Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 9001:2015

This is to certify that:

Thermo Fisher Scientific Baltics UAB
V. A.Graiciuno 8
Vilnius
LT-02241
Lithuania

Holds Certificate No: **FM 642793**

and operates a Quality Management System which complies with the requirements of ISO 9001:2015 for the following scope:

Design, development, manufacturing and sales of life science research products, including proteins, nucleic acids, nucleotides, antibodies, bio-sample preparation, cell separation reagents and associated kits, liquid chromatography (LC) silica, columns and supply of accessories for research or further manufacturing of therapeutics or in vitro diagnostics.

For and on behalf of BSI:

Andrew Launn, EMEA Systems Certification Director

Original Registration Date: 2016-03-25
Latest Revision Date: 2021-11-17

Effective Date: 2021-05-23
Expiry Date: 2024-05-22

Digitally Signed by Ceaicovschi Tudor
Date: 2022.08.05 13:17:23 EEST
Reason: MoldSign Signature
Location: Moldova

making excellence a habit.™

Certificate No: **FM 642793**

Location	Registered Activities
Thermo Fisher Scientific Baltics V. A.Graiciuno 8 Vilnius LT-02241 Lithuania	Design, development, manufacturing and sales of life science research products, including proteins, nucleic acids, nucleotides, antibodies, bio-sample preparation, cell separation reagents and associated kits, liquid chromatography (LC) silica, columns and supply of accessories for research or further manufacturing of therapeutics or in vitro diagnostics.
Thermo Fisher Scientific Baltics Molėtų pl. 5 Vilnius LT-08409 Lithuania	Manufacturing of nucleotides for research, in vitro diagnostics and further manufacturing.

Original Registration Date: 2016-03-25
Latest Revision Date: 2021-11-17

Effective Date: 2021-05-23
Expiry Date: 2024-05-22

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2016 & EN ISO 13485:2016

This is to certify that:

Thermo Fisher Scientific Baltics
V.A.Graiciuno 8
Vilnius
LT-02241
Lithuania

Holds Certificate Number: **MD 642790**

and operates a Quality Management System which complies with the requirements of ISO 13485:2016 & EN ISO 13485:2016 for the following scope:

Design, development, and manufacturing of reagents, proteins, nucleic acids, nucleotides, antibodies, associated kits, and materials intended for ex-vivo separation of human cells for in vitro diagnostics, for further manufacturing and applied market applications, including processes under aseptic condition.

For and on behalf of BSI:

Gary E Slack, Senior Vice President - Medical Devices

Original Registration Date: 2016-02-15
Latest Revision Date: 2021-11-17

Effective Date: 2021-05-23
Expiry Date: 2024-05-22

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Certificate No: **MD 642790**

Location	Registered Activities
Thermo Fisher Scientific Baltics V. A.Graiciuno 8 Vilnius LT-02241 Lithuania	Design, development, and manufacturing of reagents, proteins, nucleic acids, nucleotides, antibodies, associated kits, and materials intended for ex-vivo separation of human cells for in vitro diagnostics, for further manufacturing and applied market applications, including processes under aseptic condition.
Thermo Fisher Scientific Baltics Molėtų pl. 5 Vilnius LT-08409 Lithuania	Manufacturing of nucleotides for in vitro diagnostics and further manufacturing.

Original Registration Date: 2016-02-15
Latest Revision Date: 2021-11-17

Effective Date: 2021-05-23
Expiry Date: 2024-05-22

CERTIFICATE OF REGISTRATION

This is to certify that:

THERMO FISHER SCIENTIFIC BALTICS UAB

V. A. Graiciuno 8 | LT-02241 Vilnius - Lithuania

is in compliance with the standard

UNI EN ISO 14001:2015

and assessed according to Accredia Technical Regulations RT-09

EA Code: **12 | 19**

concerning the following kinds of products – processes – services:

Research, design, development and manufacturing including fermentation, purification, bio formulation, organic synthesis, dispensing, assembly, shipping of life science research products, including proteins, nucleic acids, nucleotides, master mixes, antibodies, bio-sample preparation and cell separation reagents and associated kits, for research and in-vitro diagnostics including processes under aseptic conditions. Production of liquid chromatography (LC) columns and supply of accessories through the phases of material reception, mixing of chemical products with absorbent elements; placing the elements in the columns, mounting of accessories, pressing, quality testing, cleaning, packing and shipping.

Originally certified by other accredited CB issued on 01/07/2012

Certificate No. **CERT-0063257**
Issue Date: **04/05/2021**

Original Certification Date: **01/07/2012**
Current Certification Date: **04/05/2021**
Certificate Expiry Date: **23/05/2024**

Certified System
SAI GLOBAL
ISO 14001
Environmental

ACCREDIA
L'ENTE ITALIANO DI ACCREDITAMENTO
SGA 054D
Membro degli Accordi di Mutuo Riconoscimento EA, IAF e ILAC
Signatory of EA, IAF and ILAC Mutual Recognition Agreements

MEMBER OF MULTILATERAL RECOGNITION ARRANGEMENT
IAF

Frank Camasta
Global Head of Technical Services
SAI Global Assurance

SAI GLOBAL

thermo scientific

PRODUCT INFORMATION

Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit #K0781, #K0782

Pub. No. MAN0012667
Rev. Date 12 October 2016 (Rev. B.00)

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

www.thermofisher.com

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

#_
Lot _
Exp. _

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit is qualified by isolating genomic DNA from 200 µL of blood following the protocols outlined in the manual. The purified genomic DNA has an $A_{260/280}$ ratio between 1.7 and 1.9. A single band of more than 30 kb is observed after agarose gel electrophoresis and ethidium bromide staining. The functional quality of purified genomic DNA is evaluated by PCR amplification of a single-copy gene and by digestion with restriction enzymes.

Quality authorized by: Jurgita Žilinskienė

CONTENTS page

COMPONENTS OF THE KIT

GeneJET Whole Blood Genomic DNA Purification Mini Kit	#K0781 50 preps	#K0782 250 preps
Proteinase K Solution	1.2 mL	5 × 1.2 mL
Lysis Solution	24 mL	120 mL
Wash Buffer WB I (concentrated)	10 mL	40 mL
Wash Buffer II (concentrated)	10 mL	40 mL
Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	30 mL	150 mL
GeneJET Genomic DNA Purification Columns pre-assembled with Collection Tubes	50	250
Collection Tubes (2 mL)	50	250

STORAGE

Proteinase K solution is stable at room temperature as long as the vial remains sealed. After the vial is opened, proteinase K should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

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

DESCRIPTION

The GeneJET™ Whole Blood Genomic DNA Purification Mini Kit is designed for rapid and efficient purification of high quality genomic DNA from whole blood and related body fluids. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard procedure takes less than 20 minutes following cell lysis and yields purified DNA greater than 30 kb in size. Isolated DNA can be used directly in PCR, qPCR, Southern blotting and enzymatic reactions. *See* Table 1 for typical genomic DNA yields from various sources.

PRINCIPLE

Samples are digested with Proteinase K in the supplied Lysis Solution. The lysate is then mixed with ethanol and loaded onto the purification column, where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared Wash Buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

Table 1. Typical genomic DNA yields from various sources.

Source	Amount	Yield, µg
Human blood	200 µL	2-10
Avian blood (chicken)	5 µL	20
Mouse blood	200 µL	2-4
Rat blood	200 µL	2
Rabbit blood	200 µL	4-7
Bone marrow	200 µL	10-65
Buffy coat	200 µL	4-13
Dried blood	100 µL	0.05-0.28
Buccal Swabs	-	0.05-0.12

IMPORTANT NOTES

- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples and perform extractions from fresh material or material that has been immediately frozen and stored at -20 °C or -70 °C.
- Add the indicated volume of ethanol (96-100%) to **Wash Buffer WB I** (concentrated) and **Wash Buffer II** (concentrated) prior to first use:

	#K0781 50 preps		**#K0782** 250 preps	
	Wash Buffer WB I	**Wash Buffer II**	**Wash Buffer WB I**	**Wash Buffer II**
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96-100%)	**30 mL**	**30 mL**	**120 mL**	**120 mL**
Total volume:	40 mL	40 mL	160 mL	160 mL

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

- Check the **Lysis Solution** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37 °C, then cool back down to 25 °C before use.
- Wear gloves when handling the **Lysis Solution and Wash Buffer I** as these reagents contain irritants.
- Typically the purified genomic DNA has an $A_{260/280}$ ratio between 1.7 and 1.9, however, when DNA concentration is lower than 20 ng/µL, deviations from the expected ratio are occasionally observed.
- Adjust the sample volume to 200 µl with 1X PBS or TE buffer (not provided).
- Centrifugation speed in rpm's is given for 24-place microcentrifuges.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

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

Buffers

For sample volume adjustment:

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

PROTOCOLS

Protocols for genomic DNA purification from buccal swabs, buffy coat, dried blood spots, body fluids, and avian blood are described on p.5-7.

A. Whole Blood Genomic DNA Purification Main Protocol

Step	Procedure
1	Add 20 µL of Proteinase K Solution to 200 µL of whole blood, mix by vortexing. Add 400 µL of Lysis Solution, mix thoroughly by vortexing or pipetting to obtain a uniform suspension. **Note.** If using less than 200 µL of blood, adjust sample volume to 200 µL with 1X PBS or TE buffer (not provided). If using larger volumes, follow the protocol on page 5.
2	Incubate the sample at 56 °C for 10 minutes while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed.
3	Add 200 µL of ethanol (96-100%) and mix by pipetting.
4	Transfer the prepared mixture to the spin column. Centrifuge for 1 min at 6,000 × g (~8,000 rpm). Discard the collection tube containing the flow-through solution. Place the column into a new 2 mL collection tube (included). ***Important: do not exceed specified relative centrifugal force.*** **Note.** Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!
5	Add 500 µL of Wash Buffer WB I (with ethanol added). Centrifuge for 1 min at 8,000 × g (~10,000 rpm). Discard the flow-through and place the column back into the collection tube.
6	Add 500 µL of Wash Buffer II (with ethanol added) to the column. Centrifuge for 3 min at maximum speed (≥20,000 × g, ≥14,000 rpm). *<u>Recommended:</u> Empty the collection tube. Place the purification column back into the tube and re-spin the column for 1 min. at maximum speed (≥20,000 × g, ≥14,000 rpm).* Discard the collection tube containing the flow-through solution and transfer the column to a sterile 1.5 mL microcentrifuge tube (not included).
7	Add 200 µL of Elution Buffer to the center of the column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8,000 × g (~10,000 rpm). **Note** • For maximum DNA yield, repeat the elution step with an additional 200 µl of Elution Buffer. • If more concentrated DNA is required or if DNA has been isolated from a small amount of starting material (e.g., 50 µL) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that lower volumes of Elution Buffer will result in lower final yield of eluted DNA.
8	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

B. DNA Purification from Large Volumes of Whole Blood

For purification of DNA from samples exceeding the standard 200 µL volume, it is necessary to burst red blood cells prior to performing the cell lysis step. Up to 500 µL of mammalian blood can be processed using following protocol:

Step	Procedure
1	Add 1 mL of ice cold nuclease free water to 500 µL of whole blood, mix thoroughly by vortexing or pipetting.
2	Incubate the sample for 5 min at room temperature.
3	Centrifuge for 5 min at 800 × g (~3,000 rpm).
4	Discard the supernatant.
5	Resuspend the pellet in 200 µL of 1 x PBS.
6	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

C. DNA Purification from Nucleated Blood

Nucleated avian or fish blood contains very large amounts of genomic DNA and therefore the volume of the staring material has to be scaled down. The DNA purification procedure follows the same protocol as mammalian blood, except that 2-10 µL of blood are used per purification.

Step	Procedure
1	Take 2-10 µL of nucleated blood.
2	Adjust the volume to 200 µL with 1 × PBS.
3	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

D. DNA Purification from Buccal Swabs

Step	Procedure
1	To collect a sample, scrape the swab 5-6 times against the inside cheek.
2	Swirl the swab for 30-60 s in 200 µL of 1 × PBS.
3	Go to step 1 of the standard Whole Blood Genomic DNA Purification Protocol (p.4).

E. DNA Purification from Bone Marrow

Step	Procedure
1	Harvest 25-200 µL of fresh or frozen bone marrow.
2	Adjust the volume to 200 µL with 1 × PBS.
3	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

F. DNA Purification from Dried Blood Spots

Step	Procedure
1	Cut out the section of filter containing the dried blood sample and place into a microcentrifuge tube.
2	Add 200 µL of 1 × PBS and incubate 5-10 min at room temperature.
3	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

G. DNA Purification from Buffy Coat

Buffy coat is a leukocyte-enriched fraction of whole blood and contains approximately 5-10 times more DNA than an equivalent volume of whole blood. Prepare the buffy coat by centrifuging whole blood at 2,500 × g for 10 min at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer containing plasma; the intermediate buffy coat layer containing concentrated leukocytes, and the bottom layer containing concentrated erythrocytes.

Step	Procedure
1	Centrifuge 1.5 mL of whole blood at 2,500 × g (~5,000 rpm) for 10 minutes at room temperature. Three layers should be visible.
2	Remove upper clear layer by aspiration.
3	Collect approximately 200 µL of intermediate layer using an automatic pipette. **Note.** If necessary, adjust the volume to 200 µL with 1× PBS.
4	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

H. DNA Purification from Urine

Step	Procedure
1	Add 0.5 mL of 0.5 M EDTA to 4.5 mL of urine (final concentration 50 mM).
2	Centrifuge 10 min at 800 × g (~3,000 rpm).
3	Discard the supernatant.
4	Resuspend the pellet in 200 µL of 1 × PBS.
5	Proceed to step 1 of the Whole Blood Genomic DNA Purification Main Protocol on p.4.

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more blood than indicated in lysis protocols. **Starting material was not completely digested.** Extend the Proteinase K digestion at 56 °C until complete lysis occurs and no particles remain visible in solution. **Sample was not thoroughly mixed with lysis buffer and Proteinase K.** The mixture has to be vortexed or pipetted immediately after adding lysis buffer. **Ethanol was not added to the lysate.** Ensure that ethanol was added to the lysate before applying the sample to the Purification Column. **Ethanol was not mixed with the lysate.** After the addition of ethanol to the lysate, mix the sample by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Ensure that ethanol was added to Wash Buffer WB I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation on p.3.
Purified DNA is degraded	**Sample was frozen and thawed repeatedly.** Avoid repeated sample freeze / thaw cycles. Use a fresh sample for DNA isolation. Perform extractions from fresh material when possible. **Inappropriate sample storage conditions.** Whole blood can be stored at 4 °C for no longer than 1-2 days. For long term storage, blood samples should be aliquoted in 200 µL aliquots and stored at -20 °C.
RNA contamination	**RNA-rich sample** With the GeneJET Genomic DNA Purification Mini Kit, the optimised buffers in combination with silica membrane technology allows for purification of essentially RNA-free gDNA without RNase treatment . However, when working with extremely transcriptionally active cell types, e.g. bone marrow, some RNA contamination might occur. If absolutely RNA-free DNA is necessary, add 20 µL of RNase A solution (10 mg/mL) to the sample prior to the addition of lysis buffer (step 1, p. 4).
Inhibition of downstream enzymatic reactions	**Purified DNA contains residual ethanol.** If residual solution is observed in the purification column after washing the column with Wash Buffer II, empty the collection tube and re-spin the column for an additional 1 min. at maximum speed (≥20,000 × g, ≥14,000 rpm). **Purified DNA contains residual salt.** Use the correct order for the Wash Buffers. Always wash the purification column with Wash Buffer WB I first and then proceed with Wash Buffer II.

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

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PRODUCT USE LIMITATION

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

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

Thermo 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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The world leader in serving science

CERTIFICATE OF ANALYSIS

K0781 GeneJET TM Whole Blood Genomic DNA Purification Mini Kit

Packaging Lot: **01296349**
Expiry Date: 31.07.2025 (DD.MM.YYYY)
Storage: at 5±3°C

Filling lots for components in package:

Lot	Quantity	Description
01280755	1.2 mL	Proteinase K Solution, 20mg/ml
01285533	30 mL	Elution Buffer
01294186	10 mL	Wash Buffer WB I(concentrated)
01285493	24 mL	Lysis Solution
01288544	10 mL	Wash Buffer II (concentrated)
01294340		GeneJET DNA Purification Columns & collection Tubes
01294481		Collection tubes 2 ml

QUALITY CONTROL

Parameter	Method	Requirement	Result
Functional testing	Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit is qualified by isolating genomic DNA from 200 µL of blood following the protocols outlined in the manual. The purified genomic DNA has an A260/280 ratio between 1.7 and 1.9. A single band of more than 30 kb is observed after agarose gel electrophoresis and ethidium bromide staining. The functional quality of purified genomic DNA is evaluated by PCR amplification of a single-copy gene and by digestion with restriction enzymes.	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

DreamTaq Hot Start DNA Polymerase

The hot-start polymerase for everyday research

New Thermo Scientific™ DreamTaq™ Hot Start DNA Polymerase offers a great balance between performance and value. Designed for consistently robust and reliable amplification, DreamTaq Hot Start DNA Polymerase can help you more easily get the results you're looking for, with virtually any template, application, or target.

Why use hot-start PCR?

- Prevents amplification of nonspecific products
- Amplifies low-abundance targets
- Provides convenient room-temperature setup

Why use DreamTaq Hot Start DNA Polymerase?

DreamTaq Hot Start DNA Polymerase is the hot-start version of our enhanced Thermo Scientific™ DreamTaq™ DNA Polymerase. Like our standard DreamTaq DNA Polymerase, this hot-start polymerase offers higher yields and longer amplicons than conventional *Taq*-based products. In addition, due to the hot-start modification, DreamTaq Hot Start DNA Polymerase has been engineered to provide increased sensitivity and specificity.

Features:

- Minimized optimization of primer annealing temperatures
- Optimized DreamTaq™ buffer, which includes 20 mM $MgCl_2$
- Ability to use same cycling conditions as used with conventional *Taq* polymerase
- Wide range of amplicon lengths
- 2X master mix formats
- Direct loading options
- Compatibility with most PCR applications

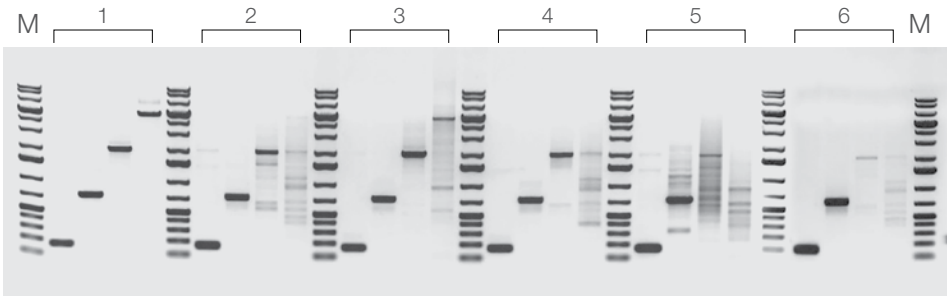


Figure 1. Robust amplification of human genomic DNA. DreamTaq Hot Start DNA Polymerase produces more product, cleaner bands, and longer amplicons than hot-start DNA polymerases from other suppliers. Amplification products (160 bp, 727 bp, 2 kb, or 5 kb) from human genomic DNA are shown in the figure above.
M: GeneRuler 1 kb Plus DNA Ladder. 1. DreamTaq Hot Start DNA Polymerase; 2. Promega GoTaq G2 Hot Start Polymerase; 3. NEB One*Taq* Hot Start DNA Polymerase; 4. TaKaRa *Taq* DNA Polymerase Hot Start Version; 5. Kapa Biosystems KAPA2G Robust HotStart PCR Kit; 6. Bioline MyTaq HS DNA Polymerase.

ThermoFisher SCIENTIFIC

thermo scientific

Technical details

- Amplifies from as little as 3 pg human genomic DNA
- Routinely amplifies up to 6 kb genomic DNA and 20 kb lambda DNA
- Generates 3´-dA overhangs
- Incorporates dUTP and modified nucleotides

Usage and applications

Choose DreamTaq Hot Start DNA Polymerase for the amplification of DNA from plasmid, viral, or complex genomic templates. Common applications include:

- Colony PCR
- Genotyping
- RT-PCR
- Generation of PCR products for TA cloning

Why use green?

The Thermo Scientific™ DreamTaq™ Green Buffer (10X) supports direct gel loading of PCR products. The two tracking dyes and a density reagent in the green buffer do not interfere with PCR performance and are compatible with downstream applications including DNA sequencing, ligation, and restriction digestion.

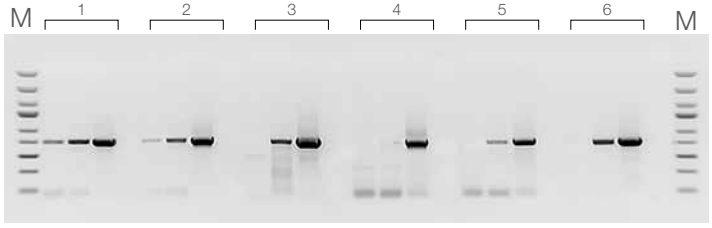


Figure 2. High sensitivity. DreamTaq Hot Start DNA Polymerase amplifies from lower template amounts than hot-start DNA polymerases from other suppliers. Each set of PCR reactions contained either 3 pg, 30 pg, or 3 ng of human genomic DNA.
M: GeneRuler Express DNA Ladder. 1. DreamTaq Hot Start DNA Polymerase; 2. TaKaRa *Taq* DNA Polymerase Hot Start Version; 3. Kapa Biosystems KAPA2G Robust HotStart PCR Kit; 4. Bioline MyTaq HS DNA Polymerase; 5. NEB One*Taq* Hot Start DNA Polymerase; 6. Promega GoTaq G2 Hot Start Polymerase.

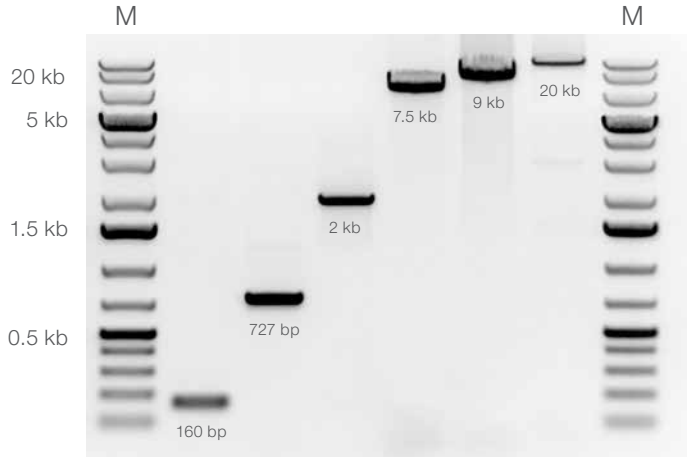


Figure 3. Consistent and reliable amplification. DreamTaq Hot Start DNA Polymerase amplifies human genomic DNA with high specificity up to 9 kb amplicons. Even longer 20 kb amplicons can be amplified with lambda DNA templates.
M: Thermo Scientific™ GeneRuler™ 1 kb Plus DNA Ladder.

Ordering information

Product	Quantity	Cat. No.
DreamTaq Hot Start DNA Polymerase	200 U	EP1701
	500 U	EP1702
	2,500 U	EP1703
	4 x 2,500 U	EP1704
DreamTaq Hot Start PCR Master Mix	200 reactions	K9011
	1,000 reactions	K9012

Product	Quantity	Cat. No.
DreamTaq Hot Start Green DNA Polymerase	200 U	EP1711
	500 U	EP1712
	2,500 U	EP1713
	4 x 2,500 U	EP1714
DreamTaq Hot Start Green PCR Master Mix	200 reactions	K9021
	1,000 reactions	K9022

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

ThermoFisher SCIENTIFIC

ThermoFisher SCIENTIFIC
The world leader in serving science

CERTIFICATE OF ANALYSIS

EP1702 DreamTaq Hot Start DNA Polymerase, 500 U

Packaging Lot: 91304106

Expiry Date: 30.06.2024 (DD.MM.YYYY)

Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
91295053	0.5 kU	DreamTaq HS DNA Polymerase
91298843	2 x 1.25 mL	10X DreamTaq Buffer

QUALITY CONTROL

Parameter	Method	Requirement	Result
Unit concentration	One unit of enzyme incorporates 10 nmoles of dNTPs into a polynucleotide fraction at 74 ºC in 30 minutes.	5.5 ± 0.5 U/µl	Conforms
Endodeoxyribonuclease Assay	No detectable conversion of supercoiled plasmid DNA to a nicked form was observed.	Not detectable	Conforms
Residual Activity Assay	No detectable extension of labeled double stranded oligonucleotide with 5'-overhangs after incubation in the presence of dNTPs.	Not detectable	Conforms
E. coli DNA	No detectable E.coli DNA was observed.	Not detectable	Conforms
Functional Assay	Performance in PCR is tested by the amplification of a 594 bp and 7.5 kb fragments of human genomic DNA.	Reactions produce specific PCR products	Conforms

ISO CERTIFICATION

Manufactured in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

Manufactured for Thermo Fisher Scientific

Clean bands. Clear results.

GeneRuler DNA ladders are designed to deliver precision and ease of use for everyday electrophoresis

Why use GeneRuler ladders?

Thermo Scientific™ GeneRuler™ DNA ladders are ideal for sizing and approximate quantification of a wide range of double-stranded DNA. These DNA ladders are available in fragment sizes ranging from 10 bp to 48.5 kb.

Why choose ready-to-use ladders?

The most popular ready-to-use GeneRuler DNA ladders are conveniently premixed with Thermo Scientific™ TriTrack™ loading buffer, which contains three tracking dyes (xylene cyanol FF, bromophenol blue, and orange G) that help monitor separation of a wide range of DNA fragment sizes.

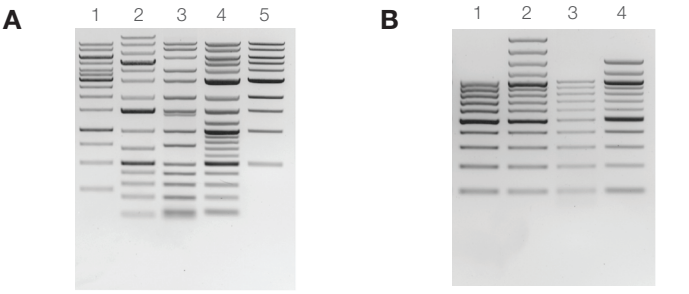
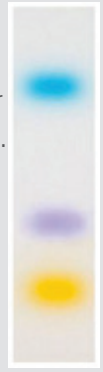


Figure 1. GeneRuler DNA ladders provide sharp and bright reference bands, with an easy-to-remember pattern. Amount of DNA ladder per lane was loaded according to each manufacturer's recommendation. **(A)** Lane 1: GeneRuler 1 kb DNA Ladder, ready-to-use; lane 2: GeneRuler 1 kb Plus DNA Ladder, ready-to-use; lane 3: Sigma-Aldrich DirectLoad™ Wide Range DNA Marker; lane 4: NEB Quick-Load™ 2-Log DNA Ladder (0.1–10.0 kb); lane 5: NEB Quick-Load™ 1 kb DNA Ladder. **(B)** Lane 1: GeneRuler 100 bp DNA Ladder, ready-to-use; lane 2: GeneRuler 100 bp Plus DNA Ladder, ready-to-use; lane 3: Sigma-Aldrich DirectLoad™ PCR 100 bp Low Ladder; lane 4: NEB Quick-Load™ Purple 100 bp DNA Ladder.

TriTrack loading buffer has three convenient tracking dyes to help easily monitor separation of DNA ladders. Moreover, these dyes do not interfere with UV visualization.



Features

- More environment-friendly manufacturing without the use of organic solvents
- Conventional and ready-to-use formats
- Wide range of ladders available (1 kb, 100 bp, 50 bp, low range, and high range)
- Sharp and bright reference bands
- Supplied with loading dye for sample DNA
- Ships at ambient temperature

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Technical details

- Mixture of chromatography-purified individual DNA fragments
- Concentration of each DNA band is determined spectrophotometrically
- Ready-to-use DNA ladders are stable at room temperature for up to six months

Usage and applications

GeneRuler DNA ladders are used for sizing and approximate quantification in traditional molecular biology techniques:

- DNA cloning
- PCR
- Reverse transcription

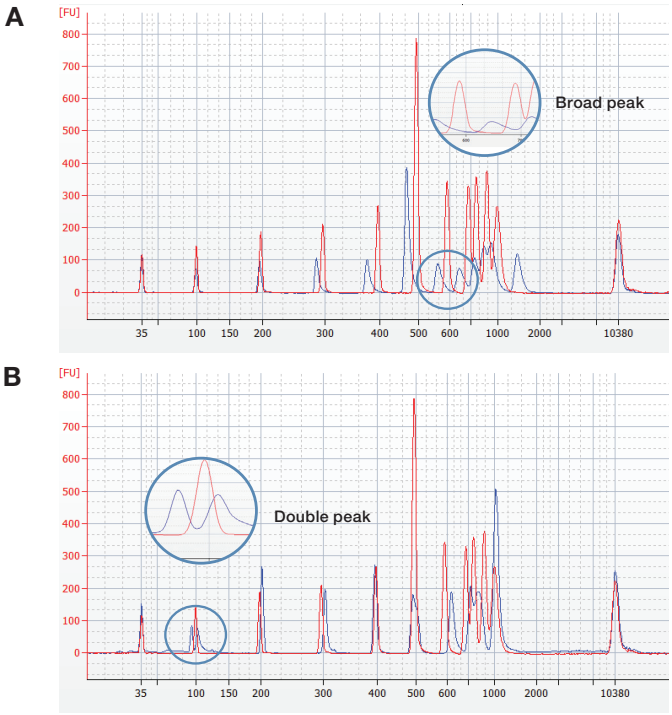

Figure 2. GeneRuler DNA ladders can offer sharp peaks during capillary electrophoresis, for exceptional purity without additional DNA fragments or dNTPs. (A) GeneRuler 100 bp DNA Ladder (red) and Promega 100 bp DNA Ladder (blue). **(B)** GeneRuler 100 bp DNA Ladder (red) and DirectLoad PCR 100 bp Low Ladder (blue).

Ordering information

GeneRuler product	Quantity	Cat. No.
100 bp DNA Ladder	50 µg/5 x 50 µg	SM0241/SM0242
100 bp DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0243/SM0244
100 bp Plus DNA Ladder	50 µg	SM0321/SM0322
100 bp Plus DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0323/SM0324
1 kb DNA Ladder	5 x 50 µg/25 x 50 µg	SM0311/SM0312
1 kb DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0314/SM0313
1 kb Plus DNA Ladder	5 x 50 µg/25 x 50 µg	SM1331/SM1332
1 kb Plus DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM1334/SM1333
50 bp DNA Ladder	50 µg/5 x 50 µg	SM0371/SM0372
50 bp DNA Ladder, ready-to-use	50 µg	SM0373
DNA Ladder Mix	5 x 50 µg/25 x 50 µg	SM0331/SM0332
DNA Ladder Mix, ready-to-use	50 µg/5 x 50 µg	SM0334/SM0333
Express DNA Ladder, ready-to-use	50 µg	SM1553
Ultra Low Range DNA Ladder, ready-to-use	50 µg	SM1213
Low Range DNA Ladder, ready-to-use	50 µg	SM1193
High Range DNA Ladder, ready-to-use*	50 µg	SM1353

* This product contains bromophenol blue and xylene cyanol FF as tracking dyes. It is not available with the TriTrack buffer.

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

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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

SM0373 **GeneRuler 50bp DNA Ladder, rtu**
Packaging Lot: **01289855**
Expiry Date: 31.05.2026 (DD.MM.YYYY)
Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
01270035	50 µg	GeneRuler 50bp DNA Ladder, rtu
01270672	1 mL	6X TriTrack DNA Loading Dye

QUALITY CONTROL

Parameter	Method	Requirement	Result
Appearance	Visual inspection.	Dark solution	Conforms
Identity	1 µg of the ladder is analyzed by agarose gel electrophoresis in parallel with the same composition ladder from a previous bulk lot.	13 fragments are visible and correspond to those from the previous bulk lot	Conforms
Deoxyribonucleases	Incubation of DNA Ladder at elevated temperature (37 °C) and analysis on agarose gel.	No changes in bands intensity, no smearing is observed after incubation	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

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CERTIFICATE OF ANALYSIS

A25742 **PowerUp™ SYBR™ Green Master Mix**

Packaging Lot: 91302107

Expire Date: 07.2023

QUALITY CONTROL

TEST	SPECIFICATION	RESULT
FUNCTIONAL TEST Functional test performed on Applied Biosystems StepOne Plus Real-Time PCR System		
Average NTC (No Template Control) reactions	≥ 37.0 CT	Pass
Average Ct for the third concentration point	20.2 – 23.2 CT	Pass
The R^2 (correlation coefficient) for the standard curve	≥ 98 %	Pass
The % PCR efficiency	85 – 115 %	Pass
The average delta Rn for the second concentration point	3.6 – 10.6	Pass
ANALYTICAL TESTS Deoxyribonucleoside triphosphate (dNTP) concentrations (measured individually)		
dATP	0.43 - 0.58 mM	Pass
dCTP	0.43 - 0.58 mM	Pass
dGTP	0.43 - 0.58 mM	Pass
dTTP	0.21 - 0.29 mM	Pass
dUTP	0.85 - 1.15 mM	Pass
Mg^{2+} concentration	4.76 - 6.44 mM	Pass
pH	8.23 - 8.53	Pass
NUCLEASE TESTS		
RNase equivalents level	≤ 1.15	Pass
DNase equivalents level	≤ 155	Pass
DNA TEST		
E. coli DNA level	≤ 4 copy	Pass

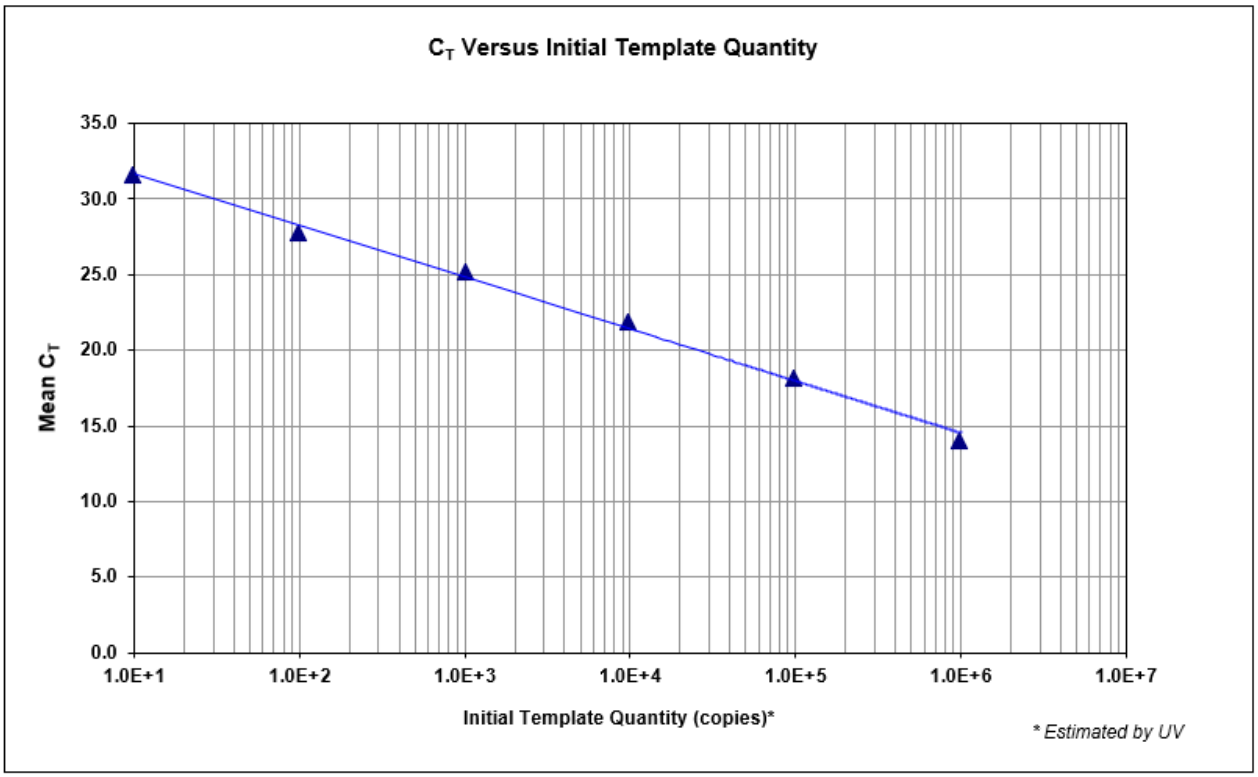
For Research Use Only. Not for use in diagnostic procedures.

ISO CERTIFICATION
Manufactured in compliance with ISO 9001 and ISO 13485 certified quality management systems.

Quality authorized by QC: J. Žilinskienė

Manufactured for Thermo Fisher Scientific

Image for product number A25742 lot number 91302107



PowerUp™ SYBR™ Green Master Mix

Universal 2X master mix for real-time PCR workflows

Catalog Numbers A25741, A25742, A25743, A25776, A25777, A25778, A25779, A25780, A25918

Pub. No. 100031508 **Rev.** D.0

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**.

This Quick Reference is intended as a benchtop reference for experienced users of PowerUp™ SYBR™ Green Master Mix. For detailed instructions, supplemental procedures, and troubleshooting, refer to the *PowerUp™ SYBR™ Green Master Mix User Guide* (Pub. No. MAN0013511).

Guidelines

Input DNA template requirements

Use 1–10 ng single-stranded cDNA or 10–100 ng gDNA per reaction.

PCR reactions

- Four replicates of each reaction are recommended.
- Reaction mixes can be prepared depending upon experimental requirements. Scale the components according to the number of reactions and include 10% overage.
- If using smaller reaction volumes, scale all components proportionally. Reaction volumes <10 µL are not recommended.

Using NTC controls

No template control (NTC) reactions can be used to identify PCR contamination. NTC reactions contain all reaction components (PowerUp™ SYBR™ Green Master Mix, primers, water) except sample, and therefore should not return a C_T value.

Methods

Set up the PCR reactions

1. Prepare the appropriate number of reactions, plus 10% overage.

Component	Volume (10 µL/well)	Volume (20 µL/well)
PowerUp™ SYBR™ Green Master Mix (2X)	5 µL	10 µL
Forward and reverse primers[1]	Variable	Variable
DNA template + Nuclease-Free Water[2]	Variable	Variable
Total	**10 µL**	**20 µL**

[1] For optimal performance in Fast and Standard modes, use 300–800 nM for each primer.
[2] Use 1–10 ng cDNA or 10–100 ng gDNA for each reaction.

2. Mix the components thoroughly, then centrifuge briefly to spin down the contents and eliminate any air bubbles.
3. Transfer the appropriate volume of each reaction to each well of an optical plate.
4. Seal the plate with an optical adhesive cover, then centrifuge briefly to spin down the contents and eliminate any air bubbles.

PCR can be performed on the reaction plate up to 24 hours after completing the set-up, when stored at room temperature.

Set up and run the real-time PCR instrument

1. Place the reaction plate in the real-time PCR instrument.

2. Set the thermal cycling conditions using the default PCR thermal cycling conditions specified in the following tables according to the instrument cycling parameters and melting temperatures of the specific primers.

   Note: Standard cycling conditions are recommended for genomic DNA templates. Use only standard cycling conditions for the 7900HT Real-Time PCR Instrument.

Table 1 Fast cycling mode (primer T_m ≥60°C)

Step	Temperature	Duration	Cycles
UDG activation	50°C	2 minutes	Hold
Dual-Lock™ DNA polymerase	95°C	2 minutes	Hold
Denature	95°C	1 second[1] or 3 seconds[2]	40
Anneal/extend	60°C	30 seconds	

[1] When using a QuantStudio™ Real-Time PCR System or a ViiA™ 7 Real-Time PCR System.

[2] When using a 7500 Fast Real-Time PCR System, StepOnePlus™ Real-Time PCR System, or StepOne™ Real-Time PCR System.

Table 2 Standard cycling mode (primer T_m ≥ 60°C)

Step	Temperature	Duration	Cycles
UDG activation	50°C	2 minutes	Hold
Dual-Lock™ DNA polymerase	95°C	2 minutes	Hold
Denature	95°C	15 seconds	40
Anneal/extend	60°C	1 minute	

Table 3 Standard cycling mode (primer T_m <60°C)

Step	Temperature	Duration	Cycles
UDG activation	50°C	2 minutes	Hold
Dual-Lock™ DNA polymerase	95°C	2 minutes	Hold
Denature	95°C	15 seconds	40
Anneal	55–60°C[1]	15 seconds	
Extend	72°C	1 minute	

[1] Anneal temperature should be set to the melting point for your primers.

3. Set the instrument to perform a default dissociation step.

   A dissociation step can be performed up to 72 hours after the real-time PCR run if the plate is stored in the dark and up to 24 hours after the real-time PCR run if the plate is exposed to light.

Table 4 Dissociation curve conditions (melt curve stage)

Step	Ramp rate	Temperature	Time
1	1.6°C/second	95°C	15 seconds
2	1.6°C/second	60°C	1 minute
3[1]	0.15°C/second	95°C	15 seconds

[1] Dissociation

Use the following settings for Applied Biosystems™ instruments:

- Experiment type: Standard curve
- Reagent: SYBR™ Green reagents
- Reporter: SYBR™
- Quencher: None
- Passive reference dye: ROX™
- Ramp speed: Standard or fast (choose the same setting as in step 2)
- Melt curve ramp increment: Continuous

4. Set the reaction volume appropriate for the type of plate being used for your PCR reaction.

5. Start the run.

Analyze results

1. View the amplification plots.

2. Calculate the baseline and threshold cycles (C_T) for the amplification curves using the instrument software.

3. Check for nonspecific amplification using dissociation curves.

4. Perform relative or absolute quantitation.

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

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

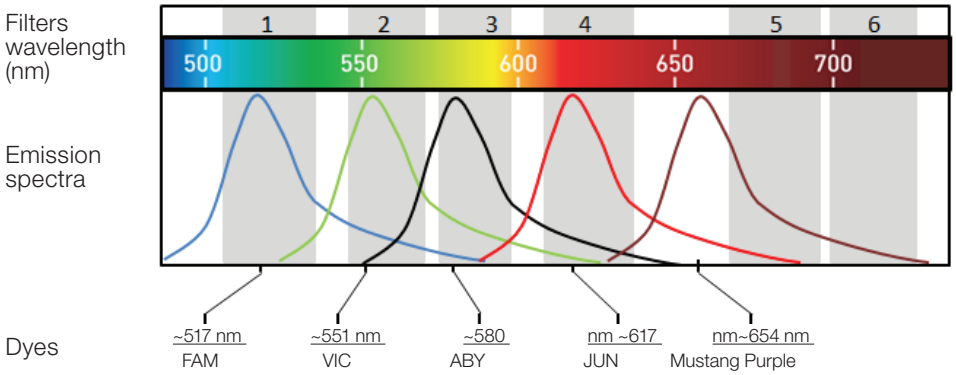


Figure 1. Fluorescence emission spectra of FAM, VIC, ABY, and JUN dyes used for multiplex real-time PCR. Grey zones represent the filters available on Applied Biosystems™ real-time PCR systems: 1 through 6 for the QuantStudio™ 7 or 12K Flex Real-Time PCR Systems; 1 through 5 for the QuantStudio™ 6 Flex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

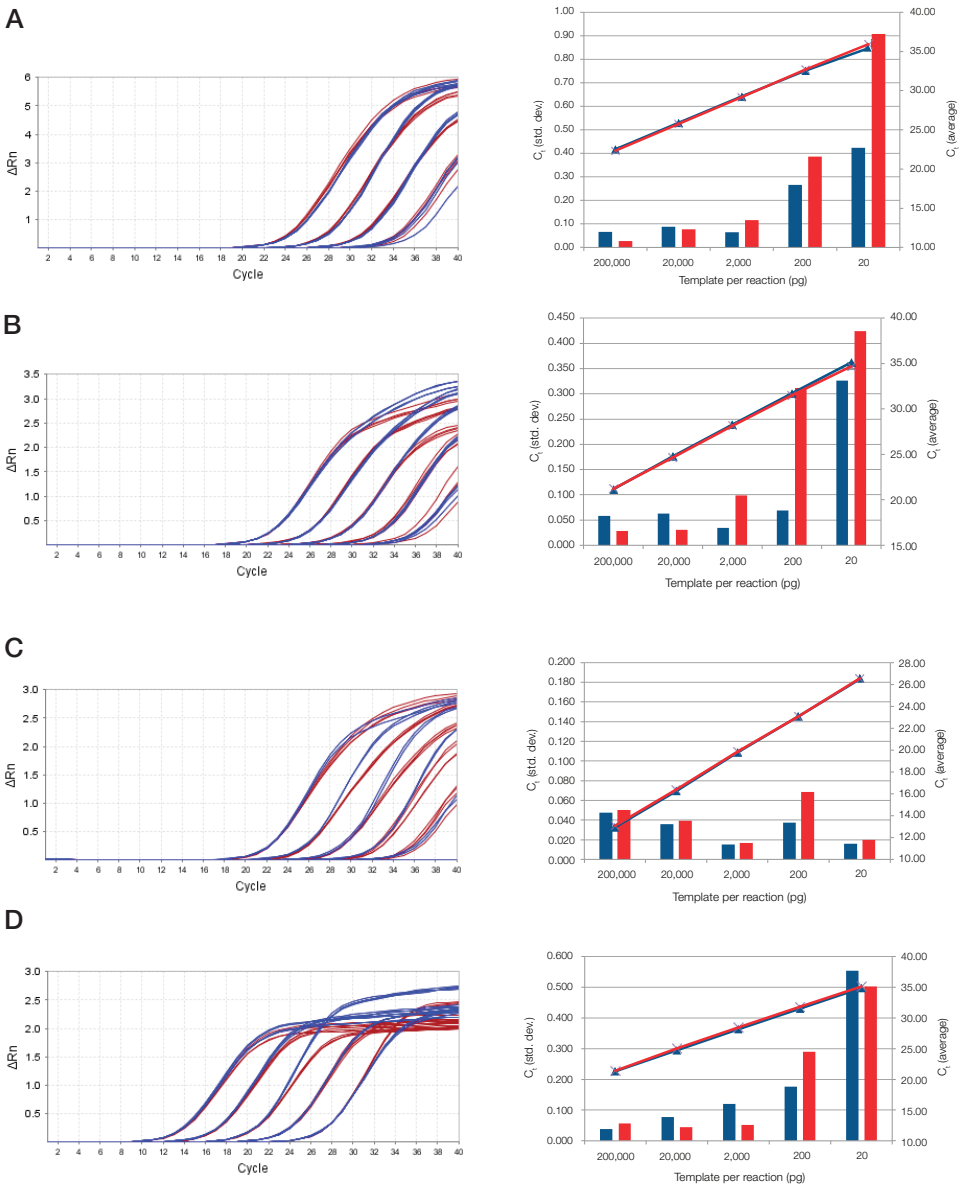

Figure 2. Comparison of singleplex and multiplex gene expression assays. (A) EGFR assay, FAM dye; **(B)** BRCA1 assay, VIC dye; **(C)** ESR1 assay, JUN dye; **(D)** RNase P assay, ABY dye. Amplification was performed on the QuantStudio 7 Real-Time PCR System using TaqMan Multiplex Master Mix. The figure 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).

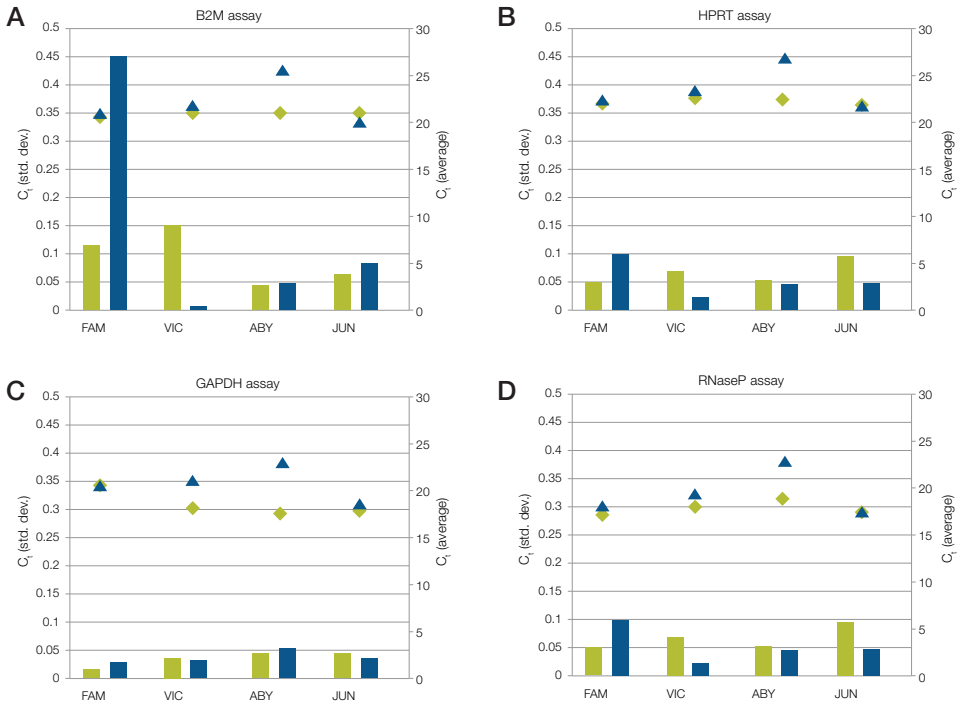

Figure 3. Comparison of our new dye combination with a dye combination from another supplier. Probes for **(A)** B2M, **(B)** HPRT, **(C)** GAPDH, and **(D)** RNase P gene expression assays were synthesized with FAM, VIC, ABY, and JUN dyes with QSY quencher (green bars and diamonds) and with another 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.

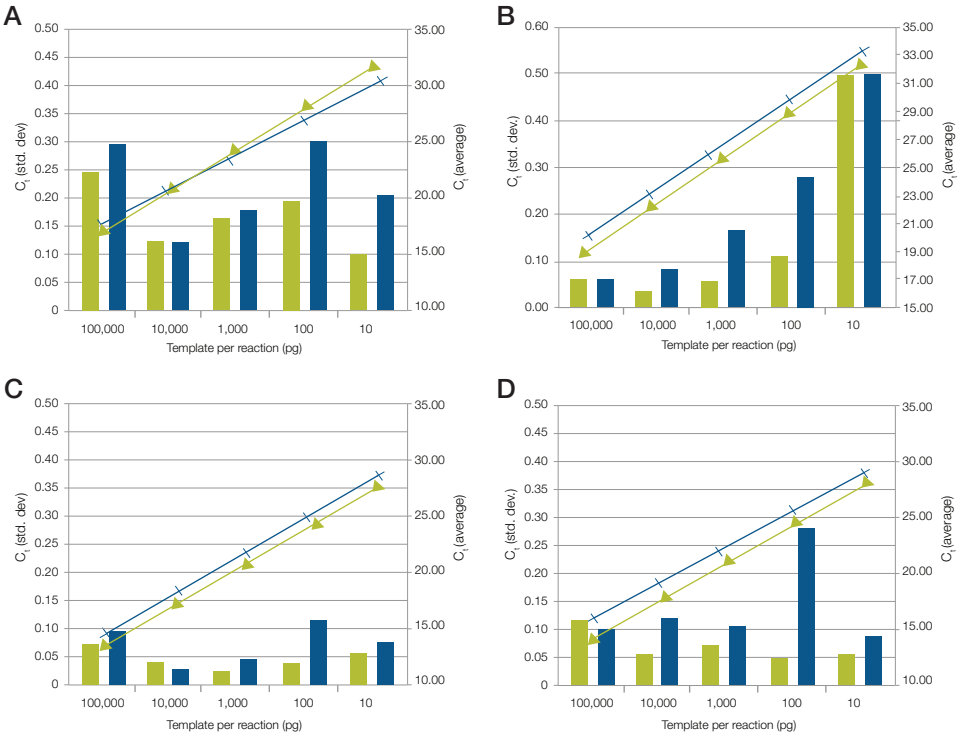

Figure 4. Comparison of TaqMan Multiplex Master Mix with another commercially available master mix. (A) B2M assay, FAM dye; **(B)** RNase P assay, VIC dye; **(C)** GAPDH assay, ABY dye; **(D)** HPRT assay, JUN dye. All assays used QSY quencher. The graph shows average standard deviation (bars) and average 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.

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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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TaqMan QSY probes

New quencher available for your qPCR probes

Applied Biosystems™ TaqMan™ QSY™ probes incorporate a proprietary nonfluorescent 3´ QSY quencher to provide maximal PCR performance in a multiplex format (Figure 1). Experience the sensitivity and specificity you know and expect from TaqMan™ Assays, with another great option for your real-time PCR assay designs.

Figure 1. QSY probe. The newly developed QSY quencher can be used in multiplex qPCR with FAM™, VIC™, ABY™, and JUN™ reporter dyes. The QSY quencher is nonfluorescent, leading to less background and improved quenching efficiency.

QSY probes are comparable to BHQ probes

Your current Black Hole Quencher™ (BHQ™) probe designs can easily be converted to QSY probes. Identical sequence designs can be used with similar performance using FAM dye (Figure 2) and improved performance using our ABY™ dye (Figure 3).

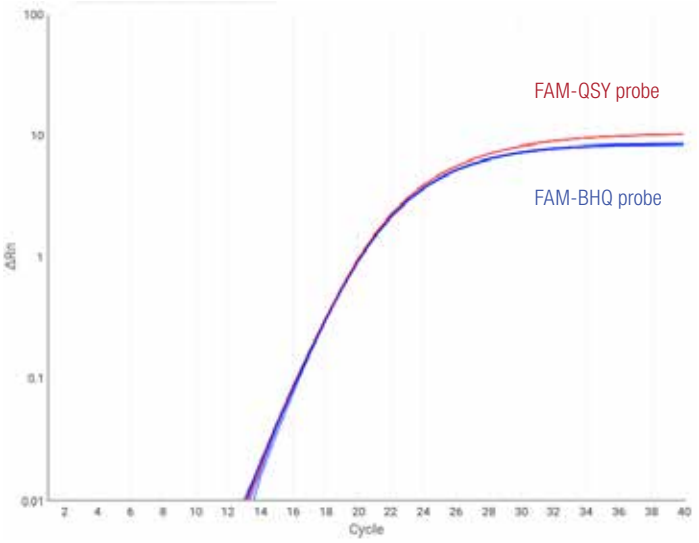

Figure 2. QSY probes have performance similar to that of BHQ probes. A FAM-QSY probe and a FAM-BHQ probe with identical oligonucleotide sequences and master mixes have similar C_t values.

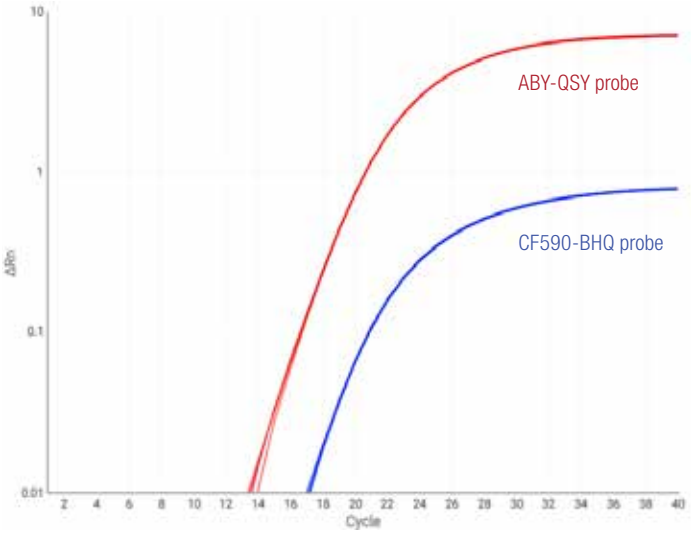

Figure 3. Improved performance in multiplex qPCR. In this multiplex experiment, the ABY-QSY probe shows a significantly lower C_t than the CF590-BHQ probe with an identical oligonucleotide sequence and master mix.

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applied biosystems

Four dye options optimized with our instruments for better sensitivity

TaqMan QSY probes can be ordered with FAM, VIC, and our proprietary ABY and JUN dyes, allowing amplification of up to 4 targets in a single reaction. All 4 dyes are optimized for the filter sets on Applied Biosystems™ real-time PCR instruments (Figure 4) and work together with minimal spectral overlap for optimal performance.

Performance without compromise

Multiplexing with TaqMan QSY probes enables cost savings and preservation of limited samples, and also yields comparable results between reactions performed in individual tubes and in 4-plex reactions, for a gene quantification experiment (Figure 5).

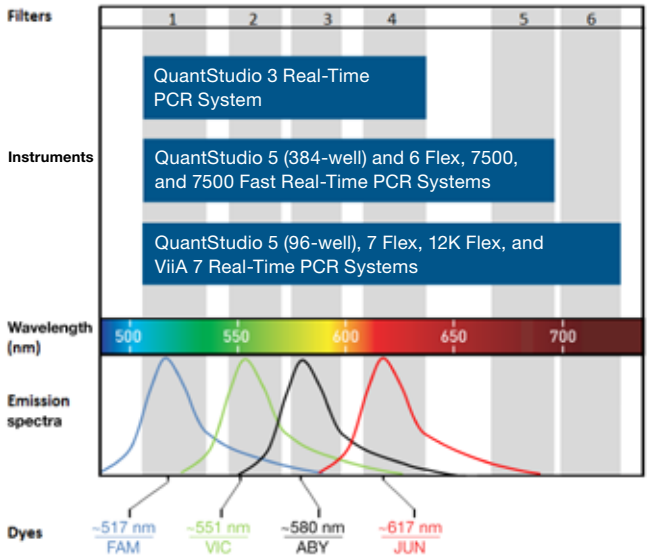

Figure 4. Fluorescence emission wavelengths used for multiplex real-time PCR. Emission spectra for FAM, VIC, ABY, and JUN dyes are shown in relation to regions of the spectrum detected by six filters available on Applied Biosystems real-time PCR instruments.

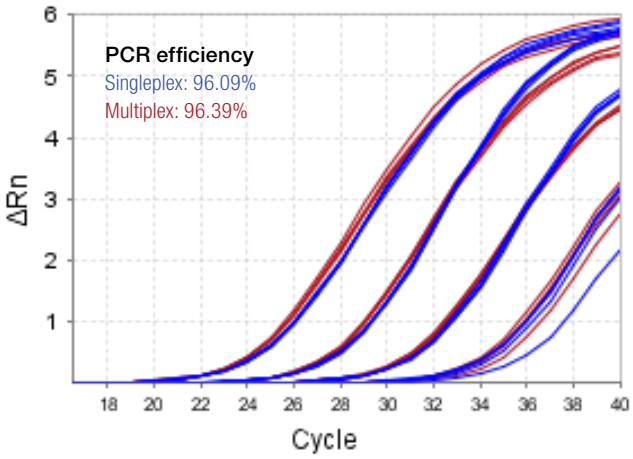

Figure 5. Comparable results for singleplex and multiplex assays. The amplification plot shows linear portions of the curves for 4 EGFR assays amplified in singleplex (blue) and 4-plex reactions (red) in a dilution series from 20,000 pg to 2 pg of reference colon cDNA per 10 µL reaction. PCR efficiencies are 96.09% for EGFR singleplex and 96.39% for EGFR 4-plex reactions.

Ordering information

Product	Quantity	Cat. No.
TaqMan QSY Probe	6,000 pmol	4482777
TaqMan QSY Probe	20,000 pmol	4482778
TaqMan QSY Probe	50,000 pmol	4482779

Product	Quantity	Cat. No.
TaqMan Multiplex Master Mix (2X)	5 mL	4461882
TaqPath 1-Step Multiplex Master Mix (4X)	5 x 1 mL	A28526
TaqPath 1-Step Multiplex Master Mix, No ROX (4X)	5 x 1 mL	A28522
Spectral Calibration Plate for Multiplex qPCR	1 plate	Various

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

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BigDye™ Terminator v3.1 Matrix Standards Kit

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

Catalog Number 4336974

Pub. No. 4363117 Rev. E

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**.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the the instrument user guide. Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The BigDye™ Terminator v3.1 Matrix Standards Kit is used to perform spectral calibrations. The matrix standard contains four sizes of DNA fragments, each size labeled with a different fluorescent dye. The matrix standard can be used to perform spectral calibrations on the following instruments:

- SeqStudio™ Flex Series Genetic Analyzer
- SeqStudio™ Genetic Analyzer
- 3500/3500xL Genetic Analyzer
- 3130/3130*xl* Genetic Analyzer

The kit contains Dye Set Z, which defines the number, dye color, and migration order of the dye peaks in the sample.

Contents and storage

Contents	Amount	Storage
BigDye™ Terminator v3.1 Matrix Standards	1 tube	Store at 2–8°C, protected from light.[1] Do not freeze.

[1] Upon receipt, the kit is stable for 6 months when stored appropriately.

Required materials not supplied

Item		Cat. No.
Hi-Di™ Formamide		4311320
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL		4346907
MicroAmp™ Optical 96-Well Reaction Plate		N8010560
MicroAmp™ Optical 384-Well Reaction Plate		4343370
Septa		
SeqStudio™ Flex and 3500 series	8-Strip Septa 3500/Flex Series (Qty 24)	4410701
	96-Well Septa 3500/Flex Series	4412614
	384-Well Septa 3500/Flex Series	4412520
SeqStudio™	Septa for SeqStudio™ Genetic Analyzer, 96 well	A35641
3130 series	Plate Septa, 96 well	4315933

Guidelines for use

- For more information on the use of matrix standards, see the instrument user guide or *DNA Fragment Analysis by Capillary Electrophoresis User Guide* (Pub. No. 4474504).
- **IMPORTANT!** Thoroughly mix the contents of the matrix standard tubes, then briefly centrifuge before use.
- To prepare the matrix standard dilution, combine the appropriate volumes of matrix standard and Hi-Di™ Formamide (Cat. No. 4311320). Dilution volumes vary depending on the specific application and instrument.
- Do not prepare the matrix standard more than 2 hours in advance.
- **IMPORTANT!** Discard any unused reagent that has been diluted in Hi-Di™ Formamide.

Prepare the standard

1. Combine the volumes of matrix standard and Hi-Di™ Formamide appropriate for the instrument. See "Component volumes and well locations for the prepared standard" on page 2.
2. Mix thoroughly, then centrifuge to bring the mixture to the bottom and eliminate air bubbles.
3. To denature the DNA fragments, incubate the mixture at 95°C for 2 minutes. Immediately place the mixture on ice.
4. Dispense the prepared standard into the appropriate wells of a reaction plate. See "Component volumes and well locations for the prepared standard" on page 2.
5. Cover the plate with septa, then centrifuge to bring the mixture to the bottom and eliminate air bubbles.
6. Assemble the plate with the retainer and base, then load on the instrument.

   Note: The SeqStudio™ Genetic Analyzer does not require a retainer and base.

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

Note: For dye set selection on the SeqStudio™ Flex and SeqStudio™ instruments, ensure that you select the **Matrix** tab before you select the dye set.

Component volumes and well locations for the prepared standard

Table 1 SeqStudio™ Flex Series Genetic Analyzer

Component	Volume		Well location for the prepared standard	
	8-capillary array	24-capillary array	96-well plate	384-well plate
Matrix standard	3 µL	6 µL	Dispense 10 µL of the prepared standard into wells of a 96-well plate: • **8-capillary array**—8 wells: A1–H1 • **24-capillary array**—24 wells: A1–H3	Dispense 5 µL of the prepared standard into wells of a 384-well plate: **24-capillary array**—24 wells (for example, A1, A3, A5; C1, C3, C5; E1, E3, E5; G1, G3, G5; I1, I3, I5; K1, K3, K5; M1, M3, M5; O1, O3, O5)
Hi-Di™ Formamide	147 µL	294 µL		
Total volume	**150 µL**	**300 µL**		

Table 2 SeqStudio™ Genetic Analyzer

Component	Volume	Well location for the prepared standard
	4-capillary array	
Matrix standard	1 µL	Dispense 10 µL of the prepared standard into wells of a 96-well plate: 4 wells: A1–D1
Hi-Di™ Formamide	49 µL	
Total volume	**50 µL**	

Table 3 3500/3500xL Genetic Analyzer

Component	Volume		Well location for the prepared standard
	8-capillary array	24-capillary array	
Matrix standard	12 µL	12 µL	**Data Collection Software v3 and later:** Dispense 10 µL of the prepared standard into wells of a 96-well plate: • **8-capillary array**—8 wells (for example, A1–H1) • **24-capillary array**—24 wells (for example, A1–H3, A4–H6, A7–H9, or A10–H12) **Note:** If you place the standard in wells that do not correspond to injection position 1, specify the starting well position in the software. **Data Collection Software v1, v1.1, and v2:** Dispense 10 µL of the prepared standard into wells of a 96-well plate: • **8-capillary array**—8 wells: A1–H1 • **24-capillary array**—24 wells: A1–H3
Hi-Di™ Formamide	238 µL	238 µL	
Total volume	**250 µL**	**250 µL**	

Table 4 3130/3130*x*/ Genetic Analyzer

Component	Volume			Well location for the prepared standard
	36-cm array	50-cm array	80-cm array	
16-capillary array				
Matrix standard	10 µL	5 µL	10 µL	Dispense 10 µL of the prepared standard into wells of a 96-well plate: **16-capillary array**—16 wells: A1–H2
Hi-Di™ Formamide	190 µL	195 µL	190 µL	
Total volume	**200 µL**	**200 µL**	**200 µL**	
4-capillary array				
Matrix standard	2.5 µL	2.5 µL	2.5 µL	Dispense 10 µL of the prepared standard into wells of a 96-well plate: **4-capillary array**—4 wells: A1–D1
Hi-Di™ Formamide	47.5 µL	97.5 µL	47.5 µL	
Total volume	**50 µL**	**100 µL**	**50 µL**	

Limited product warranty

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Revision history: Pub. No. 4363117

Revision	Date	Description
E	2 February 2022	Added the SeqStudio™ Flex Series Genetic Analyzer. Added required materials table. Consolidated "Prepare the standard" into one procedure for all instruments. Added "Component volumes and well locations for the prepared standard".
D	05 October 2021	Volumes for 3500 were corrected.
C	05 November 2018	Updated instrumentation. Updated licensing, trademarks, general style and format.
B	18 August 2009	Baseline for this revision

Applied Biosystems

BigDye® Terminator v3.1 and v1.1 Cycle Sequencing Kits

- **Enhanced robustness improves success rates, particularly with challenging templates**
- **Comprehensive chemistry solution for today's wide range of sequencing applications**
- **Improved peak-height uniformity and optimized signal balance for longer, higher quality reads**
- **Enable completion of sequencing projects more quickly and economically**

Improved Performance

BigDye® Terminator v3.1 and v1.1 chemistries provide a variety of benefits over earlier versions of BigDye chemistry. The new kits offer improved performance in sequencing difficult templates, successfully reading through dinucleotide repeats and other challenging sequence motifs. Both formulations are also designed to offer improved robustness with a wide range of template types and qualities. In addition, v3.1 and v1.1 kits generate data that has greater peak-height uniformity, which enhances basecalling accuracy and mixed-base detection. Overall, v3.1 and v1.1 kits enable longer sequencing reads and higher success rates, which lead to reduced project costs.

New Chemistries to Address Your Sequencing Needs

Like its predecessor (ABI PRISM® BigDye Terminator v3.0 Cycle Sequencing Kit), the new BigDye® Terminator v3.1 Cycle Sequencing Kit is optimized for the majority of DNA sequencing applications. The BigDye® Terminator v1.1 Cycle Sequencing Kit, which is based on the original ABI PRISM® BigDye Terminator chemistry (v1.0), is formulated for specialty applications. Together, these two new powerful and versatile chemistries meet the demands of the wide range of sequencing applications performed today.

Chemistry Options

Applications	BigDye® Terminator v3.1 Kit	BigDye® Terminator v1.1 Kit
de novo sequencing	+	✔
Resequencing	+	✔
Sequencing difficult templates	+	+
Long-read sequencing	+	✔
Sequencing across all template types (plasmids, PCR products, BACs, and fosmids)	+	✔
Mixed-base detection	+	✔
Sequencing short PCR products using rapid electrophoresis run modules	✔	+

+ Recommended ✔ Satisfactory

Table 1. Chemistry Options

Easy Integration

The dyes in the new BigDye Terminator v3.1 and v1.1 kits are the same as those in the v3.0 and v1.0/v2.0 kits respectively, and thus, no new software or instrument recalibration is required for data analysis. Therefore, researchers can easily integrate both new versions into their workflow and take advantage of the benefits these new chemistries provide.

BigDye® Terminator v3.1 Chemistry

The BigDye Terminator v3.1 Cycle Sequencing Kit is a robust, highly flexible chemistry, designed for the majority of applications, including *de novo* sequencing and resequencing. The BigDye Terminator v3.1 kit generates data with uniform peak heights and optimized signal balance to produce long, high-quality reads. Improved peak patterns also contribute to more accurate base assignments for heterozygote and mutation detection. The chemistry's robust formulation is successful with a wide variety of templates, including PCR products, plasmids, and large insert clones, such as fosmids and bacterial artificial chromosomes (BACs). The BigDye Terminator v3.1 kit provides researchers with a higher success rate than the BigDye Terminator v3.0 kit, particularly with difficult to

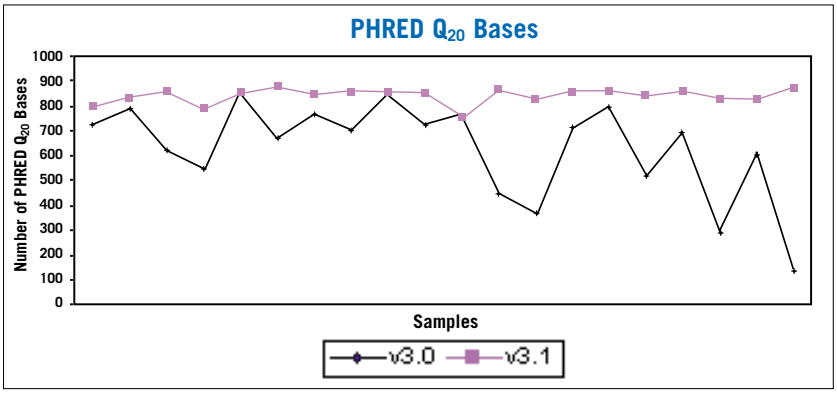

Figure 1. Longer, higher quality reads with the BigDye® Terminator v3.1 kit. The BigDye Terminator v3.1 kit generates data with an improved average number of PHRED Q_{20} bases. A library of "difficult" templates was sequenced in a customer's laboratory according to their standard protocol using the 3730*xl* DNA Analyzer. Samples using BigDye® Terminator v3.0 chemistry generated on average 629 Q_{20} bases, while samples using BigDye Terminator v3.1 chemistry generated on average 840 Q_{20} bases. (Data courtesy of Agencourt.)

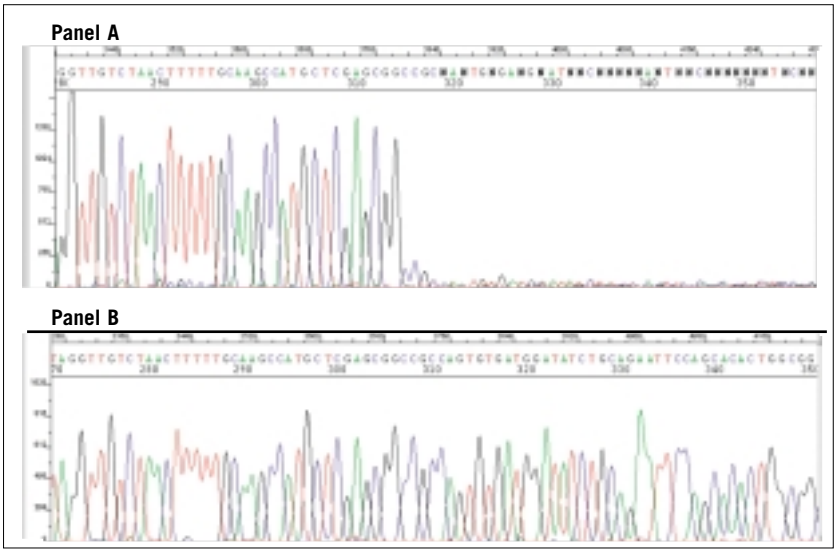

Figure 2. Improved Performance on Difficult Templates with BigDye® Terminator v3.1. A sample was run in a customer's laboratory according to their standard protocol. With the BigDye® Terminator v3.0 kit the reaction is terminated by an unknown sequence context (Panel A), while the reaction prepared with the BigDye Terminator v3.1 kit continues to read through the sample (Panel B). (Data courtesy of Agencourt.)

sequence templates, and requires only minimal changes to the current BigDye Terminator v3.0 kit protocol.

BigDye Terminator v1.1 Chemistry

The BigDye Terminator v1.1 Cycle Sequencing Kit is designed for specialty applications that require optimal basecalling adjacent to the primer. The v1.1 chemistry is an excellent choice for sequencing short PCR product templates with rapid electrophoresis run modules. With better peak-height uniformity than its v1.0 predecessor, the new v1.1 kit provides very good mixed-base detection. Like the v3.1 chemistry, the v1.1 chemistry is designed for superior robustness and provides dependable, reproducible results with a wide variety of templates. The new v1.1 protocol recommends only minimal changes to the v1.0 version.

Choosing the Right DNA Sequencing Chemistry

BigDye Terminator v3.1 and v1.1 kits allow researchers to choose the optimal chemistry for a wide range of applications. Table 1 provides guidelines for selecting the appropriate cycle sequencing kit. If your laboratory is interested in the most robust, flexible chemistry that will generate the longest reads, then you would prefer the v3.1 kit. If your lab is primarily sequencing short PCR fragments using rapid electrophor-

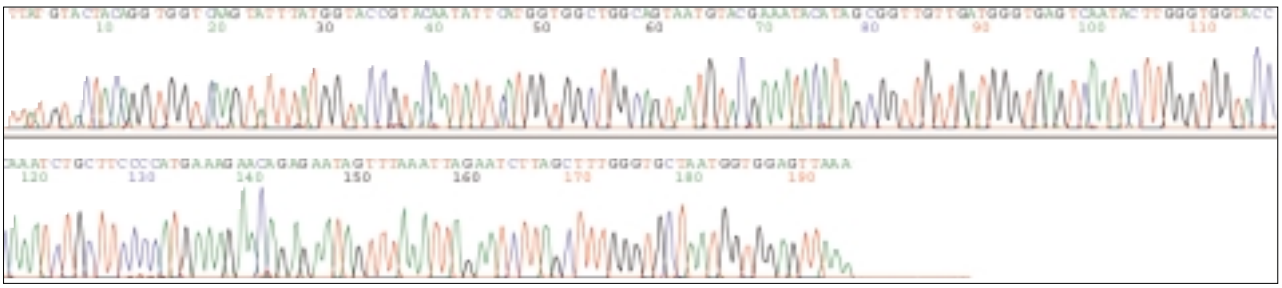

Figure 3. Short PCR Product Sequencing with BigDye® Terminator v1.1. The v1.1 kit successfully sequences a short PCR product generated from human mitochondrial DNA. The PCR product shows 100% basecalling accuracy beginning with the first base adjacent to the primer. The sample was run on the 3100 Genetic Analyzer using POP-6™ Polymer.

esis run modules, then you would prefer the v1.1 kit. Many variables contribute to DNA sequencing data quality, including template type, instrument module, total signal, peak-height uniformity, and mobility shift. All should be taken into consideration when selecting the most appropriate chemistry.

Guaranteed Performance

All BigDye sequencing reagents are tested twice for quality—first for correct formulation and then for consistent, reliable performance on our sequencing systems. Additionally, Applied Biosystems expert field and telephone support teams are readily available to answer your questions and provide whatever assistance you require.

Specifications

BigDye® Terminator v3.1 and v1.1 Cycle Sequencing Kits include all required reagents for sequencing 24, 100, 1,000, 5,000, or 25,000 single-stranded (ss) or double-stranded (ds) DNA templates. The reagents in each kit are optimized for use with the ABI PRISM® 310, 3100, and 3100–*Avant* Genetic Analyzer; the 3700, 3730, and 3730*xl* DNA Analyzer, and the 377 DNA Sequencer.

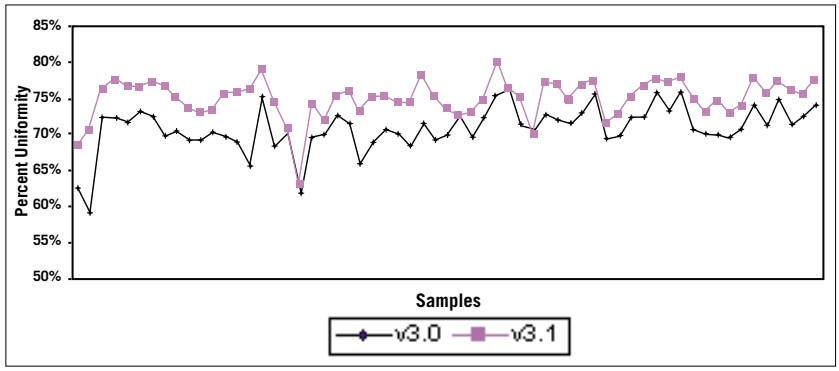

Figure 4. Improved uniformity of peak heights. BigDye® Terminator v3.1 chemistry generates data with improved peak height uniformity in customer samples. Peak height uniformity is defined as local peak height consistency of analyzed data. 100% peak height uniformity represents an idealized situation where all analyzed data peaks are of equivalent height. Improved uniformity contributes to longer, higher quality reads and more accurate mixed base detection.

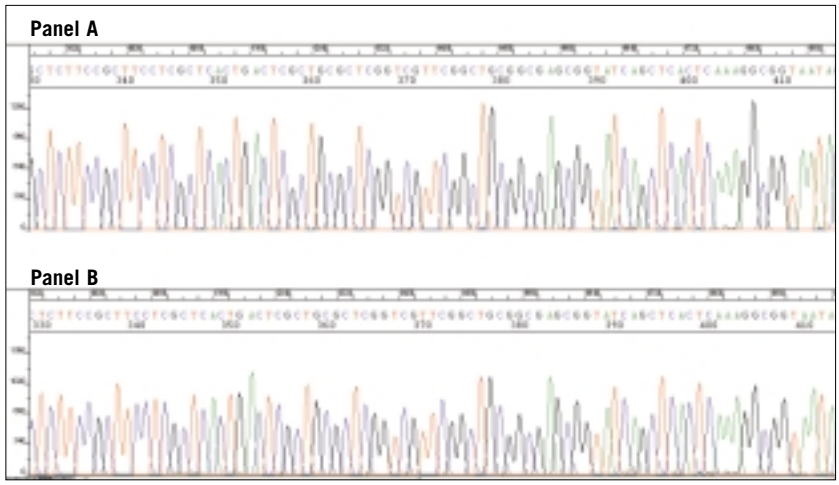

Figure 5. Improved uniformity of peak heights with BigDye® Terminator v3.1 kit. Panel A shows data from a sample sequenced using the BigDye® Terminator v3.0 kit. Panel B shows the same sample run under identical conditions with the BigDye Terminator v3.1 kit. The uniformity of the data produced with the v3.0 sample is 72% whereas the uniformity for the v3.1 sample is 76%.

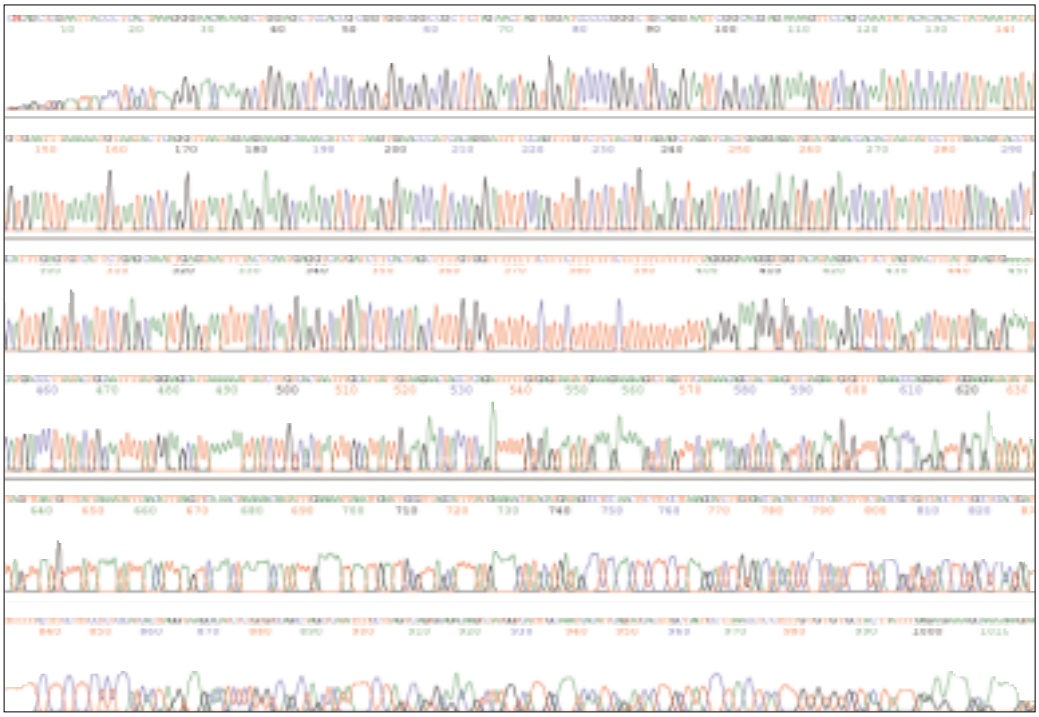

Figure 6. Longer Read Lengths with BigDye® Terminator v3.1 on the 3100 Genetic Analyzer. This figure shows accurate basecalling for more than 1,000 bases; the first ambiguity is not seen until base 1,040. The sample was run on the ABI PRISM® 3100 Genetic Analyzer with an 80 cm array using POP-4™ Polymer and the standard run module.

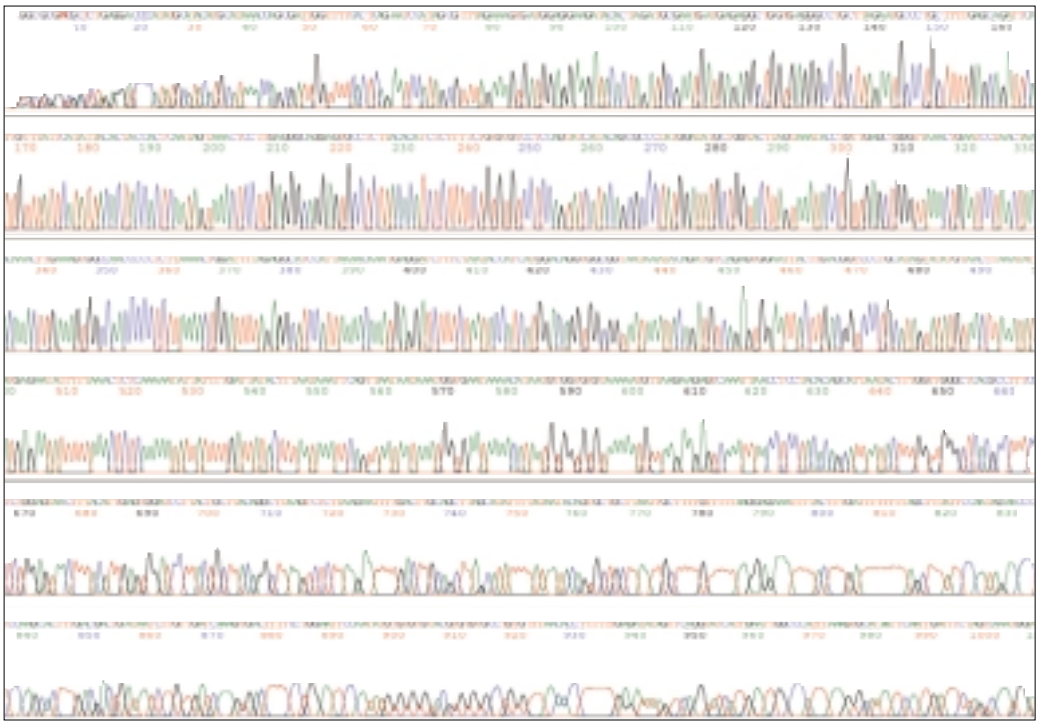

Figure 7. Longer Read Lengths with BigDye® Terminator v3.1 on the 3730*xl* Genetic Analyzer. The plasmid insert, beginning at base 23, shows accurate basecalling for more than 1,000 bases. The first ambiguity occurs at base 1,031. The sample was run on an Applied Biosystems 3730*xl* Genetic Analyzer with a 50 cm array using POP-7™ Polymer and the standard run module.

Ordering Information

BigDye® Terminator v3.1 Cycle Sequencing Kit

Ready Reactions	P/N
24	4337454
100	4337455
1,000	4337456
5,000	4337457
25,000	4337458

BigDye® Terminator v1.1 Cycle Sequencing Kit

Ready Reactions	P/N
24	4337449
100	4337450
1,000	4337451
5,000	4337452
25,000	4337453

BigDye® Terminator v3.1 Sequencing and Matrix Standards*

Description	P/N
BigDye® Terminator v3.1 Sequencing Standard	4336935
3700/3730 BigDye® Terminator v3.1 Sequencing Standard	4336943
310/377 BigDye® Terminator v3.1 Matrix Standards	4336948
3100 BigDye® Terminator v3.1 Matrix Standard	4336974
3700/3730 BigDye® Terminator v3.1 Matrix Standard	4336975

*Spatial/Spectral recalibration is not required to use v3.1 chemistry if currently using v3.0 files

BigDye® Terminator v1.1 Sequencing and Matrix Standards*

Description	P/N
BigDye® Terminator v1.1 Sequencing Standard	4336791
3700/3730 BigDye® Terminator v1.1 Sequencing Standard	4336799
310/377 BigDye® Terminator v1.1 Matrix Standards	4336805
3100 BigDye® Terminator v1.1 Matrix Standard	4336824
3700/3730 BigDye® Terminator v1.1 Matrix Standard	4336825

*Spatial/Spectral recalibration is not required to use v1.1 chemistry if currently using v1.0 or v2.0 files

BigDye® Terminator v1.1/v3.1 Sequencing Buffer (5X)

Quantity	Description	P/N
1 mL	BigDye® Terminator v1.1/v3.1 Sequencing Buffer (5X)	4336697
28 mL	BigDye® Terminator v1.1/v3.1 Sequencing Buffer (5X)	4336699
233 mL	BigDye® Terminator v1.1/v3.1 Sequencing Buffer (5X)	4336701

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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: 650.638.5800
Toll Free: 800.345.5224
Fax: 650.638.5884

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Applied Biosystems

3500 Dx and 3500xL Dx Genetic Analyzers

Flexibility for clinical research applications with the Research Use Only (RUO) Mode

Key features supporting RUO applications

- Advanced thermal system design improves temperature control for demanding DNA fragment analysis applications
- Superior signal uniformity in fragment sizing applications from instrument to instrument, run to run, and capillary to capillary
- Exceptional application flexibility—one array and one polymer are used for most applications
- No consumable-based instrument hard stops, giving you control of how much and how long consumables are utilized

3500 Dx Series system specifications for RUO applications

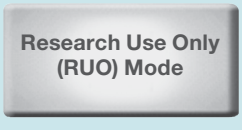
Applied Biosystems™ 3500 Dx and 3500xL Dx Genetic Analyzers can perform a number of sequencing and high-resolution fragment sizing applications for research use, including but not limited to STR/microsatellite analysis, loss of heterozygosity (LOH), and SNP confirmation and screening, as well as *de novo* sequencing and resequencing (mutation profiling).

A full range of applications may be run using a single polymer (Applied Biosystems™ POP-7™ Polymer) and the 50 cm capillary array. For even greater application versatility, Applied Biosystems™ POP-4™ and POP-6™ Polymers and the 36 cm capillary array are also available.

Secondary analysis software for RUO applications

- Applied Biosystems™ Sequencing Analysis Software with KB™ Basecaller for sequence basecalling, editing, re-basecalling, reporting, and printing
- Applied Biosystems™ Variant Reporter™ Software for mutation detection, SNP discovery, comparative sequencing, resequencing, validation, and sequence confirmation
- Applied Biosystems™ SeqScape™ Software for resequencing applications with library identification
- Applied Biosystems™ GeneMapper™ Software for microsatellite, LOH, SNP, and t-RFLP analyses
- Applied Biosystems™ GeneMapper™ *ID-X* Software for analysis of human identification data using AmpFℓSTR™ kits
- Applied Biosystems™ MicroSEQ™ ID Analysis Software for microbial sequence typing using MicroSEQ™ kits

Product	Cat. No.
Anode Buffer Container, CE-IVD	4393925
Capillary Array, 8-capillary, 36 cm, CE-IVD	4404682
Capillary Array, 24-capillary, 36 cm, CE-IVD	4404686
Capillary Array, 8-capillary, 50 cm, CE-IVD	4404684
Capillary Array, 24-capillary, 50 cm, CE-IVD	4404688
Cathode Buffer Container, CE-IVD	4408258
Conditioning Reagent, CE-IVD	4409543
POP-6 Polymer (960 samples), CE-IVD	4393711
POP-6 Polymer (384 samples), CE-IVD	4393716
POP-7 Polymer (960 samples), CE-IVD	4393713
POP-7 Polymer (384 samples), CE-IVD	4393709
POP-4 Polymer (960 samples)	4393710
POP-4 Polymer (384 samples)	4393715
Hi-Di Formamide (4 x 5 mL), CE-IVD	4404307
BigDye Terminator Sequencing Standard v1.1, CE-IVD	4462113
BigDye Terminator Sequencing Standard v3.1, CE-IVD	4404310
BigDye Terminator v3.1 Matrix Standards Kit, 3500/3500xL	4336974
BigDye Terminator v1.1 Matrix Standards Kit, 31xx and 3500	4336824
DS-33 Matrix Standard Kit (5-dye), CE-IVD	A25775
DS-33 GeneScan Install Standards, CE-IVD	A25793
DS-02 Matrix Standard Kit (5-dye, E5 dye set)	4323014
DS-32 Matrix Standard Kit (4-dye)	4345831
DS-36 Matrix Standard Kit (6-dye)	4425042
GeneScan 120 LIZ Size Standard	4322362
GeneScan 500 ROX Size Standard	401734
GeneScan 600 LIZ Size Standard v2.0, CE-IVD	A25794
GeneScan 1200 LIZ Size Standard	4379950



applied biosystems

Table 1. Sequencing modules for RUO applications.

Run modules	Throughput[1]			Configuration		Performance		
	Average run time (minutes)	Average throughput, 3500xL Dx (samples/day)	Average throughput, 3500 Dx (samples/day)	Array separation distance (cm)	Polymer type	Resolution range in ≥90% of samples	Bases collected in 90% of samples	QV20 CRL[2] in ≥90% of samples
ShortReadSeq50_POP7[3]	≤30	≥1,104	≥368	50	POP-7	40–400	≥325	≥300
RapidSeq50_POP7	≤40	≥840	≥280	50	POP-7	40–550	≥600	≥500
FastSeq50_POP7	≤65	≥504	≥168	50	POP-7	40–600	≥750	≥700
StdSeq50_POP7	≤125	≥264	≥88	50	POP-7	40–700	≥1,000	≥850
BDxShortReadSeq50_POP7	≤30	≥1,104	≥368	50	POP-7	40–400	≥325	≥300
BDxRapidSeq50_POP7	≤40	≥840	≥280	50	POP-7	40–550	≥600	≥500
BDxFastSeq50_POP7	≤65	≥504	≥168	50	POP-7	40–600	≥750	≥700
BDxStdSeq50__POP7	≤125	≥264	≥88	50	POP-7	40–700	≥1,000	≥850
RapidSeq50_POP6	≤65	≥504	≥168	50	POP-6	20–500	≥450	≥450
FastSeq50_POP6	≤90	≥368	≥122	50	POP-6	20–550	≥600	≥600
StdSeq50_POP6	≤135	≥240	≥80	50	POP-6	20–600	≥700	≥600
BDxRapidSeq50_POP6	≤65	≥504	≥168	50	POP-6	20–500	≥450	≥450
BDxFastSeq50_POP6	≤90	≥368	≥122	50	POP-6	20–550	≥600	≥600
BDxStdSeq50_POP6	≤140	≥240	≥80	50	POP-6	20–600	≥700	≥600
MicroSEQ ID 50_POP6	≤135	≥240	≥80	50	POP-6	20–600	≥700	≥600
FastMicroSEQ ID 50_POP6	≤105	≥312	≥104	50	POP-6	20–500	≥450	≥425

The specifications are reported using the BigDye Terminator v3.1 Sequencing Standard. BDx classified run modules are optimized with the 3500 Dx Series systems to obtain more usable data when sequencing reactions are purified using the BigDye XTerminator Purification Kit.

1. Throughput (samples/day) is determined by the total number of samples that can be run in 23 hours (allows time for sample preparation, instrument maintenance, and warm-up).
2. QV20 CRL is defined as the longest uninterrupted segment of bases with an average of QV ≥20, calculated over a sliding window of 21 base pairs.
3. The fast ShortReadSeq module collects 300 bp in 30 minutes for operations requiring short verification of sequence content (e.g., clone QC verification).

Table 2. Fragment analysis modules for RUO applications.

Module name	Throughput[1]			Configuration		Performance							
						General		Sizing precision[3] of 100% of alleles in ≥90% of samples			Multirun sizing[4] of 100% of alleles in ≥90% of samples		
	Average run time (minutes)	Average throughput, 3500xL Dx (samples/day)	Average throughput, 3500 Dx (samples/day)	Array length (cm)	Polymer type	Resolution range[2] in ≥90% of samples	Largest fragment collected in ≥90% of samples	50–400 bp	401–600 bp	601–1,200 bp	50–400 bp	401–600 bp	601–1,200 bp
FragmentAnalysis36_POP7	≤30	≥1,104	≥368	36	POP-7	60–400	>420	<0.15	NA	NA	<1 bp	NA	NA
Fragment Analysis36_POP4	≤35	≥936	≥312	36	POP-4	60–400	>420	<0.15	NA	NA	<1 bp	NA	NA
FragAnalysis50_POP7	40	≥840	≥280	50	POP-7	40–520	≥600	<0.15	<0.30	NA	<1 bp	<2 bp	NA
FragAnalysis50_POP6	100	≥336	≥112	50	POP-6	20–550	≥600	<0.15	<0.30	NA	<1 bp	<2 bp	NA
LongFragAnalysis50_POP7	125	≥264	≥88	50	POP-7	40–700	≥1,200	<0.15	<0.30	<0.45	<1 bp	<2 bp	<3 bp
HID36_POP4	35	≥936	≥312	36	POP-4	60–400	≥420	<0.15	NA	NA	<1 bp	NA	N/A
SNaPshot50_POP7	30	≥1,104	≥376	50	POP-7	40–120	≥120	<0.50	NA	NA	<1 bp	NA	NA

NA = specification not applicable for this parameter.

1. Throughput (samples/day) is determined by the total number of samples that can be run in 23 hours (allows time for sample preparation, instrument maintenance, and warm-up).
2. Resolution range is defined as the range of bases over which the peak spacing interval divided by the peak width at half peak height is greater than 1.
3. Sizing precision is the standard deviation of sizes for a given allele size across all capillaries in the same run.
4. Multirun sizing is a measure of the precision of the 3500 Series across multiple runs. For example, it would be expected that a 200 bp allele across 3 runs would have an average deviation of <1 bp in 90% of all samples.

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ThermoFisher SCIENTIFIC

Certificate of Analysis

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	2017-01-20
	2017-08-15

TEST	SPECIFICATION		RESULT
VISUAL INSPECTION			
Material is clear, particulate free viscous solution.			
Appearance	Clarity	Conformance	Pass
FUNCTIONAL TESTING			
Each bulk lot must pass all functional performance specifications including KB Q20 quality score.			
Read Length	>=90% of all runs pass		Pass

Manufactured in compliance with our ISO 9001, ISO 13485 certified quality management system and 21 CFR 820 quality system regulations.

Made in USA

Life Technologies Holdings Pte Ltd
Block 33, Marsiling Industrial Estate Road 3 #07-06
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For inquiries, contact us at cofarequests@lifetech.com

CE IVD

Quality Assurance
Issued 24JAN17

Declaration of Conformity

LABSYSTEMS DIAGNOSTICS
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Identification of the legal entity	**Labsystems Diagnostics Oy** **Tiilitie 3, 01720 Vantaa** **Finland** **Tel. +358-(0)20 155 7530** **Fax. +358-(0)20 155 7521**
Identification of the device(s) concerned	**NeoNat SCID-SMA Real-Time PCR Kit** *cat. no. :* 8100481

We hereby declare that the above mentioned device complies with the requirements of Council Directive 98/79/EC and the corresponding Finnish National Act 629/2010 and to the following standards.

Standards	**EN ISO 14971:2019** *Medical devices- application of risk management to medical devices.* ***EN ISO 15223-1:2016*** *Medical devices. Symbols to be used with medical device labels, labelling and information to be supplied. Part 1: General requirements.* ***EN ISO 13485:2016*** Medical devices. Quality management systems. Requirements for regulatory purposes. **EN 13612:2002** Performance evaluation of *in vitro* diagnostic medical devices **EN ISO 23640:2015** *In vitro* diagnostic medical devices. Evaluation of stability of *in vitro* diagnostic reagents **EN ISO 18113-1:2011** *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labelling) Part 1: Terms, definitions and general requirements

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Declaration of Conformity

	EN ISO 18113-2:2011 *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labelling) Part 2: In vitro diagnostic reagents for professional use
IVDD classification	**General IVDD**
Conformity assessment procedure	**Annex III of the Directive 98/79/EC**
Notified body	**Not applicable**
Signature of the authorized person	In Vantaa on the 13th April 2021 **Sameer Saral** **Chief Operations Officer**

www.labsystemsdx.com
LABSYSTEMS DIAGNOSTICS OY
VANTAA | FINLAND

Labsystems Diagnostics Oy
Address Tiilitie 3, FI-01720 VANTAA Finland
Email contact@labsystemsdx.com
Web www.labsystemsdx.com
Business ID 2494052-3
Tel +358 (0) 20 155 7530
Domicile Vantaa
VAT No FI24940523
Fax +358 (0) 20 155 7521

LABSYSTEMS DIAGNOSTICS
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REF 8100481, 8100482, 8100483

NeoNat SCID-SMA

Real-Time PCR Kit

CE

96/192/480 reactions

Instructions for use

IVD

Labsystems Diagnostics Oy
Tiilitie 3, FI-01720 Vantaa, Finland
Tel. +358-20-155 7530, Fax +358-20-155 7521
E-mail: contact@labsystemsdx.com
www.labsystemsdx.com

25.05.2022

INSTRUCTION FOR USE

The NeoNat SCID-SMA Real-Time PCR Kit is a Real-Time PCR assay for screening Severe Combined Immunodeficiency (SCID), X-linked Agammaglobulinemia (XLA) and Spinal Muscular Atrophy (SMA) in newborn's DNA from Dried Blood Spot (DBS) samples.

Product no.:

8100481 .....96 reactions

8100482.....192 reactions

8100483 ..... 480 reactions

CONTENTS Page

INTENDED USE

Labsystems Diagnostics' NeoNat SCID-SMA Real-Time PCR Kit is a Real-Time PCR kit for the screening of Severe Combined Immunodeficiency (SCID) by semi quantitative determination of *TREC* (T-cell Receptor Excision Circle), & *KREC* (Kappa-deleting Recombination Excision Circle), Spinal Muscular Atrophy (SMA) by measuring *SMN1* (Survival Motor Neuron 1), & *SMN2* (Survival Motor Neuron 2) and X-linked agammaglobulinemia (XLA) in newborn's DNA extracted from DBS samples.

This assay should only be used in conjunction with other clinical and confirmatory laboratory findings and results from this test alone should not be used to make diagnostic or treatment decisions.

INTRODUCTION

Severe Combined Immunodeficiency

Severe combined immunodeficiency is a group of rare disorders caused by mutations in different genes involved in the development and function of infection-fighting immune cells. Patients with SCID have low numbers of T-cells and their B-cells do not function. Most often, SCID is inherited in an autosomal recessive pattern, in which both copies of a particular gene - one inherited from the mother and one from the father - contain defects. X-linked SCID, which is caused by mutations in a gene on the X chromosome, primarily affects male infants [1, 2].

The SCID newborn screening test measures *TREC*s, a byproduct of T-cell development. *TREC*s are small pieces of DNA generated in T-cells as they mature. Because infants with SCID have few or no T-cells, the absence of *TREC*s may indicate SCID. Research supported by National Institute of Health and other organizations has shown that early diagnosis of SCID through newborn screening leads to prompt treatment and high survival rates [1, 2].

Molecular assay that detects the absence of *TREC*s using DBS samples has been the most effective way to diagnose SCID. There are other genetic syndromes (e.g., DiGeorge) or conditions (e.g., congenital heart disease) that can also lead to reduction in the number of circulating T-cells [1-4].

KRECs, an extrachromosomal excision products similar to *TRECs*, are formed as a result of generation of diverse B-cell receptor light chains. A rise in *KREC*s reflects the presence of newly derived bone marrow B-cells after hematopoietic cell transplantation in primary immune deficient patients. *KREC* levels in DNA isolated from human blood are undetectable in primary immunodeficiencies (PIDs) in which B-cells are absent or dysfunctional. Thus, testing newborn DBS samples for the presence of *KREC*s is potentially useful for identifying neonates with defects in early B-cell maturation. *TREC*s and *KREC*s can be measured simultaneously with Labsystems Diagnostics' NeoNat SCID-SMA Real-Time PCR Kit [5, 6].

To confirm a SCID diagnosis, a doctor will evaluate the numbers and types of T-cells and B-cells present in the blood samples as well as their ability to function [7].

X-linked Agammaglobulinemia

X-linked agammaglobulinemia (XLA) is one of the PIDs caused by mutations in Bruton tyrosine kinase (BTK) or B-cell negative SCID. XLA is characterized by severe B-cell lymphopenia and marked reduction in all classes of serum immunoglobulins. XLA can be identified by screening for *KREC*, the excised circular by-product of B-cell immunoglobulin kappa gene rearrangement [5-7].

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is a group of hereditary diseases that are caused by progressive degeneration of motor neurons - nerve cells in the brain stem and spinal cord, which control essential skeletal muscle activity and important body functions such as speaking, walking, breathing, and swallowing. The death of motor neurons leads to muscle weakness and atrophy [8, 9].

The disorder is caused by defects in both copies of the survival motor neuron 1 gene (*SMN1*) on chromosome 5q. This gene produces the survival motor neuron (SMN) protein which maintains the health and normal function of motor neurons. Low levels of SMN1 protein result in loss of function of neuronal cells in the spinal cord which in turn leads to weakness and degeneration (or atrophy) of the skeletal muscles [8-11].

There are two *SMN* genes, *SMN1* and *SMN2, which are* nearly identical and encode the same protein. However, *SMN2 gene* contains a mutation that disrupts a splice enhancer resulting in production of mainly unstable and less functional protein. Only 10-20% of SMN protein synthesized from *SMN2* gene are fully functional.

Mutations in *SMN1* are associated with spinal muscular atrophy; whereas mutations in *SMN2*, do not lead to SMA. Simultaneous detection of SMA-related mutations in both *SMN1* and *SMN2* by PCR with specific primers and probes, is more reliable way of screening for SMA, than detecting only *SMN1* mutations, because the multiplication of *SMN2* gene can compensate to some extent the negative effect of *SMN1* loss-of-function mutations. To treat SMA, SMA clinical working group unanimously recommended immediate treatment for individuals predicted to manifest SMA with the qualifying genotypes of two or three copies of *SMN2* as supported by the strong positive results arising from in the NURTURE trial [8,12].

PRINCIPLE OF THE TEST

The principle of the Labsystems Diagnostics' NeoNat SCID-SMA Real-Time PCR kit is based on the 5' nuclease amplification technique. NeoNat *SCID-SMA* Real-Time PCR kit is designed to measure *TREC, KREC*, *SMN1, SMN2 and β-globin (BG)* genes from DBS samples of newborns. The kit includes 2 plates with ready to use optimized reaction mixture for simultaneous amplification and detection of 3 genes per plate: (i) *TREC, KREC* and *β-globin* genes in SCID PCR reaction (*β-globin* is used as an internal control) and (ii) *SMN1, SMN2* and *β-globin* genes in SMA PCR reaction (*β-globin* is used as an internal control). The following steps are carried out:

1. A 3.2 mm disk is punched out from Newborn DBS specimens and DBS controls.
2. The DNA is eluted from Newborn DBS specimens and DBS control discs.
3. The DNA samples from Newborn DBS specimens and controls are added to ready to use PCR plate which contains all the reagents necessary to run the assay.
4. The PCR plate is transferred to Real-Time PCR instrument for amplification.
5. The fluorescence signal from target specific probes is measured using corresponding channels.

(Optional: procedures listed above are also performed with DBS calibrators when they are used).

This kit is validated in CFX96 Real-Time PCR instrument from Bio-Rad Laboratories and the compatability of this SCID-SMA Real-Time PCR kit is confirmed with ABI QuantStudio™ 5 Real-Time PCR System. It can be also used with other Real-Time PCR instrument with minimum of three measurement channels (FAM, HEX/VIC and CY5). The quality control criteria provided in the IFU can deviate from the user's data acquired using their own PCR instrument. Kit contains passive reference dye that is detected using the ROX channel. The threshold cycle (C_t) values are calculated after baseline correction. The result interpretation for each condition is based on C_t values of three genes (*TREC, KREC* and *β-globin for* SCID and XLA*; SMN1,SMN2 and β-globin for* SMA*)*. The test performance is monitored by four level DBS controls.
If the kit is used with other commercially available equipments, the methods shall be verified and validated as appropriate.

SCID PCR Assay

Gene	Detection Channel
TREC	FAM
KREC	VIC/HEX
β-globin	CY5
Optional ROX background correction	

In SCID PCR amplification plate, the amplification of *TREC* is measured with the FAM fluorescence channel, the amplification of *KREC* is measured with the VIC/HEX fluorescence channel and the amplification of the internal control is measured with the CY5 fluorescence channel. Depending on the qPCR instrument used, ROX channel can be used for background correction.

SMA PCR Assay

Gene	Detection Channel
SMN1	FAM
SMN2	VIC/HEX
β-globin	CY5
Optional ROX background correction	

In the SMA PCR amplification plate, the amplification of *SMN1* is measured with the FAM fluorescence channel, the amplification of *SMN2* is measured with the VIC/HEX fluorescence channel and the amplification of the internal control is measured with the CY5 fluorescence channel. Depending on the qPCR instrument used, ROX channel can be used for background for correction.

KIT CONTENTS

Note:

1. **Storage:** PCR plates and DBS calibrator and DBS controls (kit box 2) must be stored at -20 °C (-18°C - -24°C). Other reagents (kit box 1) must be stored at room temperature (RT) between +10 °C and +27 °C
2. **Shelf life**: The expiration date is printed on each component's label and on the package. Do not use reagents after the expiration date.
3. Protect all the kit components from moisture and exposure to light.

I. SCID-SMA Real-Time PCR kit Box 1 (Shipped at **RT (+10 °C - +27 °C)**)

S. No.	Content	96 Rxn/kit	192 Rxn/kit	480 Rxn/kit
1	DBS Extraction plate	1 pc	2 pcs	5 pcs
2	DBS Extraction plate lid	1 pc	2 pcs	5pcs
3	Receiver plate	1 pc	2 pcs	5 pcs
4	Holding clip	2 pc	2 pcs	2 pcs
5	96 well plate	1 pc	2 pcs	5 pcs
6	DBS Washing solution	1x60ml	2x60ml	5x60ml
7	DBS Extraction solution	1x15ml	1x30ml	1x75ml
8	Adhesive PCR plate seal	4 pcs	8 pcs	20 pcs
9	Reagent Basin	3 pcs	6 pcs	15 pcs
10	IFU	1	1	1

II. SCID-SMA Real-Time PCR kit Box 2 (To be either shipped with **dry ice or by maintaining at -20 °C (-18°C - -24°C)).**

1. **SCID PCR plate 1/2/5 pcs**

Contains dNTPs, $MgCl_2$, PCR buffer, specific primers and probes and DNA polymerase needed for the detection and quantification of *TREC*, *KREC,* and *β-globin*. **Row A (A1-A12) contains the mixture of *TREC, KREC, and* *β-globin* plasmid DNA in duplicates in concentrations from 1,000,000 to 10 copies per µl for each target (see plate layout below)*.** The data collected from these wells are used for preparing a standard curve for SCID assay.

2. SMA PCR plate 1/2/5 pcs

Contains dNTPs, $MgCl_2$, PCR buffer, specific primers and probes and DNA polymerase needed for the detection and quantification of *SMN1, SMN2, and β-globin*. **Row A (A1 -A12) contains the mixture of *SMN1, SMN2, and β-globin* plasmid DNA in duplicates in concentrations from 1,000,000 to 10 copies per µl for each target (see plate layout below)*.** The data collected from these wells are used for preparing a standard curve for SMA assay.

3. DBS Control 1/2/5 pcs **CONTROL**

The DBS control panel includes 4 different DBS spots which contain defined number of copies of *TREC, KREC, SMN1, SMN2* and *β-globin* plasmid DNA per µl of blood in the DBS. The approx. number of copies per µl of *TREC, KREC, SMN1, SMN2* and *β-globin* in DBS controls are given in the section **QUALITY CONTROL**. The place of the DBS controls on the plate are marked with C1-C4 in the plate layout table below.

4. DBS Calibrator 1/2/5 pcs **CAL**

Optional: The DBS calibrator panel includes 4 different DBS spots (Cal 1, Cal 2, Cal 3, and Cal 4) which contain TREC, KREC, SMN1, SMN2 and β-globin plasmid DNA in defined concentrations (in copies per µl of the blood in the DBS). The use of DBS calibrators is optional. The data collected from the wells containing DBS calibrators can be used as alternative to prepare standard curve. If DBS calibrators are used to prepare standard curve, the lab should establish their own standard curve acceptance criteria and assay cut-off.

Well Positions A1-12 in PCR plate already contain all the reagents required for the standard curve preparation

Plate Format

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	B	B	C	C	D	D	E	E	F	F
B	C1	C1	C2	C2	C3	C3	C4	C4				
C												
D												
E												
F												
G												
H												

Wells	Marked above as	Plasmid Calibrators
A1-A2	A A	10^6 copies/µl
A3-A4	B B	10^5 copies/µl
A5-A6	C C	10^4 copies/µl
A7-A8	D D	10^3 copies/µl
A9-A10	E E	10^2 copies/µl
A11-A12	F F	10^1 copies/µl

MATERIALS REQUIRED BUT NOT PROVIDED WITH KIT

1. Real-Time PCR instrument with at least three detection channels suitable for FAM, HEX/VIC, and CY5 measurement.
2. Disposable gloves.
3. Adjustable pipettes (20-200 µl, 1-10 µl).
4. Sterile pipette tips with filter (10 µl, 40-200 µl).
5. 96-well plate centrifuge (Any plate centrifuge that can provide the speed of 2500 rpm).
6. A 96-well plate shaking incubator with temperature control of at least 99 °C and shaking up to 1000 rpm.
7. DBS puncher
8. Tweezers

SPECIMEN COLLECTION AND HANDLING

1. Whole blood from newborn heel prick must be collected on Guthrie card with suitable filter paper and dried to form DBS. Collection of samples must be done as DBS from infants for newborn screening following CLSI document LA4-A5 [13-15].
2. The DBS must be dried in clean, dry and protected area for at least 3 hrs or overnight at RT.
3. Each DBS card must be packed separately in a bag with desiccant to prevent contamination from each other.
4. Newborn screening DBS specimens should be tested upon receipt and can be stored at ambient temperature with low humidity during handling.
5. The DBS samples are punched in 3.2 mm in diameter discs to the filter plate for DNA extraction.
6. Making a blank punch (punching in an empty filter paper) in between the samples is needed to avoid cross contamination of the DBS samples.
7. Low humidity and low temperatures (+4 - +8 °C) are suggested for short term storage of DBS. For storage of more than 2 years, low humidity and frozen conditions (-20 to -70 °C) are recommended.

PRECAUTIONS

Warning - POTENTIAL BIOHAZARDOUS MATERIAL:

All human materials used in the preparation of the calibrators/controls in the kit have been tested for the presence of the antibodies to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) as well as Hepatitis B surface antigen (HBsAg) and found to be non-reactive. As no test method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent, DBS calibrators, DBS controls and Newborn DBS samples should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood sample in the Centers for Disease Control and Prevention/National Institutes for Health Manual, “Biosafety in Microbiological and Biomedical Laboratories”, 5th Ed. 2009.

Discard all materials and samples as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at +121°C. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500 mg/l free chlorine. Allow 30 minutes for decontamination to be completed. Spills should be wiped off thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe off spills should be added to biohazardous waste matter for proper disposal. Reusable glassware must be disinfected, washed and rinsed free of detergents.

1. Avoid contact with skin and eyes when handling extraction and elution solutions.
2. Extreme precautions should be taken to prevent contamination of the reactions. Amplification procedures require highly skilled professionals.
3. Tubes containing any clinical samples and PCR plates should never be opened at the same time.
4. If the kit contents are broken/leaking, the kit components should not be used.
5. Wear disposable gloves while handling samples and kit reagents. Afterwards wash hands carefully. Never pipette by mouth.
6. Do not use the same pipette tips to handle different samples.

7. All pipettes used must be equipped with sterile filter tips.
8. Do not re-use a strip or plate even if some wells were not used.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements, are essential.
10. Do not eat, drink or smoke in dedicated work areas.
11. Disposal of all waste should be in accordance with local regulations.
12. All testing labs should strictly adhere to CLSI or equivalent regulatory guidelines [16,17]

TEST PROCEDURE

Please read the test procedure carefully before starting the experiement

DBS washing and extraction

1. **Keep row A (A1-A12) of the extraction plate empty**
2. Punch 3.2 mm disks from DBS controls (C1, C2, C3, and C4) in duplicates to wells B1-B8.
   Optional: punch 3.2 mm disks from DBS Calibrators in duplicates into the wells of 96 well DBS extraction plate.
3. Then punch 3.2 mm DBS clinical samples into wells from B9 to H12.
4. Pipette 200 µl of DBS washing solution to each well ***except wells in row A (A1-A12).*** **Keep row A (A1-A12) empty.** ***Use the plate cover to avoid contamination and evaporation of samples***.
5. Incubate the DBS extraction plate in a shaker incubator at RT at 700 rpm for 10 minutes.
6. After incubation, stack the DBS extraction plate on the receiver plate and fix them tight with the clip. Then centrifuge at 2500 rpm for 2 minutes and discard the flow through.
7. Repeat steps 4-6.
8. Pipette 65 µl of DBS extraction solution to each well **except wells in row A (A1-A12). Keep row A (A1-A12) empty.** ***Cover the plate with the plate cover to avoid contamination and evaporation of samples.***
9. Incubate the DBS extraction plate in a shaker incubator at +95 °C with shaking at 700 rpm for 15 minutes.
10. After incubation, cool the DBS extraction plate at RT for 2 minutes, place on top of a fresh 96-well plate and i**mmediately** centrifuge the sample at 2500 rpm for 2-3 minutes.
11. The volume of extracted DNA solution is ~50 µl. It is possible that some of the DBS spot extracted DNA is not filtered through the extraction plate to the 96 well plate. In that case, the extracted DNA in the DBS extraction plate can be pippetted out manually to the 96 well plate.
12. Add 50 µl of DBS washing solution to the ~50 µl of extracted DNA solution ***except wells in row A (A1-A12).*** **Keep row A (A1-A12) empty.** Mix it thoroughly by brief shaking the plate in the plate shaker. The total volume of DNA solution is approx.100 µl.

PCR amplification

13. Remove the PCR plates from the freezer and leave at RT for 5 minutes to thaw. Centrifuge briefly at 1500 rpm for 30 seconds.
14. **Leave the seal for A row (A1-A12) unopened.** ***Row A (A1-A12) is already loaded with plasmid calibrators.*** **There is a cut line at the seal between rows A & B. Carefully remove the aluminium seal from wells B1 to H12.**
15. Transfer 10 µl of the DNA solution from the plate containing extracted DNA to the wells B1-H12 of PCR plate. **Do not add anything to the wells in row A** ***(wells A1-A12 are pre-loaded with plasmid calibrators).***
16. Seal the wells from B1 to H12 with plastic PCR sealer.
17. Carefully remove the aluminium seal from wells A1-A12 of row A.
18. Re-seal the whole plate (A1-H12)* with plastic PCR sealer. Centrifuge briefly at 1500 rpm for 30 seconds.
19. Place the plate in PCR machine and run the PCR amplification protocol as mentioned below.

TEMPERATURE	TIME	CYCLES
50°C	2 min	1
95°C	5 min	1
95°C	15 sec	40
60°C	30 sec	
20°C	15 sec	1

NOTES: *Do not store and handle PCR plates in the same area as the samples, DBS controls and DBS calibrators to avoid contamination.*

The part of the plate between B1 and H12 row is sealed twice. This is to ensure the absence of cross-contamination from plasmid calibrators to other wells.

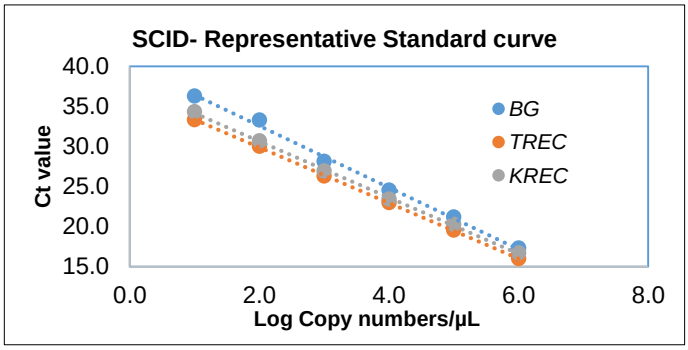
THRESHOLD CYCLE

Programming of the instruments is carried out according to the instrument's user manual. Fluorescence data are plotted against the number of cycles. The threshold cycle (C_t or C_q) serves as a tool for calculation of the starting template amount in each sample. The threshold is adjusted to a value above the baseline, but must be located in the log-linear range of the PCR curve. Before determining the C_t value, ensure that the **baseline is positioned correctly, i.e., between 3 and 15 cycles** and **adjust the threshold, if necessary**. Each laboratory should establish its own threshold.

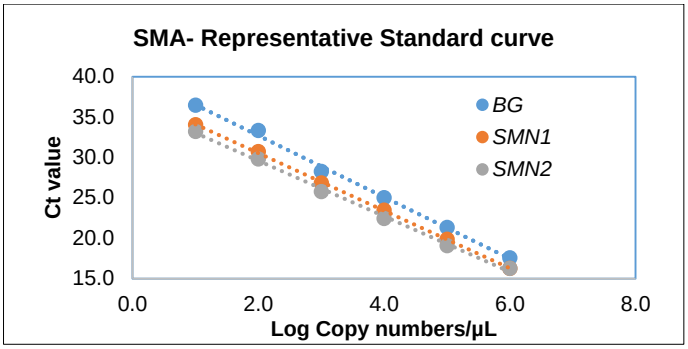
ANALYTICAL PERFORMANCE

Standard Curve

Representative plots of C_t values and concentrations of plasmid calibrators in copies/µl are shown below. This PCR kit is validated using Bio-Rad PCR instrument and all the values are automatically calculated by the Bio-Rad CFX Maestro software. Each laboratory should collect C_t values for plasmid calibrators and build own standard curve for each test plate.



ID	Copies/ µl	C_t value		
		β-globin (BG)	*TREC*	*KREC*
STD 1	10^6	19.6	18,0	19,7
STD 2	10^5	22.9	21,3	23,1
STD 3	10^4	26.3	24,6	26,5
STD 4	10^3	29.7	28,0	30,0
STD 5	10^2	33	31,3	33,3
STD 6	10^1	36.3	34,5	36,7



ID	Copies/ µl	C_t value		
		β-globin (BG)	*SMN1*	*SMN2*
STD 1	10^6	19,7	16,9	15,6
STD 2	10^5	22,9	20,2	18,9
STD 3	10^4	26,3	23,6	22,3
STD 4	10^3	29,7	27,1	25,7
STD 5	10^2	32,9	30,4	29,0
STD 6	10^1	35,9	33,6	32,5

Note: Concentration of DNA for each tested gene in unknown DBS clinical specimens is automatically calculated by the Bio-Rad PCR instrument's CFX Maestro software (using a standard curve built based on the results collected for plasmid calibrators).

Limit of Detection (LOD)/Sensitivity

The Limit of Detection was determined using Bio-Rad PCR instrument's CFX Maestro software in accordance with the CLSI EP17-A2 guidelines. The LOD for each gene is shown below [18].

Genes	Copies/µl
TREC	4
KREC	4.9
SMN1	1.2
SMN2	2.4

Linearity

Linearity of the SCID-SMA assay was evaluated using Bio-Rad PCR instrument's CFX Maestro software according to CLSI EP6-A using 4 DBS specimens. The results are summarized below [19].

Parameter	*β-globin*	*TREC*	*KREC*	*SMN1*	*SMN2*
Range (copies/µL)	10-9306	15-6559	20-14943	05-8959	11-10767
Slope	-0.99	-1.01	-0.95	-1.08	-0.99
X-intercept	4.97	5.22	5.11	5.12	5.04
R square	0.99	1	0.99	0.99	0.99

QUALITY CONTROL

1. A full set of plasmid calibrators and DBS controls must be run in duplicates in each run.
2. Each laboratory should establish mean values and acceptable ranges for C_t values of plasmid/DBS calibrators and DBS controls to assure proper performance.
3. The results should be reported only if the plasmid calibrator and control values meet the acceptance criteria.

Standard curve:

The standard curve parameters should meet the following criteria for the valid assay. This PCR kit is validated using Bio-Rad PCR instrument and the results are confirmed with Quantstudio™ 5 PCR instrument. Since each and every PCR instrument might differ in their assay values, each laboratory should standardaise the assay parameters with their own PCR instrument in accordance with the IFU.

SCID assay

Parameters	*Acceptance Criteria*
	β-globin/TREC/KREC
Efficiency	85-115%
Slope	-2.99 to -3.65
R^2	>0.9

SMA assay

Parameters	*Acceptance Criteria*
	β-globin/SMN1/SMN2
Efficiency	85-115%
Slope	-3.01 to -3.68
R^2	>0.9

Optional: DBS Calibrators

Calibration curve can be also built using the results collected from DBS calibrators.

The theoretical concentration of DNA that are spiked into the DBS calibrators in plasmid copy numbers per µl and the representative C_t values for the extracted plasmid DNA are presented in the following table

ID	Spiked plasmids, copies/ µl	C_t value				
		BG	*TREC*	*KREC*	*SMN1*	*SMN2*
Cal 1	10^6	23.5	23.3	24.4	21.9	22
Cal 2	10^5	26.8	26.6	27.9	25.2	25.4
Cal 3	10^4	30.2	29.9	31.3	28.5	28.7
Cal 4	10^3	33.5	33.4	34.5	31.7	32.1

DBS Controls:

The DBS controls should meet the following criteria for the valid assay.

SCID plate

Controls	***Acceptance Criteria***		
	β-globin, copies/ µl	*TREC,* copies/ µl	*KREC,* copies/ µl
C1	>100	>30	>30
C2	>100	>30	<15
C3	>100	<15	>30
C4	>100	<15	<15

SMA Plate

Controls	***Acceptance Criteria***		
	β-globin, copies/ µl	*SMN1,* copies/ µl	*SMN2,* copies/ µl
C1	>100	>30	>30
C2	>100	>30	<15
C3	>100	<15	>30
C4	>100	<15	<15

RESULTS INTERPRETATION

The standard curve parameters should meet the following criteria for the valid assay. This PCR kit is validated in Bio-Rad PCR instrument and the results are confirmed with Quantstudio™ 5 PCR instrument. Since each and every PCR instrument might differ in their assay values, each laboratory should standardaise the assay parameters with their own PCR instrument accordance with the IFU.

CUT-OFF VALUES

Each laboratory should establish the **cut-off** values by testing large number of normal newborn samples and available positive samples.

1. For SCID assay, concentrations of *TREC, KREC* and *β-globin* in copies/µl can be determined. The results are interpreted as follows:

- *if the value of β-globin is undetermined or lower than 1000 copies/µl, then the result is invalid*. The sample should be retested.
- If concentrations of *TREC* (in copies/µl) can not be calculated or lower than established cut-off value and *β-globin* values are >1000 copies, the sample is considered as SCID positive in screening.
- If concentrations of *KREC* (in copies/µl) can not be calculated or lower than established cut-off value and *β-globin* values are >1000 copies, the sample is considered as SCID & XLA positive in screening (20, 21).

2. For SMA assay, concentrations of *SMN1, SMN2* and *β-globin* in copies/µl can be determined. The results are interpreted as follows:

- *if the value of β-globin is undetermined or lower than 1000 copies/µl , then the result is invalid.* The sample should be tested again.
- If concentration of *SMN1* in copies/µl can not be calculated, SMN2 values can be calculated and β-globin values are >1000 copies, the sample is considered as SMA screen positive.

- **Note:** The quality of extracted DNA and/or the presence of the amplification reaction inhibitors is verified using the β-globin.
- Retesting of samples are recommended when value below or near to cut off
- SMN2 copies or ct values from RT PCR may be used to calculate the actual gene copies in the genome. The laboratory may establish and validate the SMN2 gene copies and in relation to clinical characteristics of patients with SMA (21,22).

The laboratory should use the SCID and SMA screening algorithm adopted in their country.

CLINICAL SENSITIVITY

The screening performance of SCID-SMA kit was evaluated using clinical DBS samples from normal (n=14), SCID positive (n=4), and SMA positive (n=4) newborns.

SCID - Clinical Sensitivity: 100%; clinical specificity: 100%

		SCID		
		Nomal	SCID positive	Total
Clinical diagnosis	Normal	14	0	14
	SCID	0	04	04
	Total	14	04	18

SMA - Clinical Sensitivity: 100%; clinical specificity: 100%

		SMA		
		Nomal	SMA positive	Total
Clinical diagnosis	Normal	14	0	14
	SMA	0	04	04
	Total	14	04	18

LIMITATION OF THE PROCEDURE

a) The kit is not designed to detect “leaky SCID”: a condition when a person has symptoms similar to typical SCID, but with T cell counts that aren't low enough to qualify as typical SCID.
b) The sample discs should be punched carefully as improperly collected samples can cause aberrant results.
c) This assay is sceening assay. Because no single method leads to the definitive diagnosis, the results of the present method should be interpreted in conjunction with the clinical conditionand other laboratory findings.
d) It is recommended that the assay is performed by qualified and trained laboratory technician.

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MANUFACTURER:

Labsystems Diagnostics Oy.
Tiilitie 3, FI-01720 Vantaa, Finland.
Tel. +358-20-155 7530
Fax. +358-20-155 7531
E-mail: support@labsystemsdx.com
www.labsystemsdx.com

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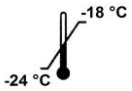

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Manufacturer


Consult instructions for use

DBS Control

DBS Calibrator


Potential biohazardous material

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NeoNat SCID SMA

REAL TIME PCR KIT

The NeoNat SCID SMA is a Real-Time PCR assay for screening Severe Combined Immunodeficiency (SCID) Syndrome and Spinal Muscular Atrophy (SMA) in newborns DNA from Dried Blood Spot (DBS) samples.

- Greater flexibility with separate multiplexing of SCID and SMA from one kit
- Kit contains all the reagents and components to perform DNA extraction from DBS
- Ready to use reaction mix and calibrators preloaded plates enable convenience for users
- Four levels of DBS controls with defined target gene copies per µL
- Compatible with multiple qPCR instruments
- Short turnaround time of ~120 mins from DNA extraction to result analysis

CE IVD RUO Research Use Only

Assay Description

- NeoNat SCID SMA Real time PCR kit is based on 5' nuclease technique
- Screens T-cell receptor excision circle (TREC) / Kappa deleting recombination excision circle (KREC) for SCID and Survival Motor Neuron 1 (SMN1) & Survival Motor Neuron 2 (SMN2) for SMA. The β-globin gene serves as an internal control

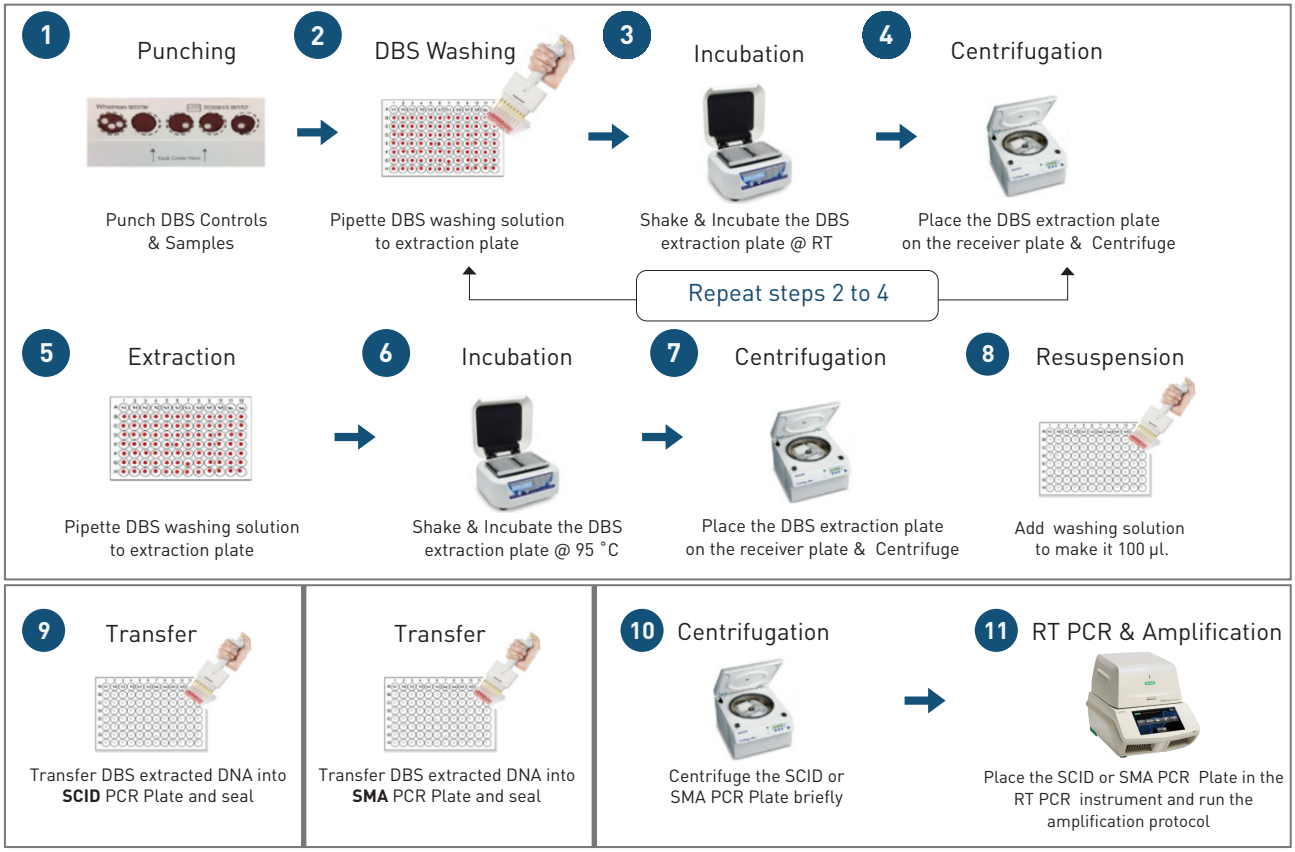
Performance

- The assay demonstrates excellent performance with 100% sensitivity and specificity for all the samples tested
- The analytical sensitivity shows a limit of detection of <5.0 copies/µL for TREC & KREC and <2.5 copies/µL for SMN1 & SMN2

Compatibility

- Kit compatible with most real-time PCR instruments in the market with at least three measurement channels (FAM, VIC/or HEX, & CY5)
- Developed and validated with Biorad CFX 96 and Thermofisher Quantstudio 5 & 6.

DNA EXTRACTION FROM DBS & PCR REACTION



Ordering Information

96 Reactions	8100481
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*Please check the availability

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