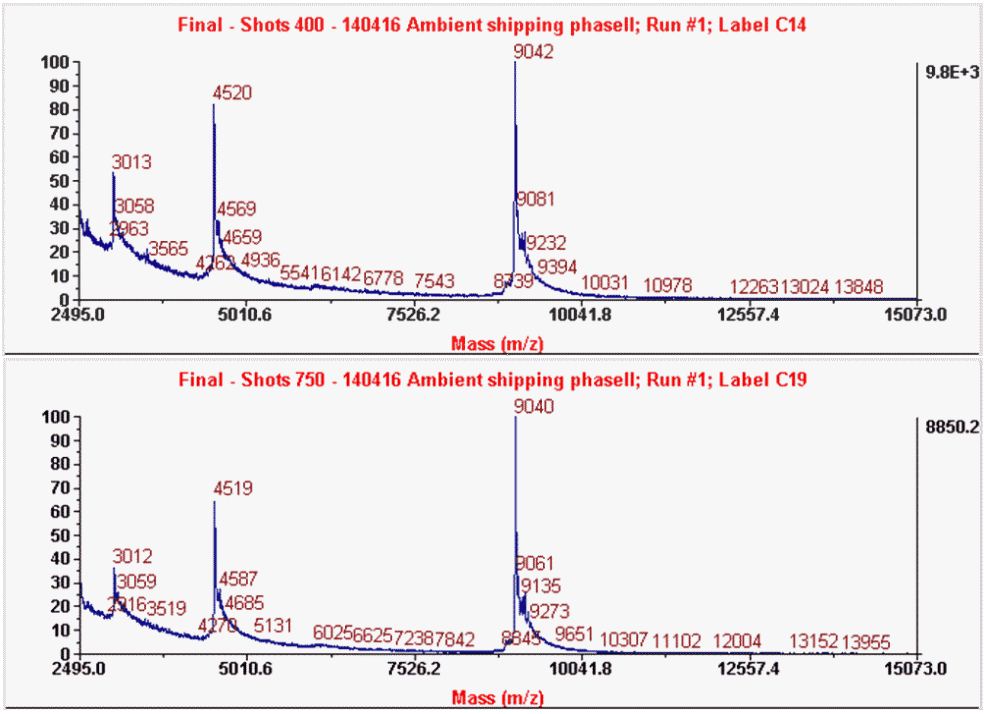

Figure 3. Simulated summer ambient shipping does not affect oligonucleotide stability—representative MALDI mass spectrometry data. The effect of simulated summer ambient shipping on oligonucleotide integrity was measured by comparing mass spectrum profiles of matched test and control samples. The profiles of the test samples are comparable to the profiles of the control samples. There was no indication of probe or primer degradation in the simulated ambient-shipped TaqMan® TAMRA™ Probe with the VIC™ dye (bottom) compared to the matched control (top).

MALDI mass spectrometry. MALDI mass spectrometry was used to generate mass profiles of the dye-labeled primers and probes. Again, matched test and control assays were analyzed and compared to each other. An example mass spectrum is shown in Figure 3. Test and control samples showed the same mass profiles, indicating that no degradation of the oligo, dye, or quencher occurred during the shipping simulation, further confirming that the simulated shipping stress did not affect product integrity.

Conclusions

The data described in this paper demonstrate that ambient shipping conditions have no effect on the quality and stability of Custom Primers and TaqMan® Probes. For each dye-labeled primer and probe tested, we were able to clearly demonstrate that ambient-temperature shipping conditions do not affect the product quality or integrity.

These results substantiate the change to ambient shipping conditions, and provide the researcher with confidence that when shipped under ambient conditions, their Custom Primers and TaqMan® Probes will exhibit no difference in function or stability compared to dry or gel ice–shipped products. In addition to ensuring our customers will continue to receive the highest quality possible, this study enables us to reduce the impact of transport of these products by 32 tons CO_2e. Our customers will see a reduction of 1,826 kg of EPS waste. Our planet will collectively see CO_2 emissions reduced by 38 tons every year.

References

1. Data derived from Bousted, I. 2006. Eco-profiles of the European Plastics Industry POLYSTYRENE (Expandable) (EPS). *Plastics Europe*, June.
2. Data derived from US Environmental Protection Agency. 2008. Climate leaders, greenhouse gas inventory protocol core module guidance: Optional emissions from commuting, business travel and product transport. US EPA document EPA-430-R-08-006. Available at http://www.epa.gov/climateleadership/documents/resources/commute_travel_product.pdf.
3. Chang H-R, Crespo J, Lee K, McGall K, MacWhorter S, Russell P, Schatz J, Seid D, Walworth C, Yu J. 2009. TaqMan® Assays shipped at ambient temperature reduce environmental impact and retain their quality and stability. Life Technologies publication 0-090071 0410. Available at http://tools.invitrogen.com/content/sfs/brochures/cms_081489.pdf.
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6. Cowland R. 2007. Developing ISTA Cold Chain Environmental Standards. Paper presented at the Dimensions '07 Conference, Orlando, Florida. Available at www.ista.org/forms/COWLAND_RAY_Dimensions07.pdf

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Safety Data Sheet

Safety Data Sheet according to Regulation (EC) No. 1907/2006 (REACH)
Classification according to Regulation (EC) No. 1272/2008 [CLP]

SECTION 1: Identification of the substance/mixture and of the company/undertaking

Product identifier

Product code	4351379
Product name	TAQMAN SNP ASSAY MTO HUMAN SM
Chemical Name	Not Applicable
REACH registration number	Formamide : 01-2119496064-35-XXXX

Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses	For research use only
Use Description Code	SU22 - Professional uses: Public domain (administration, education, entertainment, services, craftsmen), PROC15 - Use as laboratory reagent, PC21 - Laboratory chemicals, SU24 - Scientific research and development
Uses advised against	Not for consumer use.

Details of the supplier of the safety data sheet

Manufacturer / Supplier

LIFE TECHNOLOGIES EUROPE BV
KWARTSWEG 2
2665 NN BLEISWIJK
NETHERLANDS
31-(0)180 392 400
Email: MSDS@lifetech.com

Life Technologies Limited
3 Fountain Drive
Inchinnan Business Park
Paisley
PA4 9RF, UK
+44 (0)141 814 6100

24 hour Emergency Response for Hazardous Materials [or Dangerous Goods] Incident. Spill, Leak, Fire, Exposure, or Accident. Call CHEMTREC
Within the USA + Canada: 1-800-424-9300 and 1-703-527-3887
Outside the USA + Canada: 1-703-741-5970

Country Specific Emergency Number (if available):

CHEMTREC Ireland (Dublin)	+(353)-19014670 (Greeting Language: English and Irish)
CHEMTREC UK (London)	+(44)-870-8200418 (Greeting Language: English)

SECTION 2: Hazards identification

Classification of the substance or mixture

Classification according to Regulation (EC) No. 1272/2008 [CLP]

Physical hazards
Not Hazardous

Health hazards

Carcinogenicity	Category 2
Reproductive Toxicity	Category 1B
Specific target organ toxicity - Repeated exposure	Category 2

Environmental hazards
Not Hazardous

Additional information
No information available

Label elements

Labelling according to Regulation (EC) No 1272/2008 [CLP]

Hazard pictograms

Signal Word
Danger

Hazard Statements
H360 - May damage fertility or the unborn child if swallowed
H351 - Suspected of causing cancer if swallowed
H373 - May cause damage to organs through prolonged or repeated exposure

Precautionary Statements

Prevention
P201 - Obtain special instructions before use
P202 - Do not handle until all safety precautions have been read and understood
P260 - Do not breathe dust/fume/gas/mist/vapours/spray
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P281 - Use personal protective equipment as required

Response
P308 + P313 - IF exposed or concerned: Get medical advice/attention
P314 - Get medical advice/attention if you feel unwell

Storage
Not Applicable

Disposal

P501 - Dispose of contents/ container to an approved waste disposal plant

Other hazards
Not Applicable

SECTION 3: Composition/information on ingredients

Component	CAS No	EINECS-No.	Weight-%	REACH registration number	Classification according to Regulation (EC) No. 1272/2008 [CLP]
Formamide 75-12-7 (1-5%)	75-12-7	200-842-0	1-5%	01-2119496064-35-XXXX	Repr. 1B - H360 Carc. 2 - H351 STOT RE 2 - H373

SECTION 4: First aid measures

Description of first aid measures

Skin contact Wash off immediately with plenty of water for at least 15 minutes. Remove and wash contaminated clothing and gloves, including the inside, before re-use. Immediate medical attention is required.

Eye contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required.

Ingestion Never give anything by mouth to an unconscious person. Do not induce vomiting without medical advice. If swallowed, rinse mouth with water (only if the person is conscious). Risk of serious damage to the lungs (by aspiration). Get medical attention if symptoms occur.

Inhalation Remove to fresh air. If not breathing, give artificial respiration. If symptoms persist, call a doctor.

Notes to Physician Treat symptomatically.

Most important symptoms and effects, both acute and delayed
H360 - May damage fertility or the unborn child if swallowed H351 - Suspected of causing cancer if swallowed H373 - May cause damage to organs through prolonged or repeated exposure

Indication of any immediate medical attention and special treatment needed
IF exposed or concerned: Get medical advice/attention. Get medical advice/attention if you feel unwell.

SECTION 5: Firefighting measures

Extinguishing media

Suitable extinguishing media Foam. Dry powder. Dry chemical. Carbon dioxide (CO_2). Water spray.

Unsuitable extinguishing media No information available.

Special hazards arising from the substance or mixture
None known

Protective equipment and precautions for firefighters
Wear self-contained breathing apparatus and protective suit.

SECTION 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures
Avoid exposure to vapour
Avoid breathing vapours or mists
Ensure adequate ventilation
Avoid contact with skin, eyes or clothing
Use personal protection equipment
See section 8 for more information

Environmental precautions
Should not be released into the environment. Prevent product from entering drains.

Methods and material for containment and cleaning up
Soak up with inert absorbent material (e.g. sand, silica gel, acid binder, universal binder, sawdust). Sweep up and shovel into suitable containers for disposal. Clean contaminated surface thoroughly.

Reference to other sections
See section 8 for more information.

SECTION 7: Handling and storage

Precautions for safe handling
Always wear recommended Personal Protective Equipment. Wash hands before breaks and immediately after handling the product. Do not get in eyes, on skin, or on clothing. Avoid breathing vapours or mists. If during normal use the material presents a respiratory hazard, use adequate ventilation and/or wear appropriate respirator. See section 8 for more information.

Conditions for safe storage, including any incompatibilities
Keep in properly labelled containers. Keep in a dry, cool and well-ventilated place. Store in accordance with local regulations. Keep away from combustible material.

Specific end use(s)
For research use only.

SECTION 8: Exposure controls/personal protection

Control parameters

Chemical Name	EU OEL (TWA)	EU OEL (STEL)	EU Skin Notation
Formamide 75-12-7	None	None	None

Chemical Name	Austria	Belgium (TWA)	Czech Republic
Formamide 75-12-7	9 ppm 16 mg/m³	10 ppm 18 mg/m³	None

Chemical Name	Denmark (TWA)	Finland OEL (TWA)	France OEL (VME)
Formamide 75-12-7	10 ppm 18 mg/m³	10 ppm 19 mg/m³	20 ppm 30 mg/m³

Chemical Name	Germany OEL (TWA)	Ireland (TWA)	Italy OEL (TWA)
Formamide 75-12-7	None	10 ppm 18 mg/m³	None

Chemical Name	Lithuania OEL (TWA)	Netherlands OEL (MAC)	Norway
Formamide 75-12-7	10 ppm 20 mg/m³	None	10 ppm TWA 18 mg/m³ TWA 15 ppm STEL 27 mg/m³ STEL

Chemical Name	Poland	Portugal	Spain OEL (TWA)
Formamide 75-12-7	23 mg/m³ TWA Skin Notation	10 ppm TWA skin - potential for cutaneous exposure	10 ppm 19 mg/m³

Chemical Name	Sweden - Occupational Exposure Limits - TLVs (LLVs)	Switzerland	United Kingdom
Formamide 75-12-7	10 ppm TLV NGV; 20 mg/m³ TLV NGV	10 ppm TWA 18 mg/m³ TWA	20 ppm TWA; 37 mg/m³ TWA

Engineering Measures Ensure adequate ventilation, especially in confined areas.

Exposure controls

Personal protection equipment

Respiratory protection In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards.

Hand protection Glove material: Nitrile rubber with thickness (mm) :5 Break through time (hours) :>1
Recommended glove type has not been tested for use with this product.
Information is based on professional knowledge

Eye protection Tight sealing safety goggles.

Skin and Body Protection Wear laboratory coat for body protection.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

Environmental exposure controls
No special environmental precautions required.

SECTION 9: Physical and chemical properties

Information on basic physical and chemical properties

Appearance	liquid	
Odour	No data	
Odour Threshold	No data	
Molecular Weight	No data	
pH	8	
Melting point / melting range	**°C** 0-2.5	**°F** 32-36.5
Boiling point / boiling range	**°C** 100-210	**°F** 212-410
Flash point	**°C** >120	**°F** >248
Autoignition Temperature	**°C** >500	**°F** >932
Decomposition temperature	**°C** No data	**°F** No data
Evaporation rate	No data	
Flammability (solid, gas)	No data	
Upper explosion limit	No data	
Lower explosion limit	No data	
Vapour Pressure	No data	
Vapour density	No data	
Relative density	No data	
Specific gravity	No data	
Solubility	Soluble in water	
Partition coefficient: n-octanol/water	No data	
Viscosity	No data	
Explosive properties	No data	
Oxidising properties	No data	

Other information
No data.

SECTION 10: Stability and reactivity

Reactivity	None known.
Chemical stability	Stable under normal conditions.
Possibility of hazardous reactions	Hazardous reaction has not been reported.
Conditions to avoid	High temperature. Thermal decomposition of the finish can take place above (>140 °C) >284 °C.
Incompatible materials	Oxidising agent. Acids. Bases. Sulphur trioxide. Iodine.
Hazardous decomposition products	Carbon monoxide. Hydrogen cyanide (hydrocyanic acid). Nitrogen oxides (NOx).

SECTION 11: Toxicological information

Information on toxicological effects

Chemical Name	Oral LD50	Dermal LD50	Inhalation LC50
Formamide	3200 mg/kg	13500 mg/kg	3900 ppm/6H

Principal Routes of Exposure

Skin corrosion/irritation Data are conclusive but insufficient for classification

Serious eye damage/irritation Data are conclusive but insufficient for classification

Respiratory or skin sensitisation Data are conclusive but insufficient for classification

Specific target organ toxicity (STOT) – single exposure Data are conclusive but insufficient for classification

Specific target organ toxicity (STOT) – repeated exposure Target organ(s): : Cardiovascular System Hematopoietic System

Carcinogenicity Contains a known or suspected carcinogen

Germ cell mutagenicity Data are conclusive but insufficient for classification

Reproductive Toxicity May cause adverse reproductive effects - such as birth defect, miscarriages, or infertility

Aspiration Hazard Data are conclusive but insufficient for classification

SECTION 12: Ecological information

Ecotoxicity
The environmental impact of this product has not been fully investigated.

Chemical Name	Toxicity to algae	Toxicity to daphnia and other aquatic invertebrates	Toxicity to fish	Microtox Data	log Pow
Formamide	Desmodesmus subspicatus EC50>500 mg/L (72 h) Desmodesmus subspicatus EC50>500 mg/L (96 h)	Daphnia magna EC50>500 mg/L (48 h)	No data available	No data available	logPow-0.82

Persistence and degradability Readily biodegradable.

Bioaccumulative potential Material does not bioaccumulate.

Results of PBT and vPvB assessment
This mixture does not contain any substances that are assessed to be a PBT or a vPvB.

Other adverse effects
No information available.

SECTION 13: Disposal considerations

Waste treatment methods
The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.

SECTION 14: Transport information

IATA / ADR / DOT-US / IMDG
Not regulated in the meaning of transport regulations

UN number	Not Applicable
UN proper shipping name	Not Applicable
Transport hazard class(es)	Not Applicable
Packing group	Not Applicable

Environmental hazards
Not Applicable

Special precautions for user
Not Applicable

Transport in bulk according to Annex II of MARPOL and the IBC Code
Not Applicable.

SECTION 15: Regulatory information

Safety, health and environmental regulations/legislation specific for the substance or mixture

Substances of Very High Concern

Chemical Name	Weight-%	EU - REACH (1907/2006) - Article 59(1) - Candidate List of Substances for Eventual Inclusion in Annex XIV
Formamide	1-5%	Reason for inclusion Toxic for reproduction, Article 57c

Substance subject to authorisation per REACH Annex XIV
None

Restricted substances under EC 1907/2006, Annex XVII

Chemical Name	Weight-%	EU - REACH (1907/2006) - Annex XVII - Restrictions on Certain Dangerous Substances
Formamide	1-5%	Use restricted. See item 30.

Substances listed under Annex I of Regulation (EC) No 689/2008
None.

Restricted substances under Annex V of Regulation (EC) No 689/2008
None.

Substances under Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC
None.

German Water hazard classes (Wassergefährdungsklassen)

Chemical Name	Weight-%	Water hazard class (WGK)
Formamide	1-5%	hazard class 1 - slightly hazardous to water

Other International Inventories

Chemical Name	EINECS (European Union)	ELINCS (European List of Notified Chemical Substances)	ENCS (Japan)	PICCS (Philippines)
Formamide	Listed	-	Listed	Listed

Chemical Name	AICS (Australia)	South Korea (KECL)	Canada (DSL)	NDSL
Formamide	Listed	Listed	Listed	-

Chemical safety assessment
No Chemical safety assessment has been carried out.

SECTION 16: Other information

Reason for revision	Update according to Commission Regulation (EU) No 830/2015
Revision number	8
Revision date	17-Apr-2020

References

- ECHA: http://echa.europa.eu/
- TOXNET: http://toxnet.nlm.nih.gov/
- eChemPortal: http://www.echemportal.org/
- LOLI database: https://www.chemadvisor.com/loli-database

Classification and procedure used to derive the classification for mixtures according to Regulation (EC) 1272/2008 [CLP]:

Carcinogenicity	Category 2	Calculation method
Reproductive Toxicity	Category 1B	Calculation method
Specific target organ toxicity - Repeated exposure	Category 2	Calculation method

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED,INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE"

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

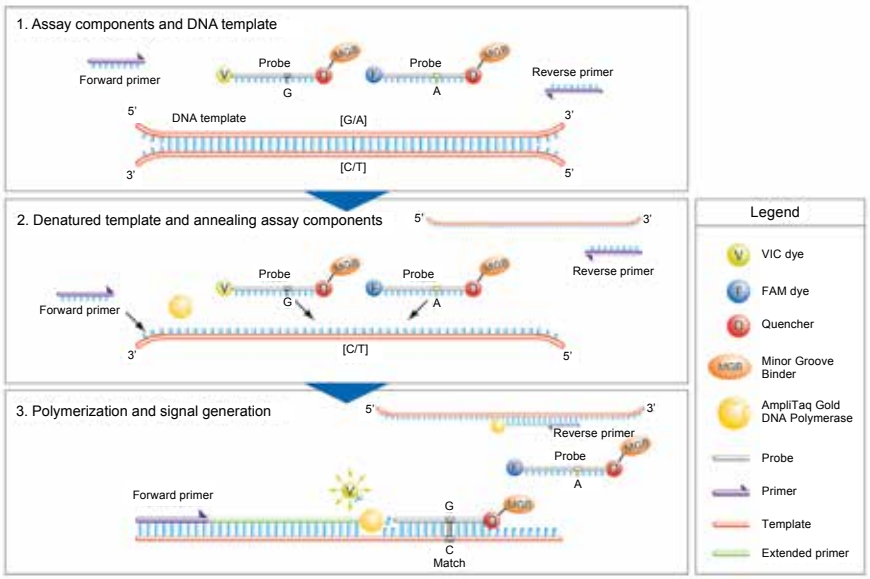

Figure 1. Allelic discrimination is achieved by the selective annealing of TaqMan MGB probes.

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.

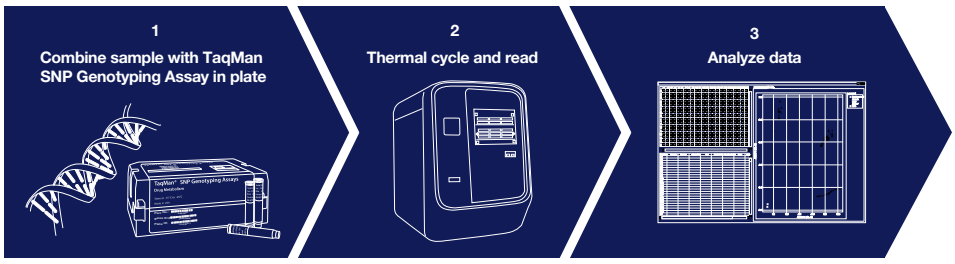

Figure 2. A simple workflow and reliable instruments combine to generate fast, high-confidence results.

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.

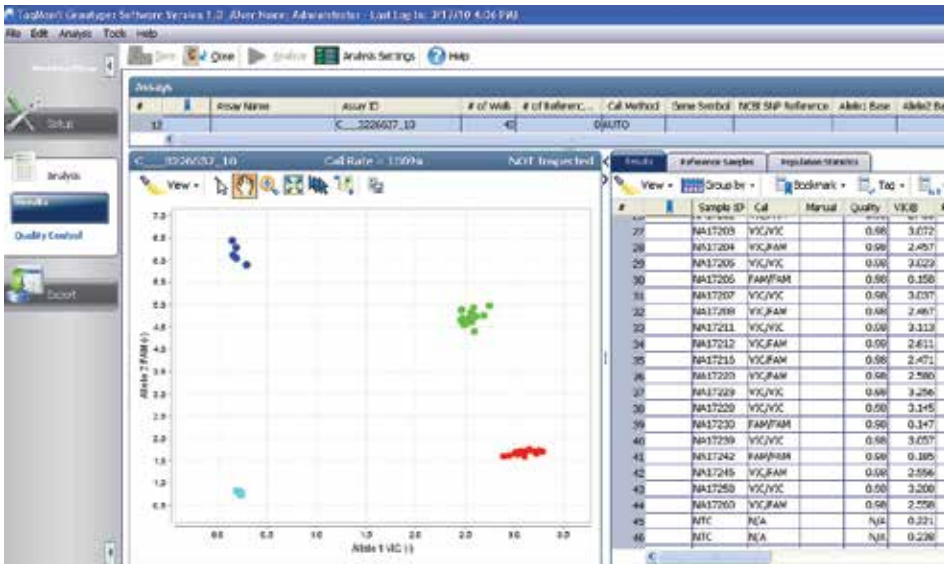

Figure 4. TaqMan Genotyper Software automatically determines sample genotypes and displays data.

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**

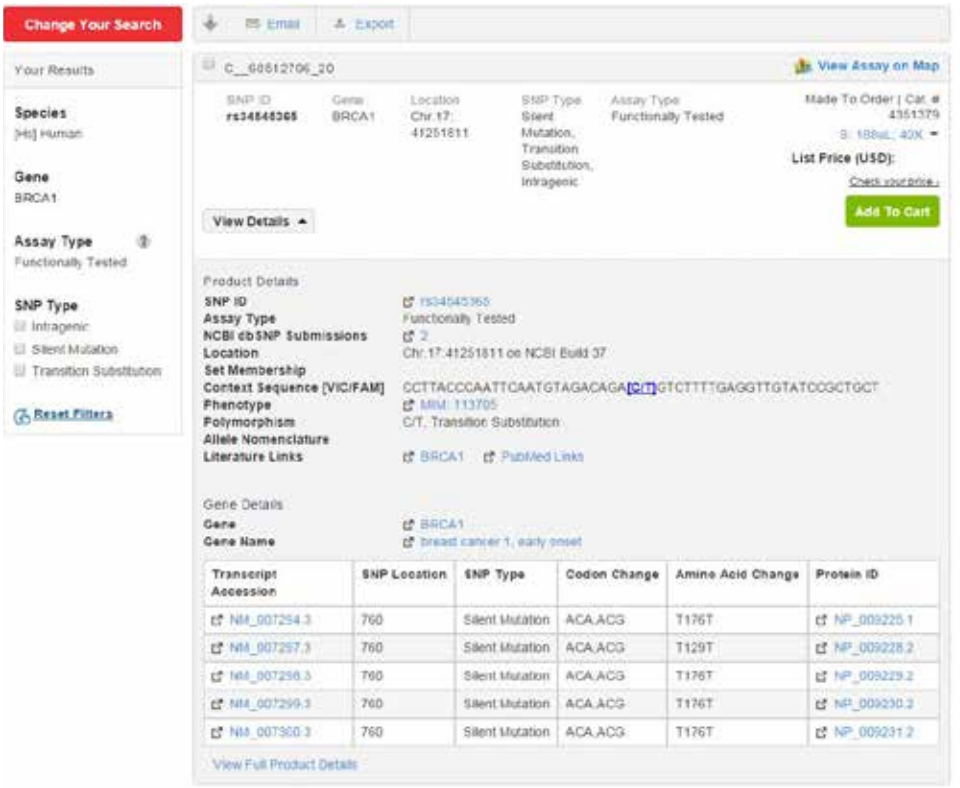

Figure 5. Our TaqMan Assay search and order tool makes online ordering easy. For convenient online ordering and multiple search options for all our genotyping assays, including keyword, batch, and location searches, visit **thermofisher.com/taqmansnp**

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.

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

ThermoFisher
SCIENTIFIC

SAFETY DATA SHEET

(In accordance with COMMISSION REGULATION (EU) No 830/2015)

SECTION 1: Identification of the substance/mixture and of the company/undertaking

Product identifier

Product code	4349763
Product name	7500 Spectral Calibration Plate with SYBR® Dye
Chemical Name	Not Applicable
REACH registration number	No registration number is given yet for this substance / substances in this mixture since the annual import quantity is less than one tonnage per annum or the transition period for its registration according to Article 23 of REACH has not yet expired.

Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses	For research use only
Use Description Code	SU22 - Public domain (administration, education, entertainment, services, craftsmen), PROC15 - Use as a laboratory reagent, PC21 - Laboratory chemicals, SU24 - Scientific research and development
Uses advised against	Not for consumer use.

Details of the supplier of the safety data sheet

Manufacturer/Supplier
LIFE TECHNOLOGIES EUROPE BV
KWARTSWEG 2
2665 NN BLEISWIJK
NETHERLANDS
31-(0)180 392 400
Email: MSDS@lifetech.com

24 hour Emergency Response for Hazardous Materials [or Dangerous Goods] Incident. Spill, Leak, Fire, Exposure, or Accident. Call CHEMTREC	Within the USA + Canada: 1-800-424-9300 and +1 703-527-3887 Outside the USA + Canada: +1 703-741-5970

Country Specific Emergency Number (if available):

CHEMTREC Ireland (Dublin)	+(353)-19014670 (Greeting Language: English and Irish)
CHEMTREC UK (London)	+(44)-870-8200418 (Greeting Language: English)

SECTION 2: Hazards identification

Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [CLP]

Physical hazards
Not Hazardous

Health hazards
Not Hazardous

Environmental Hazards
Not Hazardous

Additional information
Not Applicable

Label elements

Labelling according to Regulation (EC) No 1272/2008 [CLP]

Hazard pictograms
No Pictogram

Signal word
None

Hazard Statements
Not Applicable

Precautionary Statements

Prevention
Not Applicable

Response
Not Applicable

Storage
Not Applicable

Disposal
Not Applicable

Other hazards
Not Applicable

SECTION 3: Composition/information on ingredients

Component	CAS-No.	EINECS-No.	Weight percent	REACH registration number	Classification according to Regulation (EC) No 1272/2008 [CLP]
Glycerin 56-81-5 (7-13)	56-81-5	200-289-5	7-13	01-2119471987-18-XXXX	Not Classified

SECTION 4: First aid measures

Description of first aid measures

Skin contact Rinse with plenty of water. Immediate medical attention is not required.

Eye contact Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

INGESTION Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

Inhalation Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary.

Notes to Physician Treat symptomatically.

Most important symptoms and effects, both acute and delayed
Not Applicable

Indication of any immediate medical attention and special treatment needed
None.

SECTION 5: Firefighting measures

Extinguishing media

Suitable Extinguishing Media Water spray. Carbon dioxide (CO2). Foam. Dry chemical.

Unsuitable Extinguishing Media No information available.

Special hazards arising from the substance or mixture
Not Known.

Advice for fire-fighters
Standard procedure for chemical fires.

SECTION 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures
Ensure adequate ventilation. Always wear recommended Personal Protective Equipment. Use personal protection equipment. See Section 8 for more detail.

Environmental precautions
No special environmental precautions required. Avoid discharge into drains and waterways whenever possible.

Methods and material for containment and cleaning up
Soak up with inert absorbent material.

Reference to other sections
See section 8 for more information.

SECTION 7: Handling and storage

Precautions for safe handling
Use personal protective equipment as required. No special handling advices are necessary.

Conditions for safe storage, including any incompatibilities
Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers.

Specific end use(s)
For research use only.

SECTION 8: Exposure controls/personal protection

Control parameters

Chemical Name	EU OEL (TWA)	EU OEL (STEL)	EU Skin Notation
Glycerin 56-81-5	None	None	None

Chemical Name	Austria	Belgium (TWA)	Denmark (TWA)	Finland OEL (TWA)
Glycerin 56-81-5	None	10 mg/m³	None	None

Chemical Name	France OEL (VME)	Germany OEL (TWA)	Ireland (TWA)	Italy OEL (TWA)
Glycerin 56-81-5	10 mg/m³	200 mg/m³ exposure factor 2	10 mg/m³	None

Chemical Name	Sweden - Occupational Exposure Limits - TLVs (LLVs)	Netherlands OEL (MAC)	Spain OEL (TWA)	United Kingdom
Glycerin 56-81-5	None	None	10 mg/m³	10 mg/m³ TWA (mist)

Chemical Name	European Union	France OEL (VME)	Germany OEL (TWA)
Glycerin 56-81-5	None	10 mg/m³	200 mg/m³ exposure factor 2

Chemical Name	Italy OEL (TWA)	Portugal	Netherlands OEL (MAC)	Finland OEL (TWA)
Glycerin 56-81-5	None	None	None	None

Chemical Name	Austria	Denmark	Poland	Switzerland
Glycerin 56-81-5	None	None	None	None

Chemical Name	Ireland	Norway	Lithuania OEL (TWA)	Spain OEL (TWA)
Glycerin 56-81-5	None	None	None	10 mg/m³

Engineering measures Ensure adequate ventilation, especially in confined areas.

Exposure controls

Personal protection equipment

Respiratory protection In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards.

Hand Protection Wear suitable gloves. Glove material: Compatible chemical-resistant gloves.

Eye protection Tight sealing safety goggles.

Skin and body protection Wear suitable protective clothing.

Hygiene measures Handle in accordance with good industrial hygiene and safety practice.

Environmental exposure controls
No special environmental precautions required.

SECTION 9: Physical and chemical properties

Information on basic physical and chemical properties

Appearance	Liquid	
Odour	no data available	
pH	Mixture has not been tested	
Melting point / melting range	**°C** >0	**°F** >32
Boiling point / boiling range	**°C** >100	**°F** >212
Flash point	**°C** >90	**°F** >160
Autoignition temperature	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Decomposition temperature	**°C** Mixture has not been tested	**°F** Mixture has not been tested
Evaporation rate	No data available	
Flammability (solid, gas)	Not applicable	
Upper explosion limit	Mixture has not been tested	
Lower explosion limit	Mixture has not been tested	
Vapour Pressure	Mixture has not been tested	
Relative density	Mixture has not been tested	
Specific gravity	No data available	
Solubility	Soluble in water	
Partition coefficient: n-octanol/water	No data available	
Explosive properties	Mixture has not been tested	

OTHER INFORMATION
No data available.

SECTION 10: Stability and reactivity

Reactivity	None known.
Chemical stability	Stable under normal conditions.
Possibility of hazardous reactions	Hazardous reaction has not been reported.
Conditions to Avoid	None under normal processing.
Incompatible Materials	Strong acids. oxidising agents. Acetic anhydride. Isocyanates. Ammonia. Bases.
Hazardous decomposition products	Carbon oxides.

SECTION 11: Toxicological information

Information on toxicological effects

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Glycerin	= 12600 mg/kg Oral	No data available	>570mg/m³(Rat)

Principal Routes of Exposure, Potential health effects

Irritation Conclusive but not sufficient for classification

Corrosivity Conclusive but not sufficient for classification

Sensitisation Conclusive but not sufficient for classification

STOT - Single Exposure Conclusive but not sufficient for classification

STOT - Repeated Exposure Conclusive but not sufficient for classification

Carcinogenicity Conclusive but not sufficient for classification

Mutagenicity Conclusive but not sufficient for classification

Reproductive Toxicity Conclusive but not sufficient for classification

Aspiration Hazard Conclusive but not sufficient for classification

SECTION 12: Ecological information

Toxicity
Contains no substances known to be hazardous to the environment or not degradable in waste water treatment plants.

Chemical Name	Freshwater Algae Data	Water Flea Data	Freshwater Fish Species Data	Microtox Data	log Pow
Glycerin	No data available	Daphnia magna EC50>500 mg/L (24 h)	No data available	No data available	logPow-1.76

Persistence and degradability Inherently biodegradable.

Bioaccumulative potential Material does not bioaccumulate.

Results of PBT and vPvB assessment
This mixture does not contain any substances that are assessed to be a PBT or a vPvB.

Other adverse effects No information available.

SECTION 13: Disposal considerations

Waste treatment methods
The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.

SECTION 14: Transport information

IATA / ADR / DOT-US / IMDG
Not classified as dangerous in the meaning of transport regulations.

UN Number	Not Applicable
UN proper shipping name	Not Applicable
Transport hazard class(es)	Not Applicable
Packing group	Not Applicable

Environmental Hazards
Not Applicable

Special precautions for user
Not Applicable

Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code
Not Applicable.

SECTION 15: Regulatory information

Safety, health and environmental regulations/legislation specific for the substance or mixture

Substances of Very High Concern
None.

Restricted substances under EC 1907/2006, Annex XVII
None.

Substances listed under Annex I of Regulation (EC) No 689/2008
None.

Restricted substances under Annex V of Regulation (EC) No 689/2008
None.

Substances under Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC
None.

German Water hazard classes (Wassergefährdungsklassen)

Chemical Name	Weight percent	Germany - Water Classification (VwVwS) - Annex 1	Germany - Water Classification (VwVwS) - Annex 2 - Water Hazard Classes	Germany - Water Classification (VwVwS) - Annex 3
Glycerin	7-13		hazard class 1 - low hazard to waters	

Other International Inventories

Chemical Name	EINECS (European Union)	ELINCS (European List of Notified Chemical Substances)	ENCS (Japan)	PICCS (Philippines)
Glycerin	Listed	-	Listed	Listed

Chemical Name	AICS (Australia)	South Korea (KECL)	Canada (DSL)	NDSL
Glycerin	Listed	Listed	Listed	-

Chemical Safety Assessment
No Chemical safety assessment has been carried out.

SECTION 16: Other information

Reason for revision Update according to Commission Regulation (EU) No 830/2015
Revision number 5
Revision date 11-Dec-2017

References
• ECHA: http://echa.europa.eu/
• TOXNET: http://toxnet.nlm.nih.gov/
• eChemPortal: http://www.echemportal.org/
• LOLI database: https://www.chemadvisor.com/loli-database

Classification and procedure used to derive the classification for mixtures according to Regulation (EC) 1272/2008 [CLP]:
Not classified

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED,INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE"

7500 Real-Time PCR Systems Spectral Calibration Kit I

Catalog Number 4349180

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

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

Contents and storage

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

Related Documentation

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

Limited product warranty

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

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

1 December 2015

ThermoFisher SCIENTIFIC

Certificate of Analysis

ThermoFisher SCIENTIFIC

BigDye® XTerminator™ Kit 100 Reactions

Product No.	**4376486**
Lot No.	**2010180**
Date of Manufacture	**24NOV2020**
Expiration Date	**20MAY2021**

TEST	SPECIFICATION	RESULT
4376495, SAM™ Solution (9mL)		
PRODUCT APPEARANCE		
*Note: Solution may precipitate upon storage. See product protocol for guidance if precipitation is observed.		
Appearance	Colorless, Particulate Free Solution*	Pass
pH TEST		
pH Range	5.5 – 7.5	7.087
CONDUCTIVITY TEST		
Conductivity	<50µS/cm	8.507 µS/cm
4376492, XTerminator™ Solution (2mL)		
PRODUCT APPEARANCE		
Appearance	Light Brown, Opaque Suspension	PASS
USE TEST		
A DNA sequencing reaction prepared with BigDye® Terminator v.3.1 chemistry and 100ng of pGEM template was purified with XTerminator™ Solution/SAM™ Solution. The purified reaction was analyzed using an AB 3130xl Genetic Analyzer with a 50 cm capillary and POP-7™ polymer.		
% of capillaries exhibiting zero blobs between 60 and 100 bp	>=85%	PASS

SAM™ Solution Lot No.: 2010091
XTerminator™ Solution Lot No.: 2010112

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

Manufactured in compliance with our ISO 13485 certified quality management system.

Bedford Site: 2 Preston Court Bedford, MA, USA

Quality Assurance
Issued 30NOV2020

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

BigDye® XTerminator™ Purification Kit

- Single-well plate—No liquid transfer
- Complete dye blob removal
- Stabilized samples
- Improves any sequencing workflow
- Manual or automated protocols
- Rapid, reliable, and reproducible

The BigDye® XTerminator™ Purification Kit is a fast, simple purification method for DNA sequencing reactions that improves the sequencing workflow (Figure 1) and removes unincorporated BigDye terminators. No more dye blobs! Cleanup is complete in under 40 minutes and requires less than 10 minutes of labor.

More effective than ethanol precipitation (no disappearing pellets!), and cheaper than column purification and other commercial kits, the BigDye XTerminator Purification Kit removes dye blobs by capturing unincorporated dye terminators, salts and other charged molecules that may interfere with base calling and electrokinetic sample injection (Figures 2–4). The kit is compatible with all terminator sequencing chemistries, reaction volumes, and template types.

Simple Purification Process

Traditional purification methods, such as ethanol precipitation, require the addition of multiple reagents along with decanting and centrifuging steps.

The BigDye XTerminator Purification Kit requires the addition of only two reagents, which can be added sequentially or premixed:

- XTerminator™ Solution—Scavenges unincorporated dye terminators and free salts from the post-sequencing reaction
- SAM™ Solution—Enhances the performance of the XTerminator™ Solution and stabilizes the post-purification reactions

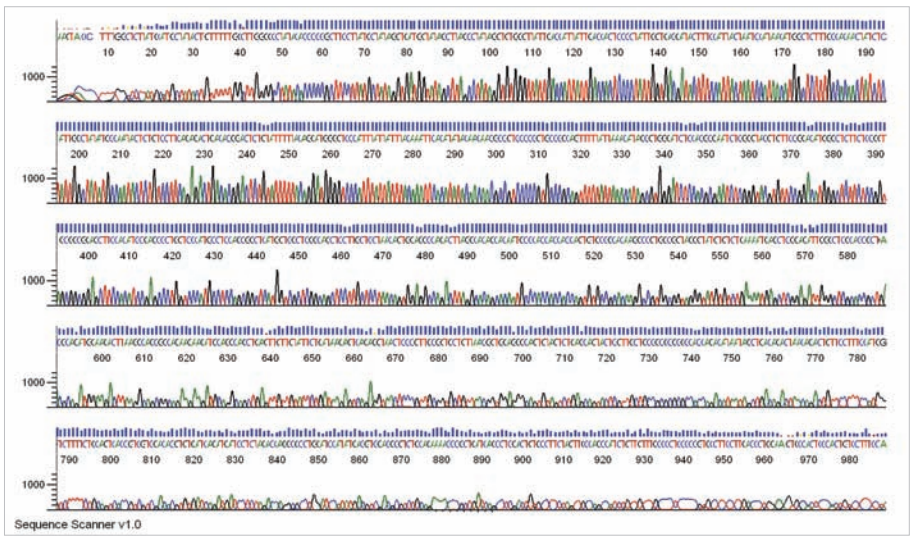


Figure 2. High Quality, Long Read Sequence Data Using the BigDye XTerminator Purification Kit. High quality dye blob-free data can be produced using BigDye XTerminator Purification Kit without sacrifice to sequence length of read. Q20 base read = 1044 bp. Data kindly provided by the DNA Technology Unit, Plant Biotechnology Institute (NRC-PBI), Saskatoon, Canada.

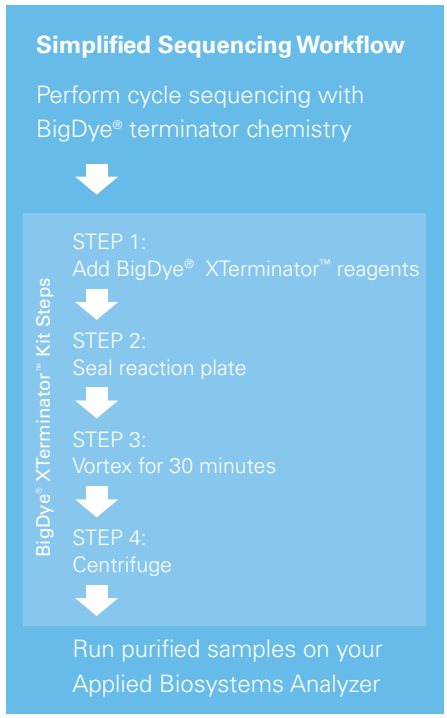


Figure 1. Simplified and faster sequencing workflow using the BigDye® XTerminator™ Purification Kit.

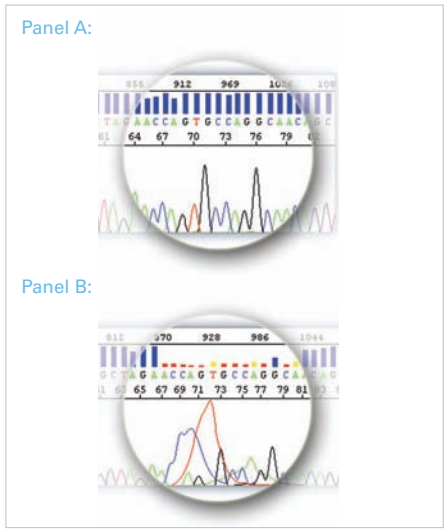


Figure 3. Purification using BigDye XTerminator Kit versus Ethanol Precipitation (A) BigDye XTerminator Purification Kit yields dye blob-free data. (B) Ethanol purification generally yields sequence data with dye blobs.

Simplified Sequencing Workflow

STEP 1. Perform cycle sequencing with BigDye® terminators.

STEP 2. After cycle sequencing, centrifuge the reaction plate briefly, then pipette the SAM™ Solution into each well, per the table below.

Note: XTerminator™ and SAM™ solutions may be premixed and added in a single step. For details, please see Big Dye® XTerminator™ Purification Kit Protocol (P/N 4374408).

Sequencing Reaction Volume	SAM Solution (µL/well)
10 µL/well (96-well plate)	45
20 µL/well (96-well plate)	90

STEP 3. Pipette the correct volume of XTerminator Solution into each well.

a. Vortex the XTerminator Solution bulk container briefly.

b. Using a wide-bore pipette tip, aspirate the solution per the table below and add to each well.

Sequencing Reaction Volume	BigDye XTerminator Solution (µL/well)
10 µL/well (96-well plate)	10
20 µL/well (96-well plate)	20

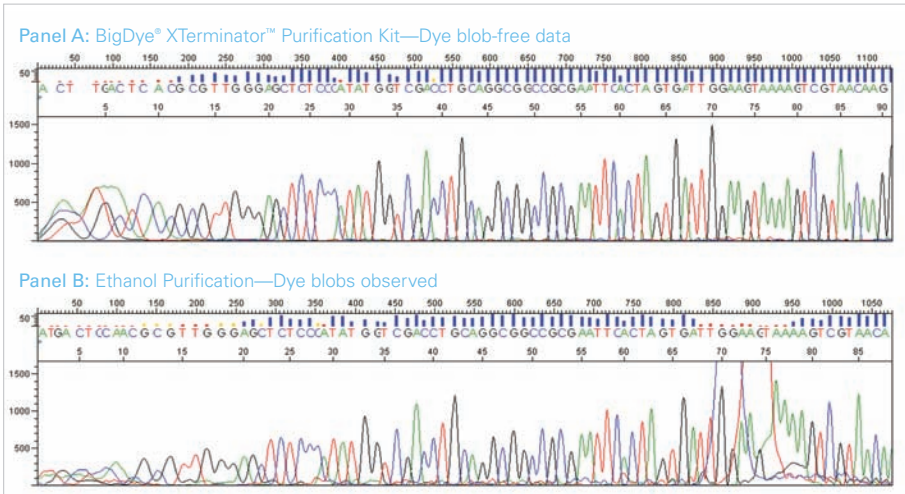


Figure 4. (A) BigDye XTerminator Purification yields high quality data and superior short fragment recovery and removes dye blobs from unincorporated dye terminators. (B) Traditional methods yield sequence data with dye blobs. Data kindly provided by the DNA Technology Unit, Plant Biotechnology Institute (NRC-PBI), Saskatoon, Canada.

c. Repeat steps "a" through "b" to pipette the complete volume of XTerminator Solution into each well.

STEP 4. Seal the plate using a heat seal or Clear Adhesive Film (P/N 4306311). Vortex for 30 minutes, then centrifuge the reaction plate briefly.

STEP 5. Place the reaction plate in the Applied Biosystems DNA analyzer. Select run module and run plate.

Note: This protocol can be automated on a Biomek® FX Laboratory Automation Workstation (Beckman Coulter). For details please see Big Dye XTerminator Purification Kit Protocol (P/N 4374408).

Software

Applied Biosystems provides downloadable run modules for use with the Big Dye XTerminator Purification Kit and Data Collection Software. These modules are designed for the 3100, 3100–*Avant*, 3130/3130*xl*, and 3730/3730*xl* Analyzers.

The software modules for the BigDye XTerminator Purification Kit are compatible with the Windows® NT, Windows® 2000, and Windows® XP operating systems and are available for download at **www2.appliedbiosystems.com/support/software/.**

Product Description	Package	Part Number
BigDye XTerminator Purification Kit	2 mL (~100 x 20 µL purifications)	4376486
	20 mL (~1,000 x 20 µL purifications)	4376487
	50 mL (~2,500 x 20 µL purifications)	4376484

Printed in the USA, 12/2006 Publication 107PR04-01

Applied Biosystems

Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.345.5224
www.appliedbiosystems.com

International Sales
For our office locations please call the division headquarters or refer to our Web site at
www.appliedbiosystems.com/about/offices.cfm

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.

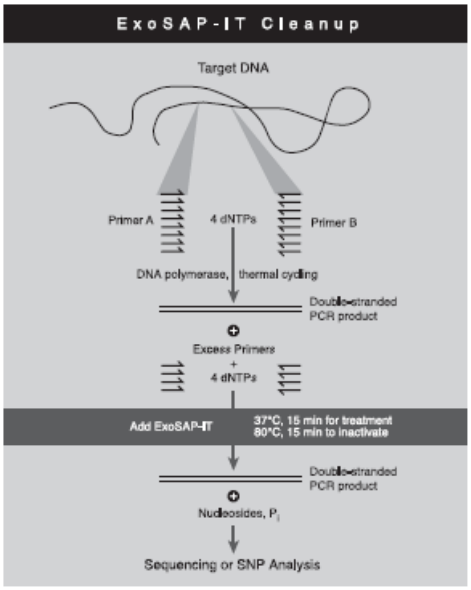
PCR cleanup protocol

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

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CERTIFICATE OF ANALYSIS

78201.1.ML ExoSAP-IT™
Packaging Lot: 01013900
Expiry Date: 30.11.2022 (DD.MM.YYYY)
Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
01010236	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

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

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CERTIFICATE OF ANALYSIS

K0702 GeneJET PCR Purification Kit

Packaging Lot: **01017618**
Expiry Date: 31.12.2023 (DD.MM.YYYY)
Storage: at 5±3°C

Filling lots for components in package:

Lot	Quantity	Description
00996508	60 mL	Binding Buffer
00990020	45 mL	Wash Buffer (concentrated)
01015366	30 mL	Elution Buffer
01013510		PCR Purification Columns

QUALITY CONTROL

Parameter	Method	Requirement	Result
Functional testing	The kit was tested in the purification of PCR products. The quality of the purified DNA was evaluated spectrophotometrically, by agarose gel electrophoresis, digestion with FastDigest restriction enzymes and automated fluorescent sequencing.	Conforms	Conforms

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

Quality authorized by QC: J. Žilinskienė

Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific GeneJET PCR Purification Kit

#K0701, #K0702

www.thermoscientific.com/onebio

#K0701
Lot **Expiry Date**
50 preps

CERTIFICATE OF ANALYSIS

The kit was tested in the purification of PCR products according to the protocol described in the manual. The quality of the purified DNA was evaluated spectrophotometrically, by agarose gel electrophoresis, digestion with Thermo Scientific FastDigest restriction enzymes and automated fluorescent sequencing.

Quality authorized by:  Jurgita Zilinskiene

CONTENTS page

COMPONENTS OF THE KIT

GeneJET PCR Purification Kit	50 preps #K0701	250 preps #K0702
Binding Buffer	12 mL	60 mL
Wash Buffer (concentrated)	9 mL	45 mL
Elution Buffer (10 mM Tris-HCl, pH 8.5)	15 mL	30 mL
GeneJET Purification Columns (preassembled with collection tubes)	50	250

STORAGE AND STABILITY

Thermo Scientific GeneJET PCR Purification Kit should be stored at room temperature (15-25 °C). For columns we recommend 4 °C storage for periods greater than 1 year. Any precipitate that forms in the buffers during storage can be redissolved by incubating briefly at 37 °C, then cooling to room temperature before use.

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

DESCRIPTION

The GeneJET™ PCR Purification Kit is designed for rapid and efficient purification of DNA from PCR and other enzymatic reaction mixtures.

The kit utilizes a proprietary silica-based membrane technology in the form of a convenient spin column, eliminating the need for tedious resin manipulations or toxic phenol-chloroform extractions.

The GeneJET PCR Purification Kit effectively removes primers, dNTPs, unincorporated labeled nucleotides, enzymes and salts from PCR and other reaction mixtures. The kit can be used for purification of DNA fragments from 25 bp to 20 kb. The recovery rates are 90-100% in a 100 bp – 10 kb DNA fragment size range (*see* Fig. 1). Each GeneJET purification column has a total binding capacity of up to 25 µg of DNA and the entire procedure takes just 5 min. The purified DNA can be used in common downstream applications such as sequencing, restriction digestion, labeling, ligation, cloning, *in vitro* transcription, blotting or *in situ* hybridization.

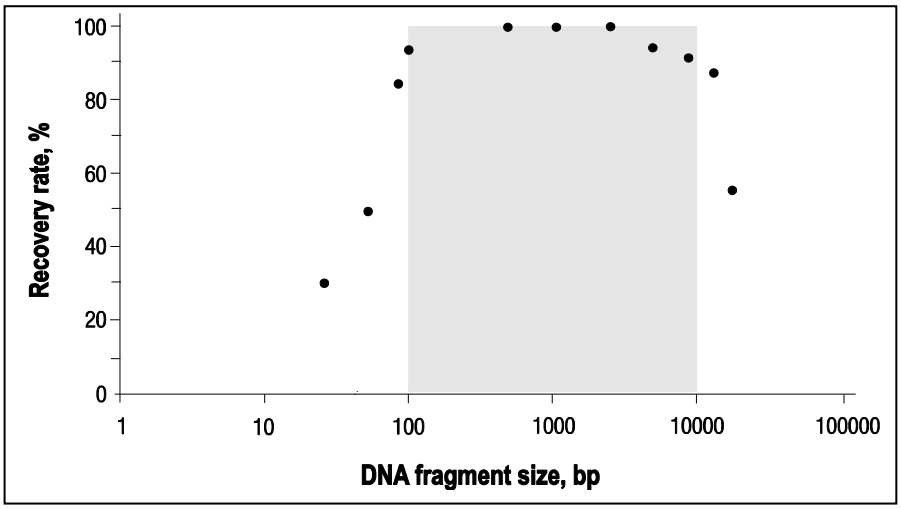

Fig. 1. Recovery dependence on DNA fragment size

PRINCIPLE

A reaction mixture containing DNA is combined with the binding buffer and added to a purification column. A chaotropic agent in the binding buffer denatures proteins and promotes DNA binding to the silica membrane in the column. As an added convenience, the binding buffer contains a color indicator that allows for easy monitoring of the solution pH for optimal DNA binding. Impurities are removed with a simple wash step. Purified DNA is then eluted from the column with the elution buffer. The recovered DNA is ready for use in downstream applications.

IMPORTANT NOTES

- Prior to the initial use of the kit, dilute the **Wash Buffer** (concentrated) with ethanol (96-100%):

	50 preps #K0701	**250 preps** #K0702
Wash Buffer (concentrated)	9 mL	45 mL
Ethanol	45 mL	225 mL
Total Volume	54 mL	270 mL

  After the ethanol has been added, mark the check box on the bottle to indicate the completed step.
- Examine the **Binding Buffer** for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37 °C and cooling to 25 °C.
- Wear gloves when handling the **Binding Buffer** as this solution contains irritants (*see* p.7 for SAFETY INFORMATION).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Ethanol 96-100%.
- Isopropanol.
- 3 M sodium acetate, pH 5.2 (may be necessary).
- Microcentrifuge.
- 1.5 or 2 mL microcentrifuge tubes.
- Heating block or water bath (may be necessary).

PURIFICATION PROTOCOLS

Note

- Read IMPORTANT NOTES on p. 3 before starting.
- All purification steps should be carried out at **room temperature**.
- All centrifugations should be carried out in a table-top microcentrifuge at **>12000 × g** (10 000-14 000 rpm, depending on the rotor type).

Protocol A. DNA purification using centrifuge

Step	Procedure
1	Add a **1:1 volume** of **Binding Buffer** to completed PCR mixture (e.g. for every 100 µL of reaction mixture, add 100 µL of Binding Buffer). Mix thoroughly. Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
2 for DNA ≤500 bp	*Optional:* if the DNA fragment is ≤500 bp, add a 1:2 volume of 100% isopropanol (e.g., 100 µL of isopropanol should be added to 100 µL of PCR mixture combined with 100 µL of Binding Buffer). Mix thoroughly. **Note.** If PCR mixture contains primer-dimers, purification without isopropanol is recommended. However, the yield of the target DNA fragment will be lower.
3	Transfer up to 800 µL of the solution from step 1 (or optional step 2) to the GeneJET purification column. Centrifuge for 30-60 s. Discard the flow-through. **Notes.** If the total volume exceeds 800 µL, the solution can be added to the column in stages. After the addition of 800 µL of solution, centrifuge the column for 30-60 s and discard flow-through. Repeat until the entire solution has been added to the column membrane. **Close the bag with GeneJET Purification Columns tightly after each use!**
4	Add **700 µL** of **Wash Buffer** (diluted with the ethanol as described on p. 3) to the GeneJET purification column. Centrifuge for 30-60 s. Discard the flow-through and place the purification column back into the collection tube.
5	Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove any residual wash buffer. **Note.** This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.
6	Transfer the GeneJET purification column to a clean 1.5 mL microcentrifuge tube (not included). Add **50 µL** of **Elution Buffer** to the center of the GeneJET purification column membrane and centrifuge for 1 min. **Note** • For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µL does not significantly reduce the DNA yield. However, elution volumes less than 10 µL are not recommended. • If DNA fragment is >10 kb, prewarm Elution Buffer to 65 °C before applying to column. • If the elution volume is 10 µL and DNA amount is ≥5 µg, incubate column for 1 min at room temperature before centrifugation.
7	Discard the GeneJET purification column and store the purified DNA at -20 °C.

Protocol B. DNA purification using vacuum manifolds

Step	Procedure
1	Perform DNA binding stage according to steps 1 - 2 in Protocol A on page 4.
2	Prepare the vacuum manifold according to the supplier's instructions. Place the GeneJET purification column(s) onto the manifold. **Close the bag with GeneJET Purification Columns tightly after each use!**
3	Transfer up to 800 µL of the solution (from step 1 or 2 as in protocol A) to the GeneJET purification column. **Note.** If the total volume exceeds 800 µL, the solution can be added to the column in stages. After each application, apply the vacuum and discard the flow-through. Repeat until the entire volume has been applied to the column membrane.
4	Apply the vacuum to draw the sample through the column. Switch off the vacuum after the entire sample has passed through the column.
5	Add **700 µL** of **Wash Buffer** (diluted with the ethanol as described on p. 3) to the GeneJET purification column.
6	Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column. Place the purification column back into the collection tube.
7	Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove any residual wash buffer. **Note.** This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.
8	Transfer the GeneJET purification column to a clean 1.5 mL microcentrifuge tube (not included). Add **50 µL** of **Elution Buffer** to the center of the GeneJET purification column membrane and centrifuge for 1 min. **Note.** • For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µL does not significantly reduce the DNA yield. However, elution volumes less than 10 µL are not recommended. • If DNA fragment is >10 kb, prewarm Elution Buffer to 65 °C before applying to column. • If the elution volume is 10 µL and DNA amount is ≥5 µg, incubate column for 1 min at room temperature before centrifugation.
9	Discard the GeneJET purification column and store the purified DNA at -20 °C.

TROUBLESHOOTING

Problem	Possible Cause and Solution
Low DNA yield	**Inefficient DNA binding** Verify that a 1:1 volume of Binding Buffer is added to the reaction mixture. Ensure the solutions are mixed well. Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the solution color is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow. **Inefficient membrane wash** Ensure that the recommended volume of ethanol has been added to the Wash Buffer (concentrated) prior first use (*see* p. 3). **Inefficient DNA elution** Add Elution Buffer directly to the center of the membrane and not to the side of the GeneJET purification column. Use 20-50 µL of Elution Buffer and ensure that the volume completely covers the surface of the membrane. Increase the Elution Buffer volume twice or perform two elution cycles when purifying larger amounts of DNA. (e.g., >15 µg). In step 5 of Protocol A (step 7 of Protocol B), ensure all residual wash buffer is removed from the membrane. Longer centrifugation time (extra minute) can aid in removal of wash buffer. **PCR reaction mixture does not contain DNA** Check for the presence and yield of the PCR product by running an aliquot of the reaction on an agarose gel.
Downstream reactions are unsuccessful	**Presence of residual ethanol** In step 5 of Protocol A (step 7 of Protocol B), ensure all residual wash buffer is removed from the membrane. Longer centrifugation time can aid in removal of wash buffer. **Inefficient membrane wash** Ensure that the collection tube is not overfilled during the wash step and that any of the wash buffer has remained in the bottom of the GeneJET purification column. Always discard the flow-through after centrifugation. **Eluate contains excess salt** Ensure that the wash step 4 of Protocol A is effective. Incubate the GeneJET purification column with the Wash Buffer for several minutes before proceeding to centrifugation.
DNA does not remain in an agarose gel well	In step 5 of Protocol A (step 7 of Protocol B), ensure all residual wash buffer is removed from the membrane. Longer centrifugation time can aid in removal of wash buffer.

SAFETY INFORMATION

Binding Buffer
Hazard-determining component of labeling: **guanidinium thiocyanate**

Xn Harmful

Risk phrases

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
R32 Contact with acids liberates very toxic gas.
R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases

S9 Keep container in a well-ventilated place.
S23 Do not breathe gas/fumes/vapour/spray.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste.
S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

Binding Buffer

Warning

Hazard statements:

H302 Harmful if swallowed.
H412 Harmful to aquatic life with long lasting effects.

Precautionary statements:

P273 Avoid release to the environment.
P264 Wash thoroughly after handling.
P270 Do no eat, drink or smoke when using this product.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330 Rinse mouth.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

1. Vogelstein, B. and Gillespie, D., Preparative and analytical purification of DNA from agarose, Proc. Natl. Acad. Sci. USA, 76, 615-619, 1979.
2. Marko, M.A., Chipperfield, R. and Birnboim, H.C., A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder, Anal. Biochem., 121, 382-387, 1982.
3. Boom, R., Sol, C.J.A., et al., Rapid and simple method for purification of nucleic acids, J. Clin. Microbiol., Mar, 495-503, 1990.

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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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CERTIFICATE OF ANALYSIS

#A27828 **MagMAX™ mirVana™ Total RNA Isolation Kit Box 2 of 2**

Quantity: 96 react.
Packaging Lot: 00302599
Storage: at Room Temperature (15 to 25 °C)

Expiry Date: 05.2016 (MM.YYYY)

The kit is composed of:

Lysis Buffer
Quantity: 115 mL
Filling Lot: 00289359

Wash Solution 1 Concentrate
Quantity: 10 mL
Filling Lot: 00289361

Wash Solution 2 Concentrate
Quantity: 12 mL
Filling Lot: 00289362

Rebinding Buffer
Quantity: 4.8 mL
Filling Lot: 00278705

PK Digestion Buffer
Quantity: 4.4 mL
Filling Lot: 00289363

Elution Buffer
Quantity: 9.6 mL
Filling Lot: 00289364

RNA Binding Beads
Quantity: 2 mL
Filling Lot: 00299967

MagMAX™ TURBO DNase™ Buffer
Quantity: 4.6 mL
Filling Lot: 00289365

Thermo Fisher Scientific Baltics UAB V.A. Graiciuno 8 Vilnius LT-02241 Lithuania tel. +370 700 55131 fax +370 700 55132 www.thermoscientific.com/onebio info.baltics@thermofisher.com

Company code 122351387, VAT code LT223513811
Company data are stored at the Register of Legal Entities of the Republic of Lithuania

QUALITY CONTROL

Parameter	Method	Requirement	Result
Functional Testing	RNA is isolated from mouse tissue and mouse plasma following the applicable manual extraction procedure described in the MagMAX™ mirVana™ Total RNA Isolation Kit User Guide (publication# MAN0011131). RNA is checked for minimal yield, purity and integrity requirements and suitability for real-time RT-PCR.	Conforms	Conforms

ISO CERTIFICATION

This product is produced in accordance to Thermo Fisher Scientific Baltics UAB, a wholly owned subsidiary of Thermo Fisher Scientific Inc. (hereinafter Thermo Fisher) certified Quality Management (ISO 9001) and Environmental Management (ISO 14001) systems. Thermo Fisher Scientific Baltics UAB is also certified according to the standards ISO 13485 (Quality management system for IVD medical devices).

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Rev. 1

QC responsible: J. Žilinskienė

Date: 24 July 2015

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