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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!

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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.

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).

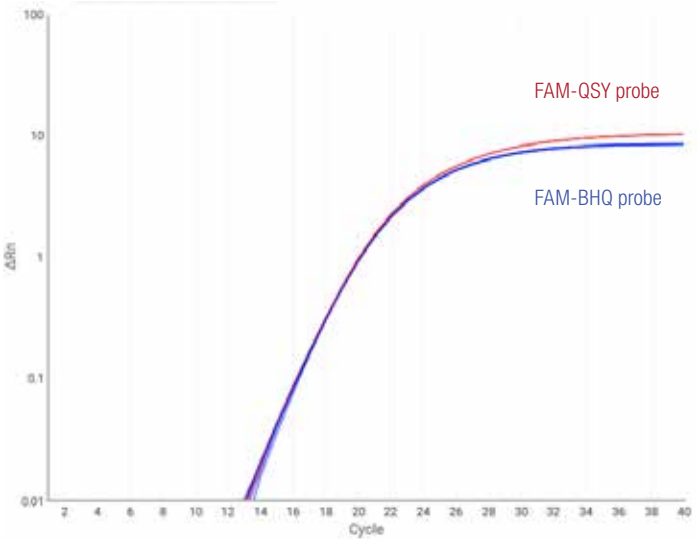

Figure 2. QSY probes have performance similar to that of BHQ probes. A FAM-QSY probe and a FAM-BHQ probe with identical oligonucleotide sequences and master mixes have similar C_t values.

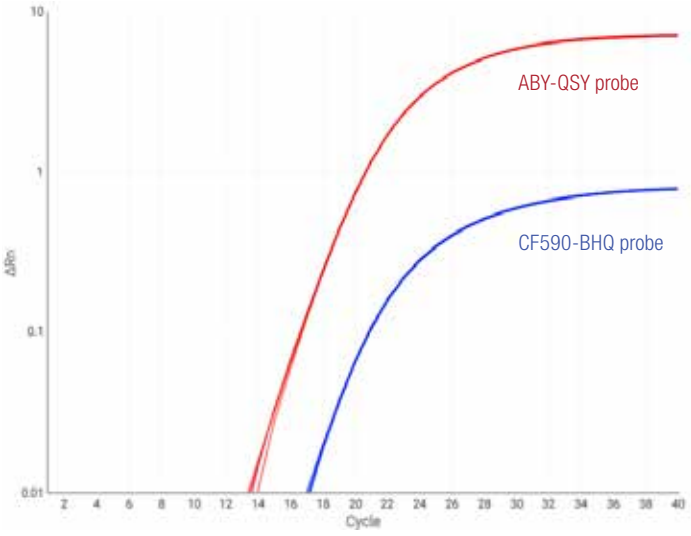

Figure 3. Improved performance in multiplex qPCR. In this multiplex experiment, the ABY-QSY probe shows a significantly lower C_t than the CF590-BHQ probe with an identical oligonucleotide sequence and master mix.

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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).

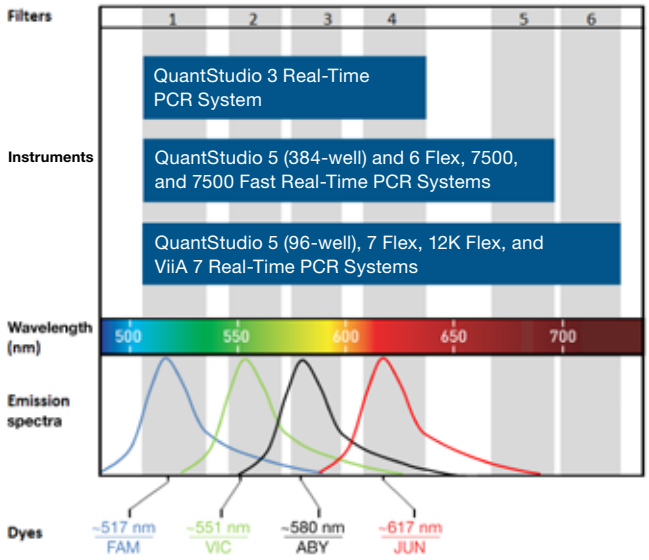

Figure 4. Fluorescence emission wavelengths used for multiplex real-time PCR. Emission spectra for FAM, VIC, ABY, and JUN dyes are shown in relation to regions of the spectrum detected by six filters available on Applied Biosystems real-time PCR instruments.

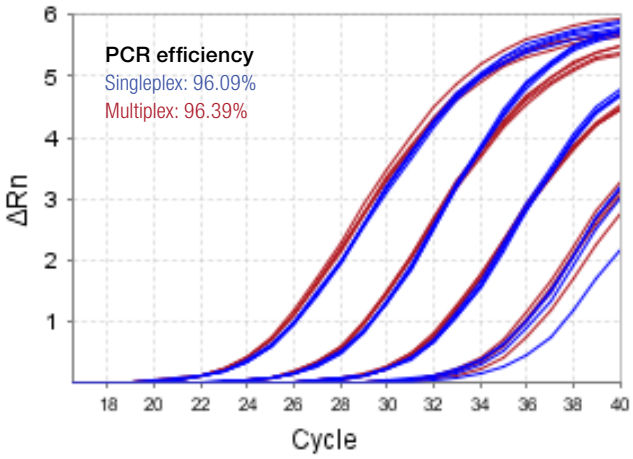

Figure 5. Comparable results for singleplex and multiplex assays. The amplification plot shows linear portions of the curves for 4 EGFR assays amplified in singleplex (blue) and 4-plex reactions (red) in a dilution series from 20,000 pg to 2 pg of reference colon cDNA per 10 µL reaction. PCR efficiencies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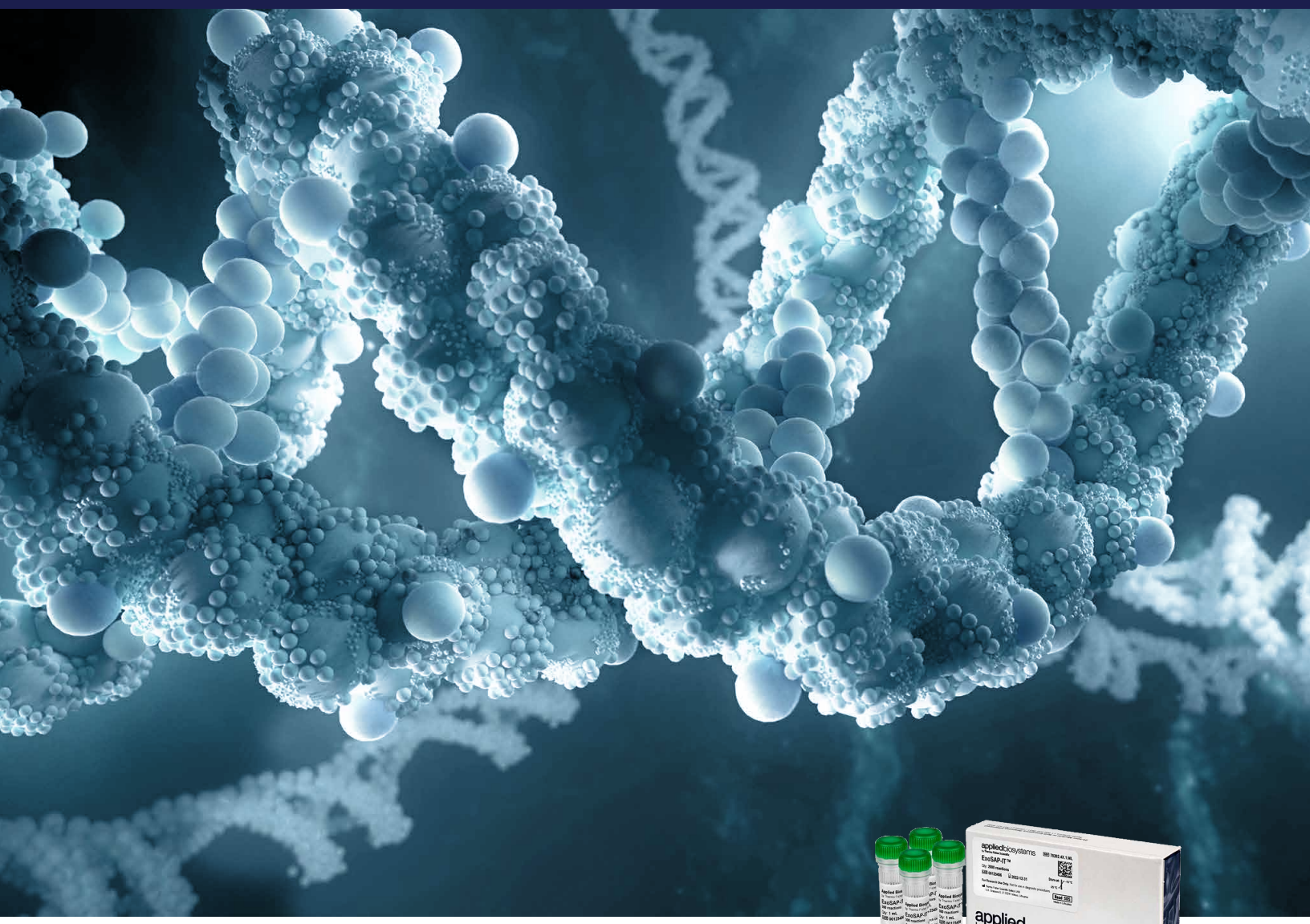

ExoSAP-IT PCR cleanup reagents

One step to superior sequencing results

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ExoSAP-IT *Express* reagent: PCR cleanup in as little as 5 minutes

Our one-step Applied Biosystems™ ExoSAP-IT™ *Express* PCR Product Cleanup Reagent enables quality sequencing results in a fraction of the time.

- **5 min protocol**—fastest enzymatic cleanup of PCR product
- **One-tube, one-step PCR cleanup**—add reagent directly to PCR product
- **Novel enzyme technology**—enzymes irreversibly inactivated in just 1 min at 80°C
- **Conserve PCR samples**—100% recovery of PCR products, regardless of amplicon length
- **Scalable**—treat PCR reaction in volumes from 5 µL to 5 mL
- **Eliminate spin columns or magnetic beads**—helps decrease time and expense while increasing yield

High-quality, accurate results

Compared with alternative PCR cleanup methods, ExoSAP-IT *Express* PCR Product Cleanup Reagent helps to ensure the availability of purified samples ready for downstream applications in just 5 minutes. This unique, highly stable one-tube solution allows for 100% recovery of DNA and longer read lengths for greater confidence, consistency, and accuracy. PCR products give superior sequencing results when treated with ExoSAP-IT *Express* reagent (Figure 1).

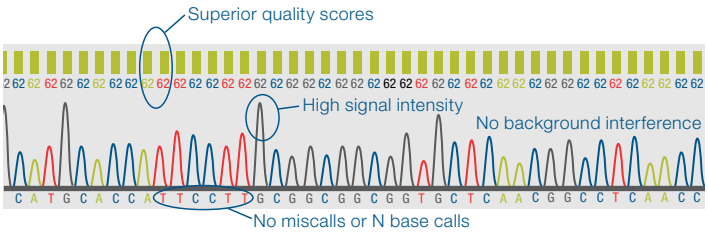

Figure 1. Sequencing results of a 1 kb PCR product treated with ExoSAP-IT *Express* reagent. Treatment with ExoSAP-IT *Express* reagent prior to sequencing eliminates miscalls and improves sequencing scores (numbers and bars above sequence; quality score >60, probability of error ≤0.0001%). Sequence shown is approximately 400 bases from the primer binding site.

Fastest PCR cleanup method

ExoSAP-IT *Express* reagent includes a novel exonuclease I that removes unincorporated primers and nucleotides with a reduced purification time. Only one pipetting step is required, simplifying the workflow and producing a sample ready for downstream applications in only 5 minutes (Figure 2).

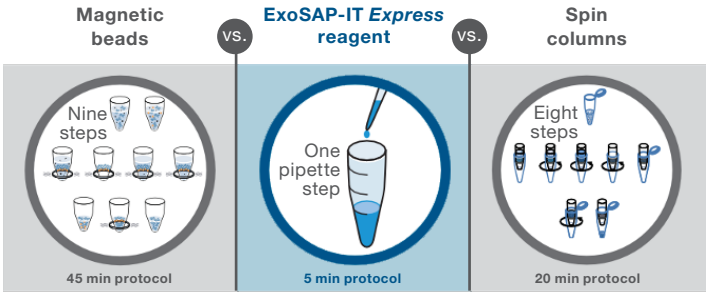

Figure 2. Comparison of cleanup methods. Use of ExoSAP-IT *Express* reagent eliminates spin columns, magnetic beads, filtration, and gel purification. With a 5 min protocol, ExoSAP-IT *Express* reagent is the fastest and easiest method for PCR cleanup, helping to minimize pipetting errors and contamination.

Conserve PCR samples—simple one-step, 100% recovery

The ExoSAP-IT *Express* enzymatic cleanup method helps minimize errors by reducing your protocol to a single pipetting step, allowing for automated or manual processing. ExoSAP-IT *Express* reagent outperforms the competition with 100% recovery of all amplicon sizes, from short to long (Table 1).

Table 1. DNA recovery after purification.

Amplicon size	Agencourt™ AMPure™ XP beads	ExoSAP-IT reagent
86 bp	10%	100%
103 bp	12%	100%
545 bp	63%	100%
1,007 bp	88%	100%

ExoSAP-IT Express reagent enables 100% recovery and provides effective cleanup of all amplicon sizes. In contrast, Agencourt AMPure XP beads were ineffective at purifying small amplicons, whether determined by image analysis or by the Invitrogen™ Quant-iT™ PicoGreen™ assay.

Overview

ExoSAP-IT reagents are a proprietary mixture of exonuclease I combined with shrimp alkaline phosphatase (SAP) in a specially formulated buffer that removes excess primers and dNTPs following a PCR reaction (Figure 3). Exonuclease I removes residual single-stranded primers and any single-stranded DNA produced during PCR. SAP removes the remaining dNTPs from the PCR mixture that may interfere with subsequent reactions.

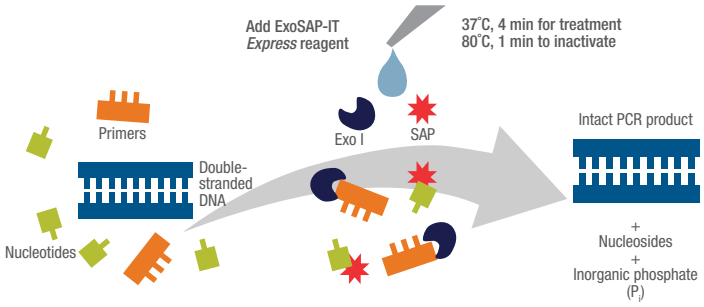

Figure 3. How ExoSAP-IT reagent works. Treat 5 µL of PCR product with 2 µL of ExoSAP-IT reagent. Treatment is carried out at 37°C followed by an incubation period at 80°C to completely inactivate both enzymes. Once contaminants are removed, your PCR products are ready for downstream applications such as Sanger sequencing, next-generation sequencing (NGS), fragment analysis, single-nucleotide polymorphism (SNP) analysis, *in vitro* transcription, or single-base extension.

The original ExoSAP-IT reagent formulation

Applied Biosystems™ ExoSAP-IT™ PCR Product Cleanup Reagent has been cited in over 10,000 publications. Thermo Fisher Scientific is the only manufacturer of ExoSAP-IT reagents, assuring your lab of product consistency and integrity.

- 30 min cleanup protocol
- Recommended for processing 1–96 samples at a time
- Best cost-per-reaction value

Also available in a high-throughput formulation

Applied Biosystems™ HT ExoSAP-IT™ *Fast* High-Throughput PCR Product Cleanup Reagent is an alternative formulation of the original ExoSAP-IT reagent, specifically designed for the unique requirements of high-throughput, automated platforms and multichannel pipettes. HT ExoSAP-IT *Fast* reagent quickly provides high-quality purified samples. This formulation offers decreased viscosity and is ideal for automated liquid handling platforms, with the same convenience and stability you have come to expect from ExoSAP-IT reagent.

- 14 min cleanup protocol
- Stable at 4°C for 1 month and at room temperature for 2 days
- Scalable for PCR cleanup ranging from a single tube up to a 384-well plate

Which ExoSAP-IT PCR cleanup reagent is right for you?

ExoSAP-IT reagents offer a unique one-tube, one-step enzymatic method for PCR cleanup. All ExoSAP-IT reagents provide 100% recovery of PCR products regardless of the fragment sizes. This PCR cleanup method removes excess primers and dNTPs and does not interfere with downstream applications. Achieve superior results with ExoSAP-IT reagent—improve accuracy with higher yields and full PCR product recovery.

Use our selection guide to determine which formulation is best for your next experiment (Table 2).

Table 2. Selection guide for ExoSAP-IT reagents.

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

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Ordering information

Product	Quantity	Cat. No.
ExoSAP-IT *Express* PCR Product Cleanup Reagent	100 reactions	75001.200.UL
	480 reactions (8-tube strips)	75001.1.EA
	500 reactions	75001.1.ML
	2,000 reactions	75001.4X.1.ML
	5,000 reactions	75001.10.ML
ExoSAP-IT PCR Product Cleanup Reagent	100 reactions	78200.200.UL
	500 reactions	78201.1.ML
	2,000 reactions	78202.4X.1.ML
	5,000 reactions	78205.10.ML
HT ExoSAP-IT *Fast* High-Throughput PCR Product Cleanup Reagent	20 reactions	7859520RXN
	1,000 reactions	785951000RXN
	5,000 reactions	785955000RXN
	480 reactions (8-tube strips)	785951EA
	5,760 reactions (12 x 8-tube strips in a tray)	785951PK
	23,040 reactions (48 x 8-tube strips in a tray)	785954PK

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

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invitrogen

Qubit 4 Fluorometer—fast answers for precious samples

Purify DNA | Purify RNA | Quantitate | Analyze

Find out if you have enough DNA or RNA for your experiment

The Invitrogen™ Qubit™ 4 Fluorometer with Wi-Fi is designed to quickly and specifically quantitate DNA or RNA.

Key features include:

- Invitrogen™ Qubit™ assay dyes bind selectively to DNA, RNA, or protein, making it more sensitive than UV absorbance
- Uses as little as 1 µL of sample, even with very dilute samples
- Fast, reliable detection of degraded RNA with the Invitrogen™ Qubit™ RNA IQ Assay
- Integrated reagent calculator to quickly generate working solution calculations
- Flexible options for exporting results: Wi-Fi, USB drive, or direct connection with a USB cable

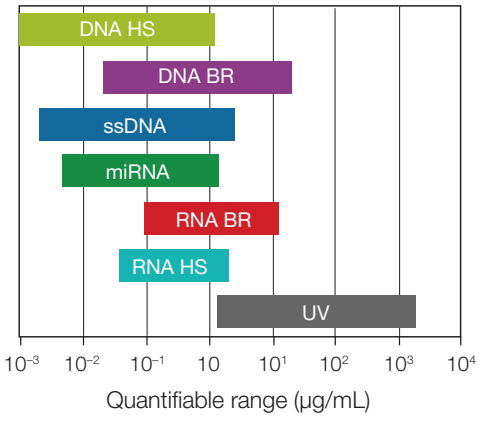

Figure 1. Comparison of quantifiable sample concentration ranges for the Qubit assays vs. UV absorbance measurements. UV absorbance readings are not selective for RNA vs. DNA.

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Features of Qubit assays

- Kits contain concentrated assay reagent, dilution buffer, and prediluted standards
- Dyes fluoresce only when bound to the target of interest, minimizing the effects of contaminants—including degraded DNA or RNA—on the result
- All assays are performed using the same general protocol with a simple mix-and-read format
- Only 2 minutes of incubation time required for DNA and RNA assays

Ordering information

Product	Initial sample concentration	Quantitation range	Quantity	Cat. No.
RNA integrity and quality kit				
Qubit RNA IQ Assay Kit*	NA	NA	75 assays	Q33221
			275 assays	Q33222
RNA quantitation kits				
Qubit RNA BR Assay Kit	1 ng/µL to 1 µg/µL	20–1,000 ng	100 assays	Q10210
			500 assays	Q10211
Qubit RNA HS Assay Kit	250 pg/µL to 100 ng/µL	5–100 ng	100 assays	Q32852
			500 assays	Q32855
Qubit microRNA Assay Kit	50 ng/mL to 100 µg/mL	1–1,000 ng	100 assays	Q32880
			500 assays	Q32881
DNA quantitation kits				
Qubit ssDNA Assay Kit	50 pg/µL to 200 ng/µL	1–200 ng	100 assays	Q10212
Qubit dsDNA BR Assay Kit	100 pg/µL to 1,000 ng/µL	2–1,000 ng	100 assays	Q32850
			500 assays	Q32853
Qubit dsDNA HS Assay Kit	10 pg/µL to 100 ng/µL	0.2–100 ng	100 assays	Q32851
			500 assays	Q32854
Qubit 1X dsDNA HS Assay Kit	10 pg/µL to 100 ng/µL	0.2–100 ng	100 assays	Q33230
			500 assays	Q33231
Protein quantitation kit				
Qubit Protein Assay Kit	12.5 µg/mL to 5 mg/mL	0.25–5 µg	100 assays	Q33211
			500 assays	Q33212
Instrument and accessories				
Qubit 4 System Verification Assay Kit			1 kit	Q33237
Qubit 4 Fluorometer with Wi-Fi			1 instrument	Q33238
Qubit 4 Quantitation Starter Kit (with Wi-Fi)			1 kit	Q33239
Qubit 4 NGS Starter Kit (with Wi-Fi)			1 kit	Q33240
Qubit 4 RNA IQ Starter Kit (with Wi-Fi)			1 kit	Q33241
Qubit Assay Tubes			500 tubes	Q32856

* Note: The Qubit RNA IQ Assay for the detection of degraded RNA can only be run on the Qubit 4 Fluorometer and cannot be performed on the original Qubit, Qubit 2.0, or Qubit 3.0 Fluorometers.

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

ThermoFisher SCIENTIFIC

Buffered Saline Solutions

For various human clinical samples

Pub. No. MAN0018562 **Rev.** 1.0

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**.

Intended Use

For *in vitro* diagnostic use

Buffered saline solutions are isotonic saline solutions used to maintain pH and osmotic balance as well as provide cells with water and essential inorganic ions. Buffered saline solutions are used in a variety of laboratory applications, such as washing cells, transporting cells, diluent of human biological samples, or for reagent preparation. Thermo Fisher Scientific offers a wide range of buffered saline solutions including those with and without; calcium chloride, magnesium chloride, magnesium sulfate, and phenol red.

Buffered saline solutions are for professional use. They are used in medical laboratories by personnel who have received specialized education and training with regard to procedures utilizing In Vitro Diagnostic products. IVD products of this type are not intended as sole determinant in a diagnostic situation. Test results are interpreted by a healthcare professional as part of the clinical management of a patient.

Principle and explanation of procedure

Isotonic solutions are generally utilized to maintain cells for a short period time in a viable condition while the cells are manipulated outside of their regular growth environment. A physiological pH (6.8 to 7.4) value can be important to maintain viability when investigating human biological samples in an in vitro diagnostic application. Isotonic solutions can also be used for rinsing and washing steps in various diagnostic procedures (1-4).

Contents and storage

All quality control testing results are reported on lot-specific Certificate of Analysis available on our website: **thermofisher.com**.

Product	Cat. No.	Storage	Shelf life[1]
Phosphate Buffered Saline (PBS) 7.4 (1X)	10010001[2] 10010002[2]	15°C to 30°C	12 months
Phosphate Buffered Saline (PBS) 7.4 (1X)	10010023[3] 10010031[2] 10010049[3] 10010072[2]	15°C to 30°C	24 months
Phosphate Buffered Saline (PBS) 7.4 (1X)	10010015[4]	15°C to 30°C	36 months
Phosphate Buffered Saline (PBS) 7.2 (1X)	20012019[4]	15°C to 30°C	36months
Phosphate Buffered Saline (PBS) 7.2 (1X)	20012027[2] 20012043[2] 20012050[2] 20012099[2]	15°C to 30°C	24 months
Phosphate Buffered Saline (PBS) 7.4 (10X)	70011036[4] 70011044[2] 70011069[2]	15°C to 30°C	24 months
Phosphate Buffered Saline (PBS) 7.2 (10X)	70013016[4]	15°C to 30°C	36 months

CE IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Product	Cat. No.	Storage	Shelf life[1]
Phosphate Buffered Saline (PBS) 7.2 (10X)	70013032[2] 70013073[2]	15°C to 30°C	24 months
Dulbecco's Phosphate Buffered Saline (DPBS) (1X) [+] Calcium Chloride [+] Magnesium Chloride	14040083[4] 14040091[4] 14040141[2] 14040133[2] 14040117[3] 14040097[2] 14040182[2] 14040216[3]	2°C to 8°C	36 months
Dulbecco's Phosphate Buffered Saline (DPBS) (1X) [-] Calcium Chloride [-] Magnesium Chloride	14190086[4] 14190094[4] 14190144[3] 14190136[3] 14190230[4] 14190250[3] 14190235[3]	15°C to 30°C	36 months
Dulbecco's Phosphate Buffered Saline (DPBS) (1X) [-] Calcium Chloride [-] Magnesium Chloride	14190342[3] 14190359[2] 14190367[3]	15°C to 30°C	12 months
Dulbecco's Phosphate Buffered Saline (DPBS) (1X) [-] Calcium Chloride [-] Magnesium Chloride	14190240[4]	15°C to 30°C	24 months
Dulbecco's Phosphate Buffered Saline (DPBS) (10X) [-] Calcium Chloride [-] Magnesium Chloride	14200059[4] 14200067[4] 14200075[2] 14200166[2]	15°C to 30°C	36 months
Dulbecco's Phosphate Buffered Saline (DPBS) (10X) [+] Calcium Chloride [+] Magnesium Chloride	14080048[4] 14080055[2] 14080098[2]	2°C to 8°C	24 months
Hanks' Balanced Salt Solution (HBSS) (1X) [+] Calcium Chloride [+] Magnesium Chloride [-] Phenol Red	14025050[4] 14025092[2] 14025076[2] 14025134[2] 14025126[2]	15°C to 30°C	36 months
Hanks' Balanced Salt Solution (HBSS) (1X) [+] Calcium Chloride [+] Magnesium Chloride [+] Phenol Red	24020083[4] 24020091[4] 24020117[2] 24020198[2]	15°C to 30°C	36 months
Hanks' Balanced Salt Solution (HBSS) (1X) [-] Calcium Chloride [-] Magnesium Chloride [-] Magnesium Sulfate [+] Phenol Red	14170120[2] 14170112[2] 14170097[2] 14170161[2]	15°C to 30°C	36 months

Product	Cat. No.	Storage	Shelf life[1]
Hanks' Balanced Salt Solution (HBSS) (1X) [-] Calcium Chloride [-] Magnesium Chloride [-] Magnesium Sulfate [-] Phenol Red	14175046[4] 14175053[4] 14175095[2] 14175079[2] 14175103[2] 14175145[2]	15°C to 30°C	36 months
Hanks' Balanced Salt Solution (HBSS) (1X) [-] Calcium Chloride [-] Magnesium Chloride [+] Phenol Red [+] Sodium Bicarbonate	14170070[4] 14170088[4]	15°C to 30°C	36 months
Hanks' Balanced Salt Solution (HBSS) (10X) [-] Sodium Bicarbonate	14060040[4]	15°C to 30°C	36 months
Hanks' Balanced Salt Solution (HBSS) (10X) [-] Calcium Chloride [-] Magnesium Chloride	14180046[4]	15°C to 30°C	36 months
Hanks' Balanced Salt Solution (HBSS) (10X) [-] Calcium Chloride [-] Magnesium Chloride [-] Sodium Bicarbonate [-] Phenol Red	14185045[4]	15°C to 30°C	24 months
Hanks' Balanced Salt Solution (HBSS) (10X) [-] Sodium Bicarbonate [-] Phenol Red	14065056[2] 14065098[2]	15°C to 30°C	24 months
Hanks' Balanced Salt Solution (HBSS) (10X) [-] Sodium Bicarbonate [-] Phenol Red	14065049[4]	15°C to 30°C	36 months
Hanks' Balanced Salt Solution (HBSS) (10X) [-] Calcium Chloride [-] Magnesium Chloride [-] Magnesium Sulfate	14185052[2] 14185098[2]	15°C to 30°C	24 months
Earle's Balanced Salt Solution (EBSS) (1X) [-] Calcium Chloride [-] Magnesium Chloride [-] Phenol Red	14155048[4] 14155063[2] 14155098[2]	15°C to 30°C	36 months
BME, Basal Medium (Eagle) (1X) [+] Earle's Salts [+] Phenol Red [-] L-glutamine [-] HEPES	41010026[4]	2°C to 8°C Protect from light	12 months
HEPES Buffer Solution 1 M	15630049[4] 15630056[4]	2°C to 8°C	24 months

[1] Shelf life is determined from Date of Manufacture. Do not use beyond the labelled expiration date.
[2] Manufacturer: Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
[3] Dual manufactured.
[4] Manufacturer: Life Technologies™ Ltd. | 3 Fountain Drive, Inchinnan Business Park | Paisley PA49RF, Scotland, United Kingdom |Tel: +44 (0)141 81416305

Precautions

Do not use the product if packaging, including bottles and vials, have been compromised and/or show evidence of microbial contamination, cloudy appearance, discoloration, drying, cracking, or other signs of deterioration.

CAUTION! Human samples are potentially biohazardous. Follow standard precautions for handling, storage and disposal.

WARNING! Do not use for injection or infusion! Please report any serious incidents in relation to the device to the manufacturer and the Competent Authority of the EU Member State in which the user and/or patient is established.

- Once opened, use Buffered Saline Solutions within 14 days for maximal growth performance.
- Avoid repeated warming/cooling and prolonged exposure to light.
- Do not use beyond labeled expiration date.
- All solutions that come into contact with clinical samples must be sterile. Always use proper aseptic techniques and work inside a laminar flow hood. Consult our **Gibco Cell Culture Basics** for aseptic handling.

Test protocol

Buffered saline solutions have many different uses in diagnostic protocols such as cell washing, diluents or as transport media. Refer to the appropriate procedures. Several procedures and applications are provided in the references.

Cellular suspensions prepared in a buffered saline solution should not be stored longer than several hours as cellular viability may decrease. Therefore, the suitability of the buffered saline solution for a specific cell type should be tested prior to use.

Quality control

Standard evaluations for cell culture media are pH, osmolality, endotoxins and sterility testing for liquid products. All quality control testing results are reported on lot specific Certificate of Analysis available on our website: **thermofisher.com**.

References

1. Lennette EH, Halonen P and Murphy FA . Laboratory Diagnosis of Infectious Disease - Principles and Practices (1988).Springer, New York, p.43.
2. WHO Manual for the laboratory diagnosis and virological surveillance of influenza, 2011.
3. Winn, W. C., & Koneman, E. W. (2006). Koneman's color atlas and textbook of diagnostic microbiology (6th ed.). Philadelphia: Lippincott Williams & Wilkins.
4. WHO Guidelines on the Establishment of Virology Laboratories in Developing Countries, 2008.

Labeling symbols

The symbols present on the product label are explained in the following table.

	MANUFACTURER		USE BY
IVD	IN VITRO DIAGNOSTIC MEDICAL DEVICE		CONSULT INSTRUCTIONS FOR USE
REF	CATALOG NUMBER		CAUTION, CONSULT ACCOMPANYING DOCUMENTS
LOT	BATCH CODE		UPPER AND LOWER LIMITS OF TEMPERATURE
STERILE A	Sterilized using aseptic processing technique		PROTECT FROM LIGHT
CE	European Mark of Conformity	EC REP	AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY

Limited product warranty

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

Manufacturer:
Life Technologies Corporation |
3175 Staley Road |
Grand Island, NY 14072

Manufacturer:
Life Technologies™ Ltd. |
3 Fountain Drive, Inchinnan Business Park |
Paisley PA49RF, Scotland, United Kingdom |
Tel: +44 (0)141 81416305

Manufacturer:
Dual manufactured products

EC REP
European Regulatory Affairs
Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN Bleiswijk
The Netherlands
Tel: +31 (0) 10 714 5000

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

Revision history: Pub. No. MAN0018473

Revision	Date	Description
1.0	12 November 2019	New document

INSTRUCTIONS

DAPI

(4′, 6-diamidino-2′-phenylindole, dihydrochloride)

62247 62248

2244.1

Number	Description
62247	**DAPI,** 10 mg
62248	**DAPI** (1 mg/ml in water)**,** 1 ml

Molecular weight: 350.25

Molar extinction coefficient: 30,600 M^{-1} cm^{-1} at 347 nm in methanol

Excitation wavelength: 341 nm

Emission wavelength: 452 nm

CAS Number: 28718-90-3

Storage: Upon receipt store product at 2-8°C protected from light. Product is shipped at ambient temperature.

Introduction

DAPI (4′, 6-diamidino-2′-phenylindole, dihydrochloride) is a dye that fluoresces blue (455 nm) when bound to double-stranded DNA and excited by exposure to 345 nm light. DAPI binds selectively to the minor groove of adenine-thymine (A-T) regions of DNA, where its fluorescence is approximately 20-fold greater than in the nonbound state.[1-3] These properties make DAPI useful for assaying DNA in solution,[4] diagnosing mycoplasmal infection of cell cultures,[5] measuring nuclear content and sorting chromosomes in flow cytometry,[6] assessing apoptosis,[7] and detecting nuclei and organellar DNA in immunofluorescent and *in situ* hybridization procedures.[2,8] DAPI is also used as a replacement for ethidium bromide to stain double-stranded DNA in agarose gels.[5,9] Because DAPI is cell permeable and fluoresces blue, it is commonly used to counterstain nuclei in histochemical methods when red-fluorescent antibodies have been used to detect specific targets.[8] Reports also indicate that DAPI binds to polyphosphates and other polyanions,[10] dextran sulfate[11] and SDS.[12]

General Procedure for Immunofluorescent Staining

A. Reagent Preparation

PBS (Wash Buffer)	Modified Dulbecco's PBS (Product No. 28374): 8 mM sodium phosphate, 2 mM potassium phosphate, 140 mM sodium chloride, 10 mM potassium chloride; pH 7.4.
DAPI Stock Solution	Dissolve DAPI in ultrapure water to 1 mg/ml. Stock solution is stable for several months and repeated use if stored protected from light at -20°C.
DAPI Working Solution	Dilute the DAPI Stock Solution 1:1,000 in ultrapure water or PBS (1 µg/ml DAPI). Filter the Working Solution to remove dye aggregates that can result in punctate signal.

B. Procedure

1. Follow standard procedures to fix sample and then probe with specific fluorescent-labeled antibodies.
2. Thoroughly wash sample with PBS to remove nonbound probe.
3. Add a sufficient volume of DAPI Working Solution to completely cover the sample. Place aluminum foil over the sample to protect it from light and incubate at room temperature for 2-10 minutes. If resulting final signal bleeds through to other fluorescent channels, decrease incubation time in next experiment.
4. Wash sample thoroughly with PBS to remove excess DAPI.
5. Mount sample with an appropriate medium and detect according to standard protocols.

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Procedure for Assaying DNA

DAPI can be used to quantitate DNA in solution. The method is relatively insensitive to pH 5-10 but is sensitive to changes in temperature and ionic strength, as well as to fluorescence quenching by divalent or heavy metal cations.[4] The fluorescence is not linear over broad concentration ranges of DNA; therefore, use an internal standard each time the assay is performed.

A. Reagent Preparation

Assay Buffer	0.1 M NaCl, 10 mM EDTA, 10 mM Tris; pH 7.0.
DAPI Stock Solution	Dissolve DAPI in ultrapure water to 1 mg/ml. Stock solution is stable for several months and repeated use if stored protected from light at -20°C.
DAPI Working Solution	0.1 µg/ml DAPI in Assay Buffer.
DNA Standards	Dilute known amount of calf thymus DNA with Assay Buffer to make a series of DNA standards with concentrations ranging from 0 to 5 µg/ml (0 to 250 ng/50 µl). Prepare replicates of each dilution so error statistics can be calculated.

B. Assay

1. Add 50 µl of each unknown sample or DNA Standard to a disposable fluorometer cuvette.
2. Add 1.5 ml DAPI Working Solution to each cuvette.
3. Cover cuvettes with foil and incubate at room temperature for 10 minutes.
4. Measure fluorescence of each solution.
5. Prepare a standard curve by plotting the mean values of the standards plotted against their concentrations.
6. Determine concentration of each unknown sample based on its fluorescence measurement relative to the standard curve.

Related Thermo Scientific Products

Visit our web site for information about other fluorescent protein labeling reagents, including fluorescein (Product No. 46409, 46410 and 53029), rhodamine (No. 46406 and 53031), and amine-reactive or sulfhydryl-reactive Thermo Scientific DyLight Fluors. We also offer a variety of labeled secondary antibodies.

References

1. Morikawa, K. and Yanagida, J. (1981). Visualization of individual DNA molecules in solution by light microscopy: DAPI staining method. *J. Biochem.* (Tokyo) **89:**693-6.
2. Lawrence, M.E. and Possingham, J.V. (1986). Direct measurement of femtogram amounts of DNA in cells and chloroplasts by quantitative microspectrofluorometry. *J. Histochem. Cytochem.* **34:**761-8.
3. Kubista, M., *et al.* (1987). Characterization of interaction between DNA and 4′, 6-diamidino-2-phenylindole by optical spectroscopy. *Biochemistry* **26:**4545-53.
4. Brunk, C.F., *et al.* (1979). Assay for nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.* **92:**497-500.
5. Russell, W.C., *et al.* (1975). A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. *Nature* **253:**461-2.
6. Hammarton, T.C., *et al.* (2003). Stage-specific differences in cell cycle control in Trypanosoma brucei revealed by RNA interference of a mitotic cyclin. *J. Biol. Chem.* **278(25):**22877-86.
7. Lai, J., *et al.* (2003). Loss of HSulf-1 up-regulates heparin-binding growth factor signaling in cancer. *J. Biol. Chem.* **278(25):**23107-17.
8. Soto, P., *et al.* (2003). SMAD2 and SMAD7 Involvement in the post-translational regulation of Muc4 via the transforming growth factor-β and interferon-γ pathways in rat mammary epithelial cells. *J. Biol. Chem.* **278(22):**20338-44.
9. Nairn, R.S., *et al.* (1982). Comparison of ethidium bromide and 4', 6'-diamidino-2-phenylindole as quantitative fluorescent stains for DNA in agarose gels. *J. Biochem. Biophys. Meth.* **6:**95-103.
10. Tijssen, J.P.F., *et al.* (1982). Localization of polyphosphates in *Saccharomyces fragilis*, as revealed by 4′, 6-diamidino-2-phenylindole fluorescence. *Biochem. Biophys. Acta* **721:**394-8.
11. Allan, R.A. and Miller, J.J. (1980). Influence of S-adenosylmethionine on 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI)-induced fluorescence of polyphosphate in the yeast vacuole. *Can. J. Micro.* **26:**912-20.
12. Kapuscinski, J. and Skoczylas, B. (1978). Fluorescent complexes of DNA with DAPI (4′, 6-diamidine-2-phenyl indole dihydrochloride) or DCI (4′, 6-dicarboxyamide-2-phenyl indole). *Nucl. Acids Res.* **5:**3775-99.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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BigDye XTerminator™ Purification Kit

Catalog Numbers 4376484, 4376485, 4376486, and 4376487

Pub. No. 4383427 Rev. C

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of the BigDye XTerminator™ Purification Kit. For detailed instructions, supplemental procedures, and troubleshooting, see the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408).

Important guidelines

- **IMPORTANT!** Keep the reagents thoroughly mixed throughout the pipetting procedure.
- For optimal results, follow "Guidelines for sequencing reactions" on page 3.
- Use wide-bore tips (with an orifice >1.0 mm) to pipet the BigDye XTerminator™ Solution.
- Use conventional tips to pipet the SAM™ Solution and Premix Solution.
- Ensure the pipette tip is below the surface of the liquid when pipetting the BigDye XTerminator™ Solution.
- Perform all vortexing steps as described in the procedure.
- Do not heat-denature or use Hi-Di™ Formamide with samples that contain BigDye XTerminator™ reagents.

Before you begin

- Inspect the SAM™ Solution. If precipitates are visible, warm the solution at 37°C, then gently mix to dissolve the precipitates. Cool the solution to room temperature before use.
- Thoroughly mix the SAM™ Solution.
- If you are using the direct injection method, ensure the appropriate BDx run module is installed and updated on your system (see the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408)).

Sequential Pipetting method: Add the reagents to the reaction plate

Sequentially add the reagents to the sequencing reactions

1. Centrifuge the cycle-sequencing reaction plate at 1,000 × *g* for 1 minute.
2. Immediately before pipetting, mix the BigDye XTerminator™ Solution by inversion 10 times or until the solution is homogeneous.
3. Add the following components (in the order indicated) to each well of the reaction plate.

   IMPORTANT! Remix the BigDye XTerminator™ Solution every minute to prevent phase separation.

Component	Volume per 5-µL reaction	Volume per 10-µL reaction	Volume per 20-µL reaction
SAM™ Solution	22.5 µL	45 µL	90 µL
BigDye XTerminator™ Solution	5 µL	10 µL	20 µL

4. Seal the plate according to your sealing method.
   - Using a MicroAmp™ Clear Adhesive Film—Proceed to "Seal the reaction plate" on page 2.
   - Using a heat seal—Apply a heat seal at 160°C for 1.5 seconds, then proceed to "Vortex, then centrifuge the reaction plate" on page 3.

Premix Pipetting method: Add the reagents to the reaction plate

Prepare the Premix Solution

Prepare fresh Premix Solution for each processing run.

1. Immediately before pipetting, mix the BigDye XTerminator™ Solution by inversion 10 times or until the solution is homogeneous.

2. Combine the following components (in the order indicated) in an appropriately-sized bottle or reagent reservoir according to one of the following tables.

Table 1 384-well plate, 5-µL reaction volume

Component	Volume	
	1 well	1 plate[1]
BigDye XTerminator™ Solution	5 µL	2,304 µL
SAM™ Solution	22.5 µL	10,368 µL
Total Premix Solution	**27.5 µL**	**12,672 µL**

[1] Includes 20% overage.

Table 2 96-well plate, 10-µL reaction volume

Component	Volume	
	1 well	1 plate[1]
BigDye XTerminator™ Solution	10 µL	1,152 µL
SAM™ Solution	45 µL	5,184 µL
Total Premix Solution	**55 µL**	**6,336 µL**

[1] Includes 20% overage.

Table 3 96-well plate, 20-µL reaction volume

Component	Volume	
	1 well	1 plate[1]
BigDye XTerminator™ Solution	20 µL	2,304 µL
SAM™ Solution	90 µL	10,368 µL
Total Premix Solution	**110 µL**	**12,672 µL**

[1] Includes 20% overage.

Add the Premix Solution to the sequencing reactions

IMPORTANT! Keep the Premix Solution thoroughly mixed throughout the pipetting procedure.

1. Centrifuge the cycle-sequencing reaction plate at 1,000 × *g* for 1 minute.

2. Immediately before pipetting, mix the Premix Solution.
   - For Premix Solution in a bottle—Cap the bottle, then mix by inversion 10 times or until the solution is homogeneous.
   - For Premix Solution in a reagent reservoir—Pipet up and down 2–3 times or until the solution is homogeneous.

3. Add the Premix Solution to each well of the reaction plate according to the following table.

IMPORTANT! Agitate the solution before each aspiration.

Plate type (reaction volume per well)	Volume of Premix Solution per well
384-well (5-µL)	27.5 µL
96-well (10-µL)	55 µL
96-well (20-µL)	110 µL

Discard any remaining Premix Solution after use.

4. Seal the plate according to your sealing method.
   - Using a MicroAmp™ Clear Adhesive Film—Proceed to "Seal the reaction plate" on page 2.
   - Using a heat seal—Apply a heat seal at 160℃ for 1.5 seconds, then proceed to "Vortex, then centrifuge the reaction plate" on page 3.

Seal the reaction plate

IMPORTANT! Apply firm pressure to the adhesive film during application to ensure a tight, leak-proof seal during vortexing.

1. Place the reaction plate on a MicroAmp™ Splash-Free 96-Well Base, then wipe off any liquid on the surface of the plate.

2. Place the adhesive film onto the reaction plate (adhesive side facing the plate).

3. While applying firm downward pressure, move the applicator slowly across the film, both horizontally and vertically.

4. Repeat step 3 five times. While applying pressure, run the edge of the applicator along all four sides of the outer border of the film.

5. Inspect the reaction plate to confirm that all wells are sealed.

The plate is properly sealed when an imprint of each well is visible on the surface of the film.

Vortex, then centrifuge the reaction plate

1. Firmly attach the plate to the vortexer.

   For set-up and operating instructions specific to your instrument, see the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408).

2. Vortex the plate for 20 minutes (96-well plate) or 30 minutes (384-well plate), using the settings indicated.

   IMPORTANT! Do not vortex beyond the specified time.

Vortexer	Plate type	Speed or setting
Thermo Scientific™ Digital Vortex Mixer	96-well	1,800 rpm
	384-well	2,000 rpm
Digital Vortex-Genie™ 2	96-well	1,800 rpm
	384-well	2,000 rpm
Eppendorf™ MixMate™	96-well	1,800 rpm
	384-well	2,600 rpm
IKA™ MS 3 Digital Orbital Shaker	96-well or 384-well	1,800 rpm (Mode B)
IKA™ Vortex 3	96-well or 384-well	Setting 5[1]
Taitec MicroMixer E-36	96-well or 384-well	Maximum
Union Scientific™ Vertical Shaker[2]	96-well or 384-well	Setting 100

[1] Use the maximum setting that does not cause the vortexer to become unstable.
[2] If needed, add additional plates to meet the mass requirements.

3. Centrifuge the plate at 1,000 × *g* in a swinging-bucket centrifuge for 2 minutes.

4. Immediately proceed to capillary electrophoresis.

STOPPING POINT If you cannot run capillary electrophoresis immediately, store the reaction plate as described (see "Guidelines for storing the reaction plate" on page 3).

Prepare the reaction plate for capillary electrophoresis

Prepare the reaction plate according to the following table.

IMPORTANT! Do not heat-denature or use Hi-Di™ Formamide with samples that contain BigDye XTerminator™ reagents.

Instrument	Action
SeqStudio™	**For 96-well plates—**Remove the adhesive film, then place a septum on the plate.
3500/3500xL	**For 96- or 384-well plates—**Remove the adhesive film, then place a septum on the plate.
3730/3730*xl*	**For plates sealed with heat seal film—**Proceed with the sealed plate.
	For plates sealed with adhesive film—Prepare the plate according to the plate type. • **For 96-well plates—**Remove the adhesive film, then place a septum on the plate. • **For 384-well plates—**Remove the adhesive film, then perform one of the following actions. – Apply a heat seal to the plate. – Transfer 10 µL of the supernatant to a new plate, then place a septum on the plate.
3130/3130*xl*	• **For 96-well plates—**Remove the adhesive film, then place a septum on the plate. • **For 384-well plates—**Remove the adhesive film, transfer 10 µL of the supernatant to a new plate, then place a septum on the plate.
310 Genetic Analyzer	**For 96-well trays—**Remove the adhesive film, transfer 10 µL of the supernatant to a new plate, then place a septum on the plate.

Run capillary electrophoresis

1. Load the reaction plate in the instrument.

2. Set up an instrument run using the appropriate conditions for your instrument.
   - If you are using the direct injection method, use a BDx run module specified in the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408).
   - If the supernatant was transferred to a new plate after purification, see your instrument user guide for the appropriate run module.

3. Start the electrophoresis run.

(Optional) Store reaction plates that have been at room temperature <48 hours as described (see "Guidelines for storing the reaction plate" on page 3).

Guidelines for storing the reaction plate

Sequencing reactions purified with the BigDye XTerminator™ Purification Kit are stable at room temperature for up to 48 hours.

Store reaction plates covered with adhesive film, septa, or heat seal film at room temperature for up to 48 hours.

Note: After removing the reaction plate from storage, centrifuge the plate at 1,000 × *g* in a swinging-bucket rotor for 2 minutes, then proceed to run capillary electrophoresis.

Guidelines for sequencing reactions

- DNA sequencing reactions that are purified with the BigDye XTerminator™ Purification Kit result in high signal strength when analyzed. If needed, decrease the amount of DNA template in the sequencing reactions to keep the fluorescence signals on-scale during analysis.

  Note: If the template concentration is decreased, the amount of any template controls must be decreased proportionately.

- Ensure the sequencing reactions meet the minimum volume requirements according to the following table.

If needed, adjust the reaction volume with UltraPure™ DNase/RNase-Free Distilled Water before purification with the kit.

Plate type	Minimum reaction volume
384-well	5 µL
96-well	10 µL[1]

[1] If you are processing >48 samples for a single capillary electrophoresis run, we recommend a minimum reaction volume of 20 µL.

Limited product warranty

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

Life Technologies Corporation | 2 Preston Court | Bedford, MA 01730
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

Revision history: Pub. No. 4383427

Revision	Date	Description
C	2 June 2020	• Updated to the current document template, with associated updates to the warranty, trademarks, and logos. • Updated compatible instruments. • Added detailed procedure to seal the reaction plate. • Made wording and formatting updates.
B	July 2007	Baseline for this revision history.

Thermo
SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific
GeneJET Viral DNA and RNA Purification Kit

#K0821

www.thermoscientific.com/onebio

#K0821

Packaging Lot __

Exp. __

CERTIFICATE OF ANALYSIS

Quality of the kit was tested by purification of RNA and DNA from 200 µL of spiked human plasma. Yield of nucleic acids was evaluated by RT-qPCR and qPCR, and compared with spiked copy number.

Quality authorized by:  Jurgita Žilinskiene

CONTENTS page

COMPONENTS OF THE KIT

GeneJET Viral DNA and RNA Purification Kit	**#K0821** 50 preps
Column Preparation Liquid (red cap)	2 × 1.4 mL
Lysis Solution*	12 mL
Wash Buffer 1 (concentrated)*	25 mL
Wash Buffer 2 (concentrated)	11 mL
Eluent (white cap)	3 × 1.25 mL
Proteinase K (green cap)	2 × 1.3 mL
Carrier RNA, dried (blue cap)	1 vial
Spin Columns preassembled with Wash Tubes	50
Wash Tubes (2 mL)	4 × 50
Handbook	1

* contains guanidine hydrochloride (guanidinium chloride).

STORAGE

When the kit is delivered, remove the Carrier RNA from the package and store in the original aluminum bag at −20°C. Other components of the kit should be stored at room temperature (15-25°C). All components are stable until the listed expiration date.
Wash Buffer 1 and Wash Buffer 2 are stable until the listed expiration date after addition of ethanol.
Note. Close the bag with Spin Columns tightly after each use!

DESCRIPTION

The Thermo Scientific **GeneJET Viral DNA and RNA Purification Kit** is designed for rapid and efficient purification of high quality viral nucleic acids from various human and animal liquid samples such as plasma, serum, whole blood, saliva, nasal and buccal swabs, urine (cells should be collected before purification), urogenital swabs and milk.
The kit utilizes a silica-based membrane technology in the form of a convenient spin column. Nucleic acids from lysed samples bind to the column membrane while impurities are effectively removed during subsequent washing and centrifugation steps. Finally, ready-to-use nucleic acids are eluted from the column. The purified viral nucleic acids are free of proteins, nucleases, and other contaminants or inhibitors of downstream applications. Isolated DNA and RNA can be directly used in PCR, qPCR or other nucleic acid based assays. Host genomic DNA or RNA co-purified from liquid cell-containing samples typically does not interfere with viral nucleic acid yields due to high capacity of the spin column membrane (up to 50 µg).
To minimize irregularities in results, the product must be used with an appropriate internal control as well as positive and negative controls throughout the process of sample preparation, amplification and detection.

PRINCIPLE

The **GeneJET™ Viral DNA and RNA Purification Kit** uses well-established nucleic acid isolation and purification technique comprised of following steps:

1	**Sample lysis** The sample is lysed by incubation with Lysis Solution and Proteinase K under denaturating conditions at elevated temperatures (56°C). The Lysis Solution and Proteinase K inactivate both RNases and DNases, ensuring protection of viral nucleic acids against degradation.
2	**Binding viral nucleic acids to the spin column membrane** The lysed sample is transferred to a spin column where released viral nucleic acids immediately bind to the silica-based filter in the presence of chaotropic salts. The remaining lysate is removed by centrifugation.
3	**Removing remaining contaminants** The remaining contaminants are removed during three wash steps using Wash Buffer 1 and Wash Buffer 2, whereas pure nucleic acids remain bound to the membrane.
4	**Elution of pure viral nucleic acids** Pure viral nucleic acids are released from the spin column filter using Eluent. The purified nucleic acids are ready for subsequent use in downstream applications.

IMPORTANT NOTES

- Ensure the integrity of the kit components upon the delivery. Contact technical service or your local distributor in case of damage. Do not use damaged kit components.
- The **Lysis Solution** and **Wash Buffer 1** contain irritants. Always wear gloves and follow standard safety precautions when handling these reagents. For more information refer to SAFETY INFORMATION (page 12) and Material Safety Data Sheets.
- All sample material and waste should be regarded as potentially infectious. Wear the proper protection when handling samples and waste. Avoid any skin or eye contact! Work under laminar air flow conditions if possible until samples are lysed. Disinfect all work surfaces thoroughly after the procedure. Follow proper waste disposal guidelines as recommended by local authorities.
- The following steps should be taken in order to avoid cross-contamination: always change pipette tips between liquid transfers (aerosol-barrier pipette tips recommended); open only one tube at a time; use disposable gloves and discard if contaminated.
- Always use RNase-free equipment.
- Use only a freshly prepared mixture of Carrier RNA and Lysis Solution when beginning a new extraction procedure!
- Before beginning the procedure, a new Spin Column must be prepared by adding 50 µl of Column Preparation Liquid into the center of the column membrane. Do not centrifuge the column after addition of Column Preparation Liquid.

SAMPLE HANDLING

- If possible, use only fresh sample material.
- **Plasma, serum, whole blood** samples can be stored at 2-8°C for up to 24 hours, or at −20°C or −70°C for long term storage.
- **Urogenital** swabs can be stored at 2-8°C for up to 48 hours. For longer term storage cells should be collected by centrifugation and stored at −20°C or −70°C
- **Nasal and buccal swabs** can be stored at 2-8°C for up to 48 hours.
- **Urine samples** should be stored at 2-8°C for up to 12 hours (with 0.5 M EDTA added to 50 mM final concentration), or at −20°C or −70°C for long term storage (cells should be collected by centrifugation). For viral RNA purification, it is recommended to collect cells by centrifugation immediately after sample collection.
- Do not freeze/thaw samples more than once.
- Equilibrate samples to room temperature (20±5°C) before use. Remove precipitates from plasma/serum samples, if any, by centrifugation for 5 min at 3,000 × g.
- Use EDTA or citrate treated plasma samples.

PREPARING REAGENTS AND BUFFERS

Add the indicated volume of ethanol (96-100%) to the concentrated **Wash Buffer 1** and concentrated **Wash Buffer 2** prior to first use:

	#K0821, for 50 preps	
	Wash Buffer 1	**Wash Buffer 2**
Concentrated wash buffer	25 mL	11 mL
Ethanol (96-100%)	**15 mL**	**44 mL**
Total volume	40 mL	55 mL

- Ensure all working solutions are prepared according to the recommendations in the protocol.
- After preparing each solution, mark the bottle to indicate that this step has been completed.
- Check all solutions in the kit for any salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37°C, and then equilibrate to room temperature (20±5°C).
- For swabs or collected cells, reconstitute cells to recommended sample volume using PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)
- It is the user's responsibility to use appropriate controls during the procedure.

CARRIER RNA

Usage of Carrier RNA is important for efficient recovery of viral nucleic acids for two reasons. First, Carrier RNA facilitates binding of viral nucleic acids to the silica membrane, especially when there are only a small number of viral nucleic acid molecules in the sample. Additionally, in the rare event when there are a small number of active RNase molecules, large amounts of Carrier RNA reduce the probability of viral RNA being degraded under chaotropic conditions. If Carrier RNA is not added to the Lysis Solution, reduced viral nucleic acid yields may result.

PREPARATION OF CARRIER RNA

Carrier RNA is provided in a dried state packed in a moisture-impermeable aluminum bag. Prior to the first use, reconstitute the dried **Carrier RNA** by adding **300 µL of Eluent**. Allow the freshly reconstituted Carrier RNA to incubate for 5 min at room temperature, then mix thoroughly and briefly centrifuge the vial. Use immediately or store at −20°C. Do not freeze-thaw the reconstituted Carrier RNA more than 10 times. If only few samples will be processed at a time, divide the Carrier RNA solution into 50 µl aliquots (using nuclease-free tubes) and store at −20°C or −70°C.

CALCULATING THE REQUIRED QUANTITY OF CARRIER RNA

When starting a new procedure, always use a freshly prepared mixture of Carrier RNA and Lysis Solution. To calculate the correct quantity of Carrier RNA and Lysis Solution required to process multiple samples, use the following table. Supplement Lysis Solution with the required quantity of Carrier RNA and mix by pulse-vortexing or pipetting.

No. samples	Vol. Lysis Solution, mL	Vol. Carrier RNA, µL	No. samples	Vol. Lysis Solution, mL	Vol. Carrier RNA, µL
1	0.22	5.5	**13**	2.86	71.5
2	0.44	11.0	**14**	3.08	77.0
3	0.66	16.5	**15**	3.30	82.5
4	0.88	22.0	**16**	3.52	88.0
5	1.10	27.5	**17**	3.74	93.5
6	1.32	33.0	**18**	3.96	99.0
7	1.54	38.5	**19**	4.18	104.5
8	1.76	44.0	**20**	4.40	110.0
9	1.98	49.5	**21**	4.62	115.5
10	2.20	55.0	**22**	4.84	121.0
11	2.42	60.5	**23**	5.06	126.5
12	2.64	66.0	**24**	5.28	132.0

Note:

Required volumes (µL) of Carrier RNA are calculated using following formula:

$N \times 0.22$ mL = Y mL

Y mL $\times$ 25.0 µL/mL = Z µL

Where: N – number of samples to be processed;

Y – calculated volume (mL) of Lysis Solution ;

Z – volume (µL) of Carrier RNA to add to Y mL of Lysis Solution .

INTERNAL CONTROL

The presence of an internal control throughout the extraction and purification procedure may be necessary for certain assays. Please refer to the user manual provided with the downstream detection assay for further directions on how to use an internal control.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and sterile, nuclease-free pipette tips with aerosol barrier
- Vortex
- Ethanol (96-100%)
- Microcentrifuge
- Thermomixer
- Disposable gloves
- Measuring cylinder
- Nuclease-free microcentrifuge tubes of an appropriate size for preparing mixtures of Carrier RNA and Lysis Solution
- Microcentrifuge tubes for sample lysis and elution.
- RNase-free tubes should be used for RNA elution.

PROTOCOLS

Before starting

- Read the user manual; make sure all the directions are followed as indicated.
- Make sure all working solutions and samples have been prepared according to recommendations (page 5-6).
- Ensure all necessary equipment and additional materials are available before beginning the procedure (page 6).
- Organize proper handling of potentially infectious waste before beginning the procedure.
- Follow recommended safety instructions, as you will be working with potentially infectious material.
- Attention and care must be taken during the entire process.
- All centrifugation steps must be performed at room temperature.

A. Viral nucleic acid purification main protocol

This protocol is for viral DNA and RNA purification from 200 µL of EDTA- or citrate- treated plasma, serum, blood or milk samples. For other sample types refer to additional protocols (page 9). Following procedure provides instruction for processing one sample. When using larger than 200 µL (up to 400 µL) sample volumes refer to protocol E (page 10).

Step	Procedure
1	Add 50 µL of Column Preparation Liquid to the center of Spin Column membrane, so that the membrane is entirely moistened. **Note** • Before starting the procedure, each new Spin Column must be prepared by treating it with Column Preparation Liquid. Column treatment maximizes binding of the nucleic acids to the membrane, resulting in more consistent yields. • Do not centrifuge the prepared column. The prepared column should be stored at room temperature until it is used for sample processing.
2	Load 200 µL of sample to an empty 1.5 mL lysis tube. Add 200 µL of Lysis Solution (supplemented with Carrier RNA), and 50 µL of Proteinase K, mix thoroughly by vortexing or pipetting. Incubate the sample for 15 min at 56°C in a thermomixer. Leave thermomixer turned on for Eluent preheating during later steps of the procedure. Centrifuge for 3-5 s at full speed to collect any sample solution from the inside of the lid. **Note** • Supplement Lysis Solution with Carrier RNA prior to use (page 6)! Use the appropriate internal control as required in a downstream assay user's manual. Do not add the internal control directly to plasma samples. Do not add Proteinase K directly to Lysis Solution.
3	Add 300 µL of ethanol (96-100%) and mix by pipetting or vortexing. Incubate the sample at room temperature for 3 min. Centrifuge for 3-5 s at full speed to collect drops from the inside of the lid.
4	Transfer the lysate to the prepared Spin Column preassembled within the wash tube. Centrifuge the column for 1 min at 6,000 × g. Discard the Wash Tube containing flow-through. Place the Spin Column into a new 2 mL Wash Tube.

	Notes. • Ensure that a new Spin Column has been prepared as described in step 1! • Close the bag with Spin Columns tightly after each use**!**
5	Add 700 µL of Wash Buffer 1 supplemented with ethanol to the Spin Column. Centrifuge the column for 1 min at 6,000 × g. Discard the Wash Tube containing flow-through. Place the Spin Column into a new 2 mL Wash Tube. **Note.** • Supplement concentrated Wash Buffer 1 with ethanol prior to the first use (Page 5)!
6	Add 500 µL of Wash Buffer 2 supplemented with ethanol to the Spin Column. Centrifuge the column for 1 min at 6,000 × g. Discard the Wash Tube containing flow-through. Place the Spin Column into a new 2 mL Wash Tube. **Note.** • Supplement concentrated Wash Buffer 2 with ethanol prior to the first use (Page 5)!
7	Add 500 µL of Wash Buffer 2 supplemented with ethanol to the Spin Column. Centrifuge the column for 1 min at 6,000 × g. Discard the Wash Tube containing flow-through. Place the Spin Column into a new 2 mL Wash Tube.
8	Centrifuge the column for 3 min at 16,000 × g. Discard the Wash Tube containing remaining flow-through.
9	Place the Spin Column into a new 1.5 mL elution tube. Add 50 µL of Eluent preheated to 56°C to the center of Spin Column membrane. Incubate for 2 min at room temperature. Centrifuge the column for 1 min at 13,000 × g. Discard the Spin Column. **Note**. • Lower volume of Eluent (30 -40 µL) can be used in order to concentrate eluted nucleic acids. Larger elution volumes (up to 100 µL) can also be used but may result in dilution of the viral nucleic acid sample.
10	Keep the elution tube containing pure viral nucleic acids. Use the purified nucleic acids immediately or store at −20 °C or -70 °C . **Notes.** • qPCR and RT-qPCR inhibition might occur, if lower than 50 µL volume of Eluent is used for elution. • For further use in downstream qPCR applications, use 1 - 10 µL of viral DNA per 25 µL reaction volume. For reverse transcription (RT), use 1 - 10 µL of viral RNA per 20µL cDNA synthesis reaction volume.

B. Nucleic acid purification from nasal and buccal swabs

Step	Procedure
1	To collect a sample, scrape the swab 5-6 times against the inside cheek or nose.
2	Swirl the swab for 2-3 min in 200 µL of 1 x PBS or TE buffer.
3	Go to step 1 of the standard Viral Nucleic Acid Purification Protocol (page 7).

C. Nucleic acid 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). Note: Urine samples may contain insoluble salt precipitates that can reduce nucleic acid yields, thus limiting sample volume used for purification.
2	Centrifuge 10 min at 800 × g (~3,000 rpm).
3	Discard the supernatant.
4	Resuspend the pellet in 200 µL of 1x PBS.
5	Go to step 1 of the standard Viral Nucleic Acid Purification Protocol (page 7). Note. Urine samples larger than 4.5 mL are not recommended.

D. Nucleic acid purification from saliva

Step	Procedure
1	To collect cells, centrifuge the saliva sample for 5 min at 3,000 × g.
2	Resuspend cells in 200 µL of 1 x PBS or TE buffer.
3	Go to step 1 of the standard Viral Nucleic Acid Purification Protocol (page 7).

E. Viral nucleic acid purification protocol for larger sample volumes (up to 400 µL)

Important: Lysis Solution and ethanol are the only components that have to be scaled up.

Step	Procedure
1	Add 50 µL of Column Preparation Liquid to the center of Spin Column membrane, so that the membrane is entirely moistened. **Note** • Before starting the procedure, each new Spin Column must be prepared by treating it with Column Preparation Liquid. Column treatment maximizes binding of the nucleic acids to the membrane, resulting in more consistent yields. • Do not centrifuge the prepared column. The prepared column should be stored at room temperature until it is used for sample processing.
2	Load the sample to an empty 2.0 mL lysis tube. **Add the same volume (1:1) of Lysis Solution** (supplemented with Carrier RNA), and 50 µL of Proteinase K, mix thoroughly by vortexing or pipetting. Incubate the sample for 15 min at 56°C in a thermomixer. Leave thermomixer turned on for Eluent preheating during later steps of the procedure. Centrifuge for 3-5 s at full speed to collect any sample solution from the inside of the lid. **Note** • Supplement Lysis Solution with Carrier RNA prior to use (page 6)! Use the appropriate internal control as required in a downstream assay user's manual. Do not add the internal control directly to plasma samples. Do not add Proteinase K directly to Lysis Solution.
3	Add ethanol (96-100%). **Use 150 µL ethanol for every 100 µL of initial sample volume.** Mix by pipetting or vortexing. Incubate the sample at room temperature for 3 min. Centrifuge for 3-5 s at full speed to collect drops from the inside of the lid.
4	Transfer the lysate to the prepared Spin Column preassembled within the wash tube. **Do not load more than 700 µL of lysate on the column (For larger volumes, load the remaining volume of the lysate to the same column and centrifuge for a second time).** Centrifuge the column for 1 min at 6,000 × g. Discard the flow-through. Place the Spin Column into a new 2 mL Wash Tube. **Note.** Close the bag with Spin Columns tightly after each use!
5	Go to step 5 of the standard Viral Nucleic Acid Purification Protocol (page 7).

TROUBLESHOOTING

Problem	Possible cause and solution
Low nucleic acid yield	**Improper spin column preparation.** Make sure the Spin Column has been prepared properly by adding Column Preparation Liquid as described on page 5. **Ethanol was not added to the lysate.** Make sure that ethanol was added to the lysate before applying the sample to the purification column. **Ethanol was not added to Wash Buffers 1 and 2.** Make sure that ethanol was added to Wash Buffers 1 and 2 prior to the first use. **Low percentage ethanol used.** Use only 96-100% ethanol. Do not use denatured or 95% ethanol. **Carrier RNA was not added to the lysate.** Reconstitute carrier RNA in Eluent and mix with Lysis Solution as described on page 5. **Degraded carrier RNA.** Do not freeze-thaw the reconstituted Carrier RNA more than 10 times. Store at −20°C to −70°C. **Viral nucleic acid eluate too dilute.** Use recommended 30-50µL of Eluent. **Improper elution conditions.** Apply preheated Eluent into the center of Spin Column membrane as described on page 5.
Column clogging	**Starting material was not completely disrupted.** Reduce the amount of starting material and increase disruption time. **Precipitates were not removed.** When using plasma samples, remove visible kryoprecipitates by centrifugation for 5 min at 3000 × g.
Degraded RNA	**RNase contamination.** To avoid RNase contamination, wear gloves during all procedure and change gloves frequently. Use sterile, disposable RNase free pipette tips. Remove RNase contamination from non-disposable items and work surfaces. **Poor quality of samples.** Always use fresh samples or samples handled as recommended on page 4. For lysis, process the sample quickly to avoid degradation.

Lysis Solution

Xn Harmful

Hazard-determining component of labeling: **Guanidinium chloride**

Risk phrases

R22	Harmful if swallowed.
R38	Irritating to skin.
R41	Risk of serious damage to eyes.
R52/53	Harmful to aquatic organisms may cause long-term adverse effects in the aquatic environment.

Safety phrases

S23	Do not breathe gas/fumes/vapour/spray.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
S60	This material and its container must be disposed of as hazardous waste.
S61	Avoid release to the environment. Refer to special instructions/safety data sheets.

Wash Buffer 1

Xn Harmful

Hazard-determining component of labeling: **Guanidinium chloride**

Risk phrases

R22	Harmful if swallowed.
R36/38	Irritating to eyes and skin.

Safety phrases

S23	Do not breathe gas/fumes/vapour/spray.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37	Wear suitable protective clothing and gloves.
S60	This material and its container must be disposed of as hazardous waste.

Proteinase K

Xn Harmful

Hazard-determining component of labeling: **Proteinase, Tritirachium album serine**

Risk phrases

R42 May cause sensitization by inhalation.

Safety phrases

S23 Do not breathe gas/fumes/vapor/spray.
S36 Wear suitable protective clothing.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S60 This material and its container must be disposed of as hazardous waste.

REFERENCES

1. Boom, R., C.J.A. Sol, M.M.M. Salimans, C.L. Jansen, P.M.E.W. Dillen, and J. van der Noordaa. 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28:495–503.

PRODUCT USE LIMITATION

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

Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

Safety Data Sheet

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

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

Product identifier

Product code 10977035
Product name DNASE/RNASE-FREE DISTILLED WATER

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

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

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

Details of the supplier of the safety data sheet

Manufacturer / Supplier

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

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

Country Specific Emergency Number (if available):
CHEMTREC Ireland (Dublin) +(353)-19014670 (Greeting Language: English and Irish)
CHEMTREC UK (London) +(44)-870-8200418 (Greeting Language: English)

SECTION 2: Hazards identification

Classification of the substance or mixture

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

Physical hazards
Not Hazardous

Health hazards
Not Hazardous

Environmental hazards
Not Hazardous

Additional information
Not Applicable

Label elements

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

Hazard pictograms
None

Signal Word
None

Hazard Statements
Not Applicable

EU Specific Hazard Statements
Not Applicable

Precautionary Statements

Prevention
Not Applicable

Response
Not Applicable

Storage
Not Applicable

Disposal
Not Applicable

Other hazards
This mixture does not contain any substances that are assessed to be a PBT or a vPvB

SECTION 3: Composition/information on ingredients

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

SECTION 4: First aid measures

Description of first aid measures

Skin contact	Rinse skin with water. Immediate medical attention is not required.
Eye contact	Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Ingestion	Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.
Inhalation	Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary.
Notes to Physician	Treat symptomatically.

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

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

SECTION 5: Firefighting measures

Extinguishing media

Suitable extinguishing media	Water spray. Carbon dioxide (CO_2). Foam. Dry chemical.
Unsuitable extinguishing media	No information available.

Special hazards arising from the substance or mixture
Not known

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

SECTION 6: Accidental release measures

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

Environmental precautions
No special environmental precautions required.

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

Reference to other sections
See section 8 for more information.

SECTION 7: Handling and storage

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

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

Specific end use(s)
For research use only.

SECTION 8: Exposure controls/personal protection

Control parameters

Exposure Limits Contains no substances with occupational exposure limit values.

Engineering Measures Ensure adequate ventilation, especially in confined areas.

Exposure controls

Personal protection equipment

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

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

Eye protection Tight sealing safety goggles.

Skin and Body Protection Wear suitable protective clothing.

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

Environmental exposure controls
No special environmental precautions required.

SECTION 9: Physical and chemical properties

Information on basic physical and chemical properties

Appearance	liquid	
Odour	No data	
Odour Threshold	No data	
Molecular Weight	No data	
pH	6-8	
Melting point / melting range	**°C** No data	**°F** No data
Boiling point / boiling range	**°C** No data	**°F** No data
Flash point	**°C** No data	**°F** No data
Autoignition Temperature	**°C** No data	**°F** No data
Decomposition temperature	**°C** No data	**°F** No data
Evaporation rate	No data	
Flammability (solid, gas)	No data	
Upper explosion limit	No data	
Lower explosion limit	No data	
Vapour Pressure	No data	
Vapour density	No data	
Relative density	No data	
Specific gravity	No data	
Solubility	No data	
Partition coefficient: n-octanol/water	No data	
Viscosity	No data	
Explosive properties	No data	
Oxidising properties	No data	

Other information
No data.

SECTION 10: Stability and reactivity

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

SECTION 11: Toxicological information

Information on toxicological effects

There is no evidence available indicating acute toxicity.

Principal Routes of Exposure

Skin corrosion/irritation	Data are conclusive but insufficient for classification
Serious eye damage/irritation	Data are conclusive but insufficient for classification
Respiratory or skin sensitisation	Data are conclusive but insufficient for classification
Specific target organ toxicity (STOT) – single exposure	Data are conclusive but insufficient for classification
Specific target organ toxicity (STOT) – repeated exposure	Data are conclusive but insufficient for classification
Carcinogenicity	Data are conclusive but insufficient for classification
Germ cell mutagenicity	Data are conclusive but insufficient for classification
Reproductive Toxicity	Data are conclusive but insufficient for classification
Aspiration Hazard	Data are conclusive but insufficient for classification

SECTION 12: Ecological information

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

Persistence and degradability	No information available.
Bioaccumulative potential	No information available.

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

Other adverse effects
No information available.

SECTION 13: Disposal considerations

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

SECTION 14: Transport information

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

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

Environmental hazards
Not Applicable

Special precautions for user
Not Applicable

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

SECTION 15: Regulatory information

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

Substances of Very High Concern
None.

Substance subject to authorisation per REACH Annex XIV
None

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

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

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

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

German Water hazard classes (Wassergefährdungsklassen)
Not classified.

Other International Inventories
No information available

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

SECTION 16: Other information

Reason for revision	Update according to Commission Regulation (EU) No 830/2015
Revision number	3
Revision date	28-Oct-2019

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

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

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

dNTP Set, molecular biology grade

Catalog Number R0181, R0182, R0186

Pub. No. MAN0013132 **Rev.** C.00

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

Contents and storage

Cat. No.	Contents	Amount	Storage
R0181	dATP, dCTP, dGTP, dTTP	4 x 0.25 mL, 100 mM	-25 °C to -15 °C
R0182	dATP, dCTP, dGTP, dTTP	4 x 1 mL, 100 mM	
R0186	dATP, dCTP, dGTP, dTTP	4 x 5 mL, 100 mM	

Description

The set consists of 100 mM aqueous solutions of dATP, dCTP, dGTP and dTTP each supplied in a separate vial. The nucleotide solutions are titrated to pH 7.3-7.5 with NaOH. Since the nucleotides are provided separately, the dNTP Set offers maximum flexibility in preparation of reaction mixes for different applications.

Applications

For use in PCR, real-time PCR, high fidelity and long PCR, LAMP-PCR, cDNA synthesis, RT-PCR, RDA, MDA, DNA labeling, and DNA sequencing.

General Characteristics

dATP $C_{10}H_{13}N_5O_{12}P_3Na_3$; MW = 557.2;
λ_{max}=259 nm; ε=15.4×10^3 M^{-1}cm^{-1} at pH 7.0;

dCTP $C_9H_{13}N_3O_{13}P_3Na_3$; MW = 533.1;
λ_{max}=271 nm; ε=9.1×10^3 M^{-1}cm^{-1} at pH 7.0.

dGTP $C_{10}H_{13}N_5O_{13}P_3Na_3$; MW = 573.2;
λ_{max}=253 nm; ε=13.7×10^3 M^{-1}cm^{-1} at pH 7.0.

dTTP $C_{10}H_{14}N_2O_{14}P_3Na_3$; MW = 548.1;
λ_{max}=267 nm; ε=9.6×10^3 M^{-1}cm^{-1} at pH 7.0.

Important Note

Mix well each dNTP solution prior to use.

Preparation of different concentration dNTP mixtures

dNTP mixture to be prepared	Volumes of dNTP Set, µL				Water, nuclease-free, µL	Total volume of dNTP mixture, µL
	100 mM dATP	100 mM dGTP	100 mM dCTP	100 mM dTTP		
2 mM of each dNTP	10	10	10	10	460	500
	100	100	100	100	4600	5000
	250	250	250	250	11500	12500
10 mM of each dNTP	10	10	10	10	60	100
	100	100	100	100	600	1000
	250	250	250	250	1500	2500
25 mM of each dNTP	10	10	10	10	–	40
	100	100	100	100	–	400
	250	250	250	250	–	1000

Getting 0.2 mM dNTP in PCR

Volume of PCR Mixture	dNTP Mixture to be added to PCR		
	2 mM	10 mM	25 mM
25 µL	2.5 µL	0.5 µL	0.2 µL
50 µL	5 µL	1 µL	0.4 µL
100 µL	10 µL	2 µL	0.8 µL

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

PRODUCT INFORMATION

Thermo Scientific GeneRuler 50 bp DNA Ladder

Pub. No. MAN0013016
Rev. Date 12 February 2018 (Rev. C.00)

Components	#SM0371	#SM0372
GeneRuler 50 bp DNA Ladder, 0.5 µg/µL	50 µg (for 100 applications)	250 (5 x 50) µg (for 500 applications)
6X TriTrack DNA Loading Dye	1 mL	2 × 1 mL

Store at -25°C to -15°C

www.thermofisher.com

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

Description

Thermo Scientific™ GeneRuler™ 50 bp DNA Ladder is designed for sizing and approximate quantification of wide range double-stranded DNA on agarose and polyacrylamide gels. The ladder is composed of thirteen chromatography-purified individual DNA fragments (in base pairs): 1000, 900, 800, 700, 600, **500**, 400, 300, **250**, 200, 150, 100, 50. It contains two reference bands (500 and 250 bp) for easy orientation.
The ladder is dissolved in TE buffer.

Storage Buffer

10 mM Tris-HCl (pH 7.6), 1 mM EDTA.

6X TriTrack DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 0.15% orange G, 60% glycerol and 60 mM EDTA.

Protocol for Loading

Loading mixture for the 5 mm gel lane*:

Components	Gels	
	Agarose	**Polyacrylamide**
DNA ladder (0.5-1 µg)	1-2 µL	1-2 µL
6X TriTrack DNA Loading Dye	1 µL	0.5 µL
Deionized water	4-3 µL	1.5-0.5 µL
	6 µL	3 µL

Step 1: Mix gently

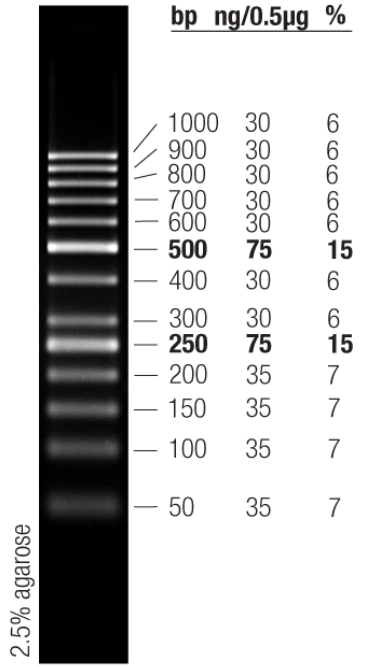
Step 2: Load on the gel

*For gels with other lane widths, the components of the mixture should be scaled either up or down. Use 0.2-0.4 µL (0.1-0.2 µg) of DNA ladder per 1 mm of lane.

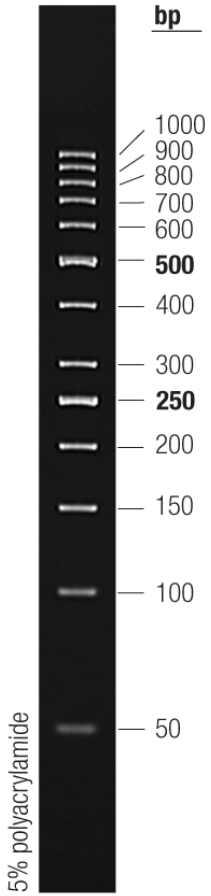
Recommendations

- Do not heat before loading.
- Dilute your DNA sample with the 6X TriTrack DNA Loading Dye (#R1161, supplied with the ladder): mix 1 volume of the dye solution with 5 volumes of the DNA sample;
- Load the same volumes of the DNA sample and the DNA ladder;
- For quantification, adjust the concentration of the sample to equalize it approximately with the amount of DNA in the nearest band of the ladder.
- For DNA band visualization with SYBR™ Green and other intercalating dyes, do not add the dyes into the sample, use gel staining after electrophoresis or include dyes into agarose gel to avoid aberrant DNA migration.
- **Important note:** For DNA bands visualization with GelRed™ use gel staining after electrophoresis to avoid aberrant DNA migration.

GeneRuler 50 bp DNA Ladder



0.5 µg/lane, 8 cm length gel,
1X TBE, 5 V/cm, 1 h



0.5 µg/lane, 20 cm length gel,
1X TAE, 8 V/cm, 3 h

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

pUC19 DNA/MspI (HpaII) Marker, 23

Catalog Number SM0221, SM0222

Pub. No. MAN0012992 **Rev.** C.00

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

Contents and storage

Cat. No.	Contents	Amount	Storage
SM0221	pUC19 DNA/MspI (HpaII) Marker, 23	50 µg (for 100 applications), 0.5 µg/µL	-25 °C to -15 °C
	6X DNA Loading Dye	1 mL	
SM0222	pUC19 DNA/MspI (HpaII) Marker, 23	250 (5 x 50) µg (for 500 applications), 0.5 µg/µL	
	6X DNA Loading Dye	2 x 1 mL	

Description

pUC19 DNA was completely digested with MspI, purified and dissolved in a storage buffer.
The DNA Marker contains the following 13 discrete fragments (in base pairs): 501, 489, 404, 331, 242, 190, 147, 111, 110, 67, 34, 34, 26.

Storage Buffer

10 mM Tris-HCl (pH 7.6), 1 mM EDTA.

6X DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.03 % bromophenol blue, 0.03 % xylene cyanol FF, 60 % glycerol and 60 mM EDTA.

Protocol for Loading

Loading mixture for the 5 mm gel lane*:

Components	Gels	
	Agarose	**Polyacrylamide**
DNA ladder (0.5-1 µg)	1-2 µL	1-2 µL
6X DNA Loading Dye	1 µL	0.5 µL
Deionized water	4-3 µL	1.5-0.5 µL
	6 µL	3 µL

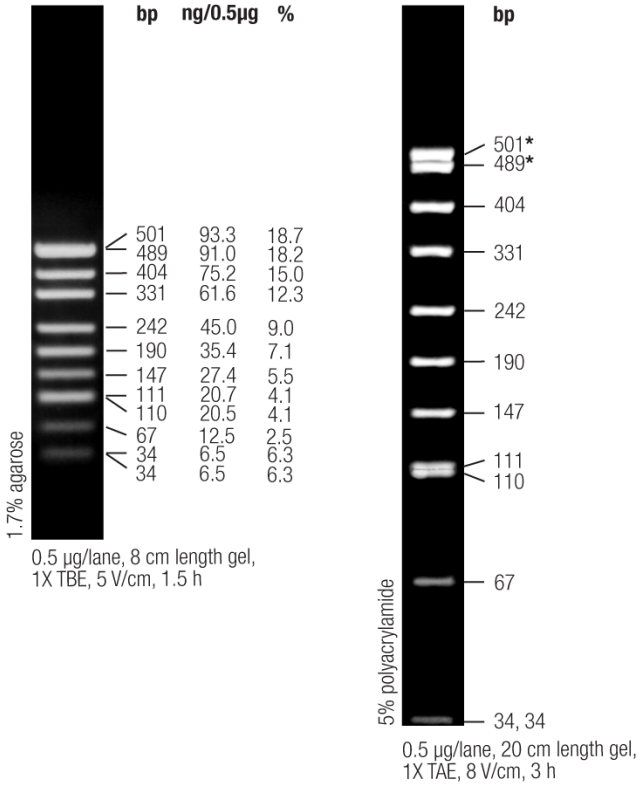
Step 1: Mix gently
Step 2: Load on the gel

*For gels with other lane widths, the components of the mixture should be scaled either up or down. Use 0.2-0.4 µL (0.1-0.2 µg) of DNA ladder per 1 mm of lane.

Recommendations

- Do not heat before loading
- Dilute your DNA sample with the 6X DNA Loading Dye (#R0611, supplied with the ladder): mix 1 volume of the dye solution with 5 volumes of the DNA sample;
- For DNA band visualization with SYBR™ Green and other intercalating dyes, do not add the dyes into the sample, use gel staining after electrophoresis or include dyes into agarose gel to avoid aberrant DNA migration.
- **Important note:** For DNA bands visualization with GelRed™ use gel staining after electrophoresis to avoid aberrant DNA migration.

pUC19 DNA/MspI (HpaII) Marker, 23



*The 501 and 489 bp bands migrate anomalously (1, 2, 3).
26 bp fragment is not visible and comprises 1.0 %

References

1. Stellwagen, N.C., Anomalous electrophoresis of deoxyribonucleic acid restriction fragments on polyacrylamide gels, Biochemistry, 22, 6186-6193, 1983.
2. Lane, D., et al., Use of gel ratardation to analyze protein – nucleic acid interactions, Microbiological Reviews, 56, 509-528, 1992.
3. Stellwagen, N.C., Conformational isomers of curved DNA molecules can be observed by polyacrylamide gel electrophoresis, Electrophoresis, 21, 2327-2334, 2000.

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SYBR® Gold Nucleic Acid Gel Stain

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
SYBR® Gold nucleic acid gel stain	500 µL	Solution in high-quality, anhydrous DMSO *	• ≤–20°C • Desiccate • Protect from light	When stored as directed, stain stock solution is stable for 6 months to 1 year.

* DMSO stock solution is a 10,000X concentrate.

Number of labelings: Sufficient dye is provided to stain at least 100 agarose or polyacrylamide minigels.

Approximate fluorescence excitation/emission maxima: 300, 495/537 nm, bound to nucleic acid

Introduction

Molecular Probes SYBR® Gold nucleic acid gel stain is the most sensitive fluorescent stain available for detecting double- or single-stranded DNA or RNA in electrophoretic gels, using standard ultraviolet transilluminators—surpassing even the sensitivity of our SYBR® Green gel stains in this application.[1] SYBR® Gold stain is a proprietary unsymmetrical cyanine dye that exhibits >1000-fold fluorescence enhancement upon binding to nucleic acids and has a high quantum yield (~0.6) upon binding to double- or single-stranded DNA or to RNA.[1] Excitation maxima for dye–nucleic acid complexes are at ~495 nm in the visible and ~300 nm, in the ultraviolet (Figure 1). The emission maximum is ~537 nm. SYBR® Gold stain is >10-fold more sensitive than ethidium bromide for detecting DNA and RNA in denaturing urea, glyoxal, and formaldehyde gels, even with 300 nm transillumination.[1] For detecting glyoxalated RNA, SYBR® Gold stain is 25–100 times more sensitive than ethidium bromide (Figure 2) and is by far the most sensitive stain available for this application.[1] SYBR® Gold stain has also been shown to be much more sensitive than SYBR® Green II stain for detecting single strand conformation polymorphism (SSCP) products.[2] SYBR® Gold stain penetrates thick and high percentage agarose gels rapidly, and even formaldehyde agarose gels do not require destaining, due to the low intrinsic fluorescence of the unbound dye. The presence of the dye in stained gels at standard staining concentrations does not interfere with restriction endonucleases, T4 DNA ligase, Taq polymerase, or with Southern or Northern blotting.[1] Dye is readily removed from nucleic acids by ethanol precipitation, leaving pure templates available for subsequent manipulation or analysis.

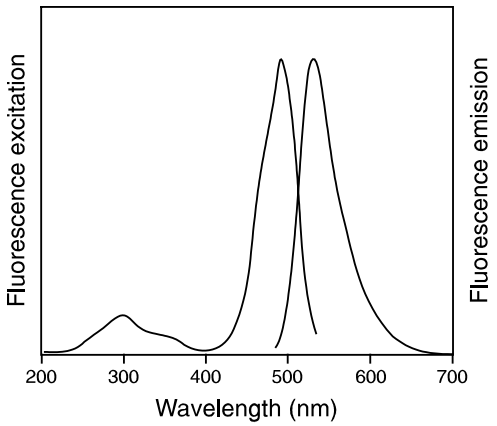

Figure 1. Excitation and emission spectra of SYBR® Gold nucleic acid gel stain bound to double-stranded DNA.

Before You Begin

Materials Required but Not Provided

- TE, TBE, or TAE buffer
- SYBR® photographic filter (S7569)
- Ethanol
- Sodium acetate or ammonium acetate

Working with the SYBR® Gold Gel Stain

Before opening, each vial should be allowed to warm to room temperature and then briefly centrifuged in a microfuge to deposit the DMSO solution at the bottom of the vial. Be sure the dye solution is fully thawed before removing an aliquot.

Staining reagent diluted in buffer can be stored protected from light either at 4°C for several weeks or at room temperature for three or four days. Staining solutions prepared in water are less stable than those prepared in buffer and must be used within 24 hours to ensure maximal staining sensitivity. In addition, staining solutions prepared in buffers with pH below about 7.0 or above 8.5 are less stable and show reduced staining efficacy. We recommend storing aqueous working solutions in plastic rather than glass, as the stain may adsorb to glass surfaces.

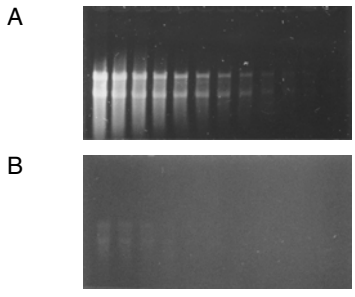

Figure 2. Comparison of glyoxalated RNA stained with SYBR® Gold stain and with ethidium bromide. Identical twofold dilutions of glyoxalated *E. coli* 16S and 23S ribosomal RNA were separated on 1% agarose minigels using standard methods [2] and stained for 30 minutes with SYBR® Gold stain in TBE buffer (A) or 0.5 µg/mL ethidium bromide in 0.1 M ammonium acetate (B). Both gels were subjected to 300 nm transillumination and photographed with Polaroid 667 black-and-white print film, through a SYBR® photographic filter (S7569) for the gel stained with SYBR® Gold dye and through an ethidium bromide gel stain photographic filter for the gel stained with ethidium bromide.

Caution

No data are available addressing the mutagenicity or toxicity of SYBR® Gold nucleic acid gel stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

Disposal

As with all nucleic acid reagents, solutions of SYBR® Gold stain should be disposed of in accordance with local regulations.

Experimental Protocol

The protocol below describes how to stain minigels with SYBR® Gold stain after electrophoresis. To stain agarose gels or polyacrylamide minigels, immerse the entire gel in staining solution. To stain large or extremely fragile polyacrylamide gels, leaving the gel on one of the gel plates and overlaying the gel with dye is probably a more practical procedure. When employing the dye overlay procedure, be sure to turn the stained gel upside down on the transilluminator prior to photography, as most glass plates will block at least some of the ultraviolet light, resulting in poor excitation of dye–nucleic acid complexes. Casting gels containing SYBR® Gold stain is not recommended, as the dye causes severe electrophoretic mobility retardation of nucleic acids in the gel.

Staining Minigels with SYBR® Gold Stain

1.1 Dilute the stock SYBR® Gold stain 10,000-fold to make a 1X staining solution.

- Dilute into TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5–8.0), TBE (89 mM Tris base, 89 mM boric acid, 1 mM EDTA, pH 8.0), or TAE (40 mM Tris-acetate, 1 mM EDTA, pH 7.5–8.0) buffer.
- Staining with SYBR® Gold stain is somewhat pH sensitive. For optimal sensitivity, verify that the pH of the staining solution at the temperature used for staining is between 7.0 and 8.5.

1.2 Incubate the gel in 1X staining solution for 10–40 minutes.

- Place the gel in the staining container, such as a petri dish, the lid of a pipet-tip box, or a polypropylene container.
- Add enough staining solution to completely cover the gel. A 50 mL volume is generally sufficient for staining most standard minigels. To stain large agarose gels, scale up the volume of staining solution in proportion to the increased gel volume and ensure that the entire gel is fully immersed during staining.
- Protect the staining solution from light by covering it with aluminum foil or by placing it in the dark.
- Prewashes of gels are not required, even for gels containing urea, formaldehyde, or glyoxalated samples. Removal of the glyoxal is also not necessary.

1.3 Agitate the gel gently at room temperature.

- The optimal staining time is typically 10-40 minutes, depending on the thickness of the gel and the percentage of agarose or polyacrylamide.

- No destaining is required.
- The staining solution may be stored in the dark and can be reused 3–4 times, although best results are obtained from fresh staining solution.

Viewing and Photographing the Gel

2.1 Illuminate the stained gel.

- Blue-light transilluminators, such as Invitrogen's Safe Imager™ blue-light transilluminator also show excellent sensitivity with SYBR® Gold stained gels.
- Stained gels may also be viewed with 300 nm ultraviolet or 254 nm epi- or transillumination.
- Stained gels may also be visualized and anaylzed with laser scanners. Maximum visible-light excitation is 495 nm.

2.2 Photograph the gel.

- Gels may be photographed using Polaroid 667 black-and-white print film and a SYBR® photographic filter (S7569). When using Polaroid film and this filter, we find that when exciting gels at 300 nm using the FOTO/UV® 450 transilluminator (FotoDyne, Inc., Hartland, WI), a 0.5-1.0 second exposure with an f-stop of 5.6 is generally optimal. Optimal photographic conditions should be determined empirically for other light sources.
- With 254 nm epi-illumination, exposures of ~1 minute may be required for maximal sensitivity when using Polaroid film and the SYBR® filter.
- Generally, optimal exposure times for SYBR® Gold dye-stained gels are shorter than those required for identical gels stained with the SYBR® Green gel stains, due to the higher quantum yield of SYBR® Gold stain.
- Gels stained with the SYBR® Gold dye can also be documented using CCD cameras or laser scanner systems equipped with appropriate optical filters. Generally filters designed for use with the SYBR® Green gel stains are adequate. Optimal exposure times or other instrument settings will have to be determined empirically.

Removing SYBR® Gold Stain from Nucleic Acids

The SYBR® Gold stain can be efficiently removed from nucleic acids by simply precipitating the DNA or RNA with ethanol. More than 97% of the dye is removed by a single precipitation step. More than 99% of the dye is removed if ammonium acetate is used as the salt in the precipitation procedure.

3.1 Add one of the following salts to the nucleic acid sample, to the indicated final concentra tion: 200 mM NaCl, 300 mM sodium acetate (pH 5.2) or 2.0 M ammonium acetate. Mix gently.

3.2 Add two volumes of ice-cold absolute ethanol and mix well. Incubate at 0°C (on ice) for 30 minutes.

3.3 Pellet nucleic acids by centrifuging for at least 15 minutes at 10,000–12,000 × g.

3.4 Remove the supernatant and wash the pellet with 70% ethanol.

3.5 Centrifuge again to pellet nucleic acids.

3.6 Allow the pellet to air dry and resuspend as desired.

References

1. Anal Biochem 268, 278 (1999); **2.** Personal communication, Chris Weghorst, Ohio State Universtity; **3.** Proc Natl Acad Sci USA 74, 4835 (1977).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
S11494	SYBR® Gold nucleic acid gel stain *10,000X concentrate in DMSO*	500 µL
S7569	SYBR® photographic filter	each

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
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Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific DreamTaq Hot Start Green DNA Polymerase

Pub. No. MAN0015973
Rev. Date 02 August 2016 (Rev. A.00)

#__

Lot: __ Expiry Date: __

Ordering Information

Catalog No.	DreamTaq Hot Start DNA Polymerase, 5 U/µL	10X DreamTaq Green Buffer*
EP1711	200 U	1.25 mL
EP1712	500 U	2 × 1.25 mL
EP1713	2500 U	10 × 1.25 mL
EP1714	4 × 2500 U	40 × 1.25 mL

* includes 20 mM $MgCl_2$

Store at –20°C

www.thermofisher.com

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

DESCRIPTION

Thermo Scientific™ DreamTaq™ Green DNA Polymerase is a combination of DreamTaq™ Hot Start DNA Polymerase and 10X DreamTaq Green Buffer. DreamTaq™ Hot Start DNA Polymerase is an enhanced hot start *Taq* DNA polymerase optimized for most PCR applications. It ensures higher sensitivity, specificity, and yields compared to conventional hot start *Taq* DNA polymerase. It is capable of amplifying long amplicons such as 6 kb genomic DNA and 20 kb λ DNA.

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

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

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

FEATURES

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

APPLICATIONS

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

CONCENTRATION

5 U/µL

DEFINITION OF ACTIVITY UNIT

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

10X DREAMTAQ GREEN BUFFER

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

The 10X DreamTaq Green Buffer includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The colored buffer does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, ligation, and restriction digestion. For applications that require PCR product analysis by absorbance or fluorescence excitation, we recommend using the colorless 10X DreamTaq Buffer (#B65) or purifying the PCR product prior to analysis.

INHIBITION AND INACTIVATION

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

PROTOCOL

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

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

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

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

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

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

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

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

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

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

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

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

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

GUIDELINES FOR PRIMER DESIGN

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

- Use PCR primers that are 15–30 nucleotides long.
- Optimal GC content of the primer is 40–60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.

(continued on reverse page)

- Avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3'-end.
- Avoid self-complementary primer regions, and complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementarity between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

ESTIMATION OF PRIMER MELTING TEMPERATURE

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

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

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

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

COMPONENTS OF THE REACTION MIXTURE

Template DNA

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

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

$MgCl_2$ concentration

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

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

dNTPs

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

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

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

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

Primers

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

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

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

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

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

Denaturation

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

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

Primer annealing

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

Extension

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

Number of cycles

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

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

Final extension

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

TROUBLESHOOTING

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

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

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

Residual Activity Assay

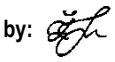
No detectable extension of labeled double stranded oligonucleotide with 5'-overhangs after incubation in the presence of dNTPs.

E. coli DNA Assay

No detectable *E.coli* DNA was observed.

Functional Assay

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

Quality authorized by:  Jurgita Zilinskiene