Applied Biosystems

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

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

Improved Performance

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

New Chemistries to Address Your Sequencing Needs

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

Chemistry Options

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

+ Recommended ✔ Satisfactory

Table 1. Chemistry Options

Easy Integration

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

BigDye® Terminator v3.1 Chemistry

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

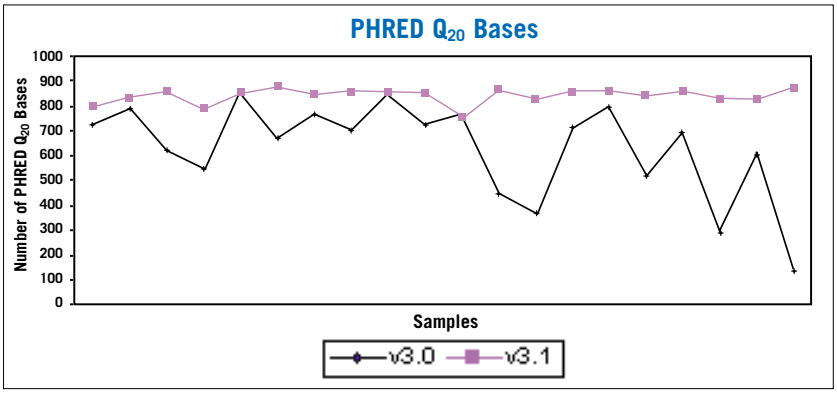

Figure 1. Longer, higher quality reads with the BigDye® Terminator v3.1 kit. The BigDye Terminator v3.1 kit generates data with an improved average number of PHRED Q_{20} bases. A library of "difficult" templates was sequenced in a customer's laboratory according to their standard protocol using the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

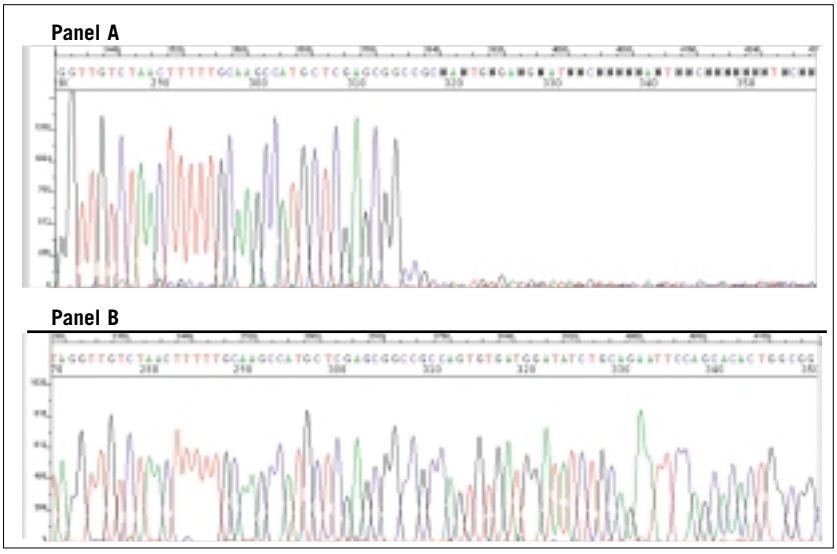

Figure 2. Improved Performance on Difficult Templates with BigDye® Terminator v3.1. A sample was run in a customer's laboratory according to their standard protocol. With the BigDye® Terminator v3.0 kit the reaction is terminated by an unknown sequence context (Panel A), while the reaction prepared with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

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

BigDye Terminator v1.1 Chemistry

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

Choosing the Right DNA Sequencing Chemistry

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

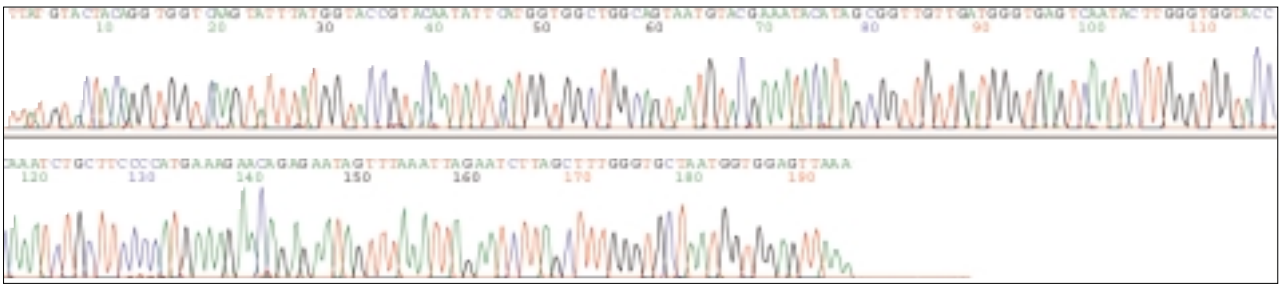

Figure 3. Short PCR Product Sequencing with BigDye® Terminator v1.1. The v1.1 kit successfully sequences a short PCR product generated from human mitochondrial DNA. The PCR product shows 100% basecalling accuracy beginning with the first base adjacent to the primer. The sample was run on the 3100 Genetic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

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

Guaranteed Performance

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

Specifications

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

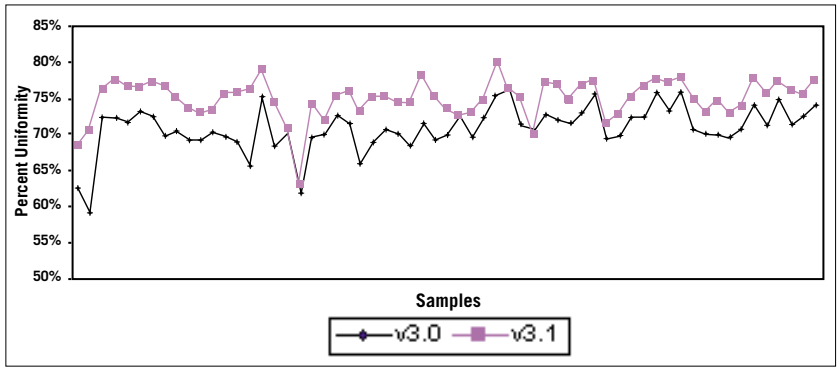

Figure 4. Improved uniformity of peak heights. BigDye® Terminator v3.1 chemistry generates data with improved peak height uniformity in customer samples. Peak height uniformity is defined as local peak height consistency of analyzed data. 100% peak height uniformity represents an idealized situation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

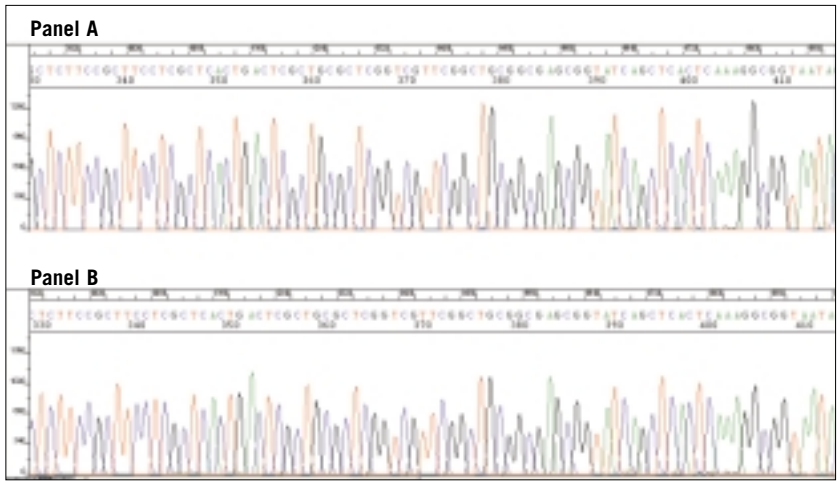

Figure 5. Improved uniformity of peak heights with BigDye® Terminator v3.1 kit. Panel A shows data from a sample sequenced using the BigDye® Terminator v3.0 kit. Panel B shows the same sample run under identical conditions with the BigDye Terminator v3.1 kit. The uniformity of the data produced with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

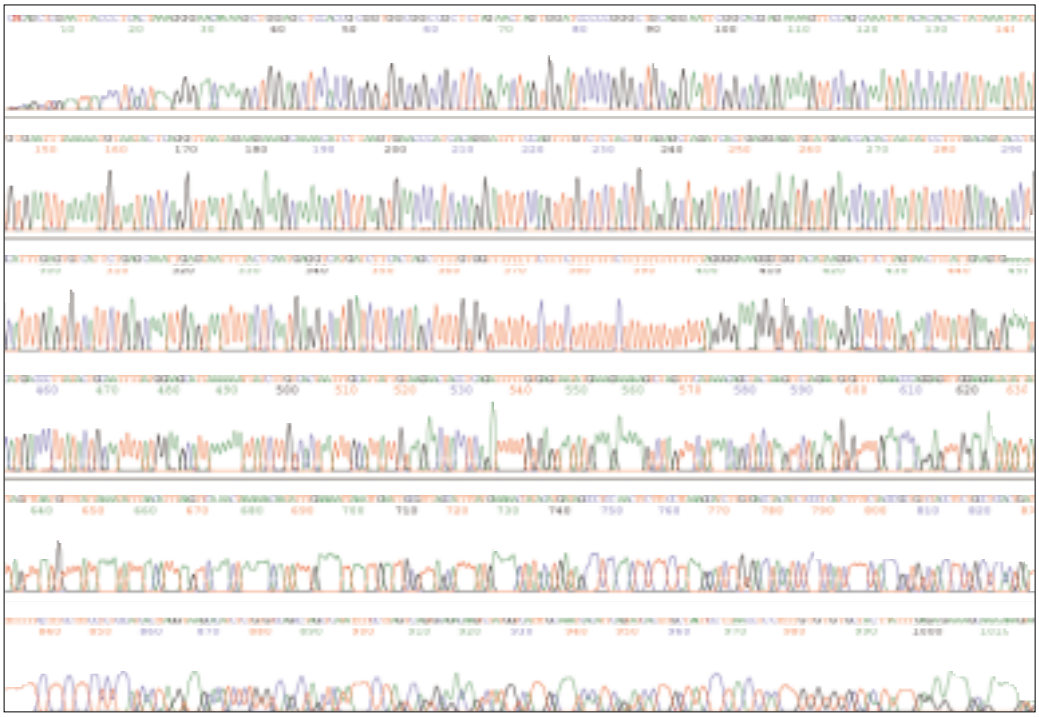

Figure 6. Longer Read Lengths with BigDye® Terminator v3.1 on the 3100 Genetic Analyzer. This figure shows accurate basecalling for more than 1,000 bases; the first ambiguity is not seen until base 1,040. The sample was run on the ABI PRISM® 3100 Genetic Analyzer with an 80 cm array using POP-4™ Polymer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

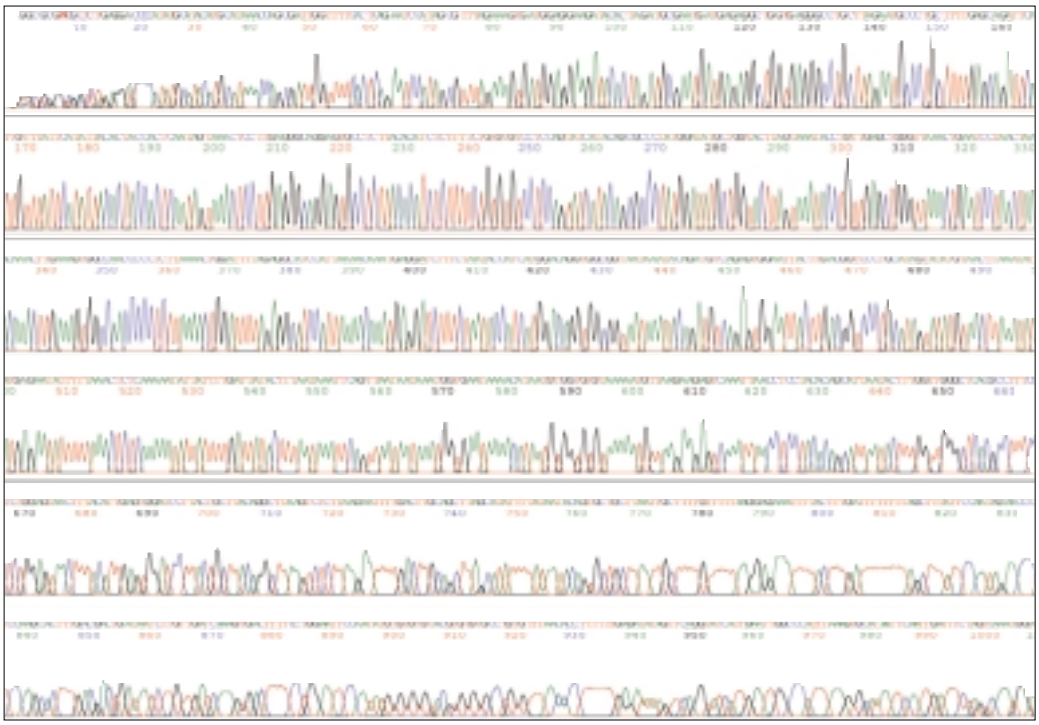

Figure 7. Longer Read Lengths with BigDye® Terminator v3.1 on the 3730*xl* Genetic Analyzer. The plasmid insert, beginning at base 23, shows accurate basecalling for more than 1,000 bases. The first ambiguity occurs at base 1,031. The sample was run on an Applied Biosystems 3730*xl* Genetic Analyzer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

Ordering Information

BigDye® Terminator v3.1 Cycle Sequencing Kit

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

BigDye® Terminator v1.1 Cycle Sequencing Kit

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

BigDye® Terminator v3.1 Sequencing and Matrix Standards*

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

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

BigDye® Terminator v1.1 Sequencing and Matrix Standards*

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

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

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

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

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Applied Biosystems

Using ExoSAP-IT™ *Express* PCR Product Cleanup to generate high quality BigDye™ Terminator v3.1 Cycle Sequencing Kit Data

Publication Number MAN0017063 **Revision** A.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**.

Overview

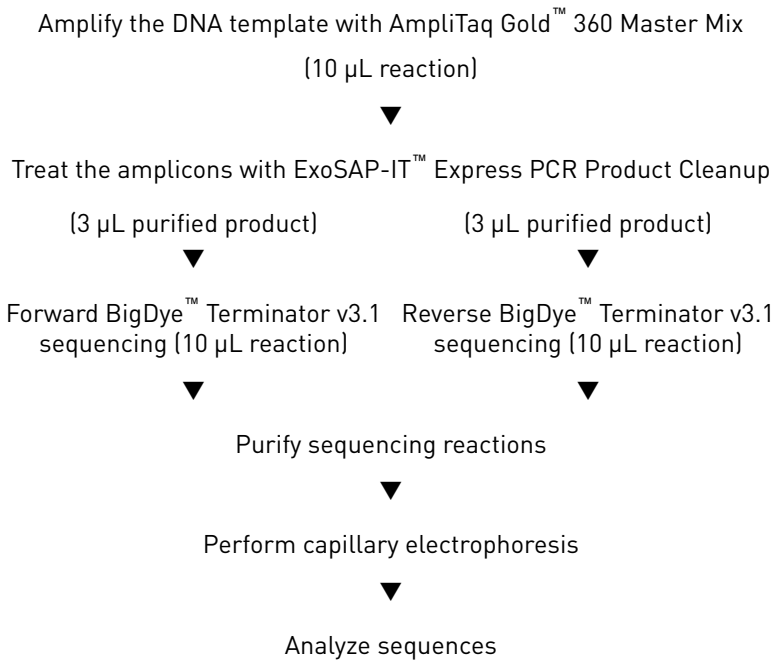
This document provides a protocol for generating high-quality sequence data using:

- BigDye™ Terminator v3.1 Cycle Sequencing Kit
- Applied Biosystems™ Genetic Analyzers
- ExoSAP-IT™ *Express* PCR Product Cleanup

Certain components of the protocol workflow such as reagent kits and other protocols for preparation of reagents may not be available through Thermo Fisher Scientific.

For sequencing short amplicons, use the alternative protocol, *Generating high-quality data using the BigDye™ Direct Cycle Sequencing Kit* (Pub. no. MAN0014436), which has been optimized to reduce loss of 5′ sequences.

Workflow



Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Reagents	
BigDye™ Terminator v3.1 Cycle Sequencing Kit	4337456
AmpliTaq Gold™ 360 Master Mix	4398881
BigDye XTerminator™ Purification Kit	4376486
UltraPure™ DNase/RNase-Free Distilled Water	10977-015
Hi-Di™ Formamide **Note:** Not required for BigDye XTerminator™ Purification Kit purification.	4311320 or 4440753
ExoSAP-IT™ *Express* PCR Product Cleanup	75001
DNA Suspension Buffer, RNAse DNAse Free (10 mM Tris/0.1 mM EDTA, pH 8.0)	Teknova, Inc. T0223
PCR and sequencing primers (HPLC-purified recommended)	Primers can be designed, chosen, and ordered with the Primer Designer™ Tool at **http://www.thermofisher.com/primerdesigner**
Reagents for Centri-Sep™ purification (*optional*)	

Item	Source
Sodium Dodecyl Sulfate (SDS)	15525-017
Centri-Sep™ 96-Well Plates	4367819
Reagents for ethanol/EDTA purification (*optional*)	
0.5M EDTA, pH 8.0 for molecular biology	AM9260G
Ethanol, absolute, for molecular biology	Major Laboratory Suppliers (MLS)
Laboratory supplies	
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical 96-Well Reaction Plate	8010560
Plate Septa, 96 well	4315933
25 mL Reagent Reservoir, Pyrogen-free, RNase/DNase certified, sterile	VistaLab Technologies, Inc. 3054-1002
Digital Vortex-Genie™ 2 or equivalent	Scientific Inducstries, Inc. SI-A536
Centrifuge with swinging bucket (with PCR plate adapter)	MLS
Compatible thermal cyclers [1]	
GeneAmp™ PCR System 9700	Contact your local sales office
Veriti™ Thermal Cycler	
Compatible genetic analyzers	
3130/3130*xl* Genetic Analyzer	Contact your local sales office
3500/3500xL Genetic Analyzer	
3730/3730*xl* DNA Analyzer [2]	

[1] If you use a different thermal cycler, you may need to optimize the thermal cycling conditions.

[2] Standard heat seal consumables can be used in place of MicroAmp™ plates and film for these instruments.

DNA and primer requirements

Input DNA requirements

The quality of the DNA can significantly influence the length of the fragment that can be amplified and the reproducibility of amplification from one sample to another. Even if the fragment successfully amplifies, poor quality DNA can result in decreased signal or increased background fluorescent noise from the sequencing reactions.

For optimal results, use 10 to 20 ng/µL of template DNA with spectrophotometer absorbance ratios ($A_{260/280}$) between 1.8 to 2.0.

Factors affecting template quality

- **Type and amount of source material** – Influences the effectiveness and sensitivity of PCR amplification and the quality of sequencing results. The number of sequencing targets relative to the number of primer molecules can influence the efficiency and read-length of the sequencing reaction.
- **Contamination** – Can inhibit PCR amplification and cycle sequencing. Potential contaminants include:
  - Protein, RNA, or chromosomal DNA
  - Excess PCR primers, dNTPs, enzyme, and buffer components
  - Remaining salts, organic chemicals such as phenol, chloroform, and ethanol, or detergents.
  - Heparin—can partially or completely inhibit PCR amplification and cycle sequencing. The Dynabeads™ DNA DIRECT™ Blood Kit and the QIAamp™ Blood Kit (QIAGEN™, GmbH) successfully remove heparin from heparin blood samples, leaving genomic DNA ready for PCR amplification.

Note: Use a DNA isolation kit that is specifically designed for formalin-fixed, paraffin-embedded (FFPE) tissue and ensure that amplicon sizes are appropriate for the length of DNA fragment size that can be isolated.
Smaller amplicons compatible with FFPE-fragmented DNA can be designed using the free Primer Designer™ Tool found at **http://www.thermofisher.com/primerdesigner**.

Determining template quality and quantity

Use a spectrophotometer to determine DNA quality and to check for protein contamination. Optimum absorbance ratios ($A_{260/280}$) are between 1.8 and 2.0.

If DNA and/or RNA contamination is suspected, run your sample on an agarose gel. A single band should be present for high-quality DNA.

For DNA quantification, A_{260} values can be converted into µg/µL using Beer´s Law:

- Concentration of single-stranded DNA = A_{260} × 33 µg/µL.
- Concentration of double-stranded DNA = A_{260} × 50 µg/µL.

Optical density (OD) measurements are used to determine template concentration. Highly concentrated (OD >1.0) or very dilute (OD <0.05) DNA samples can lead to inaccurate OD measurements. Dilute or concentrate the DNA if needed to obtain an OD value between 0.05 to 1.

Note: OD measurement is not a reliable method to determine template concentration following enzymatic PCR purification protocols. Instead, estimate PCR product purity and concentration using an agarose gel or a flourescence-based method like the PicoGreen™ reagent for use on the Qubit™ quantification platform.

Primer guidelines

The method of primer purification and choice of M13 tailed- or non-tailed sequencing primers can have a significant effect on the ease of reaction set up and the quality of the sequencing data that is obtained in dye terminator cycle sequencing reactions.

- Use HPLC-purification for all primers to minimize cycle sequencing noise and provide longer sequencing reads.
- Use M13 sequencing primers to simplify the sequencing workflow when sequencing multiple PCR products and to reduce the loss of valuable 5′ unresolvable bases. With M13 sequencing primers, you make single forward and reverse reaction mixes, instead of multiple, primer-specific reaction mixes.

  Note: The M13 forward or reverse sequence must be incorporated at the 5′ end of the PCR primer to use the M13 sequencing primers.

Primer Designer™ Tool

Primer Designer™ Tool is a free online tool to search for the appropriate PCR/Sanger primer pair from a database of >650,000 pre-designed primer pairs for resequencing the human exome. Go to: **http://www.thermofisher.com/primerdesigner** for more information, including a direct link to purchase the designed primers online.

Prepare and store primers

1. Resuspend all PCR and sequencing primer stocks at 100 µM concentration in DNA buffer (10 mM Tris/0.1 mM EDTA, pH 8.0) and store them at –20°C.
2. Create individual amplicon-specific PCR primer pools of 0.8 µM PCR primers using UltraPure™ DNase/RNase-Free Distilled Water to minimize excess salt contribution that can inhibit subsequent reactions. Store working solutions at –20°C.

Amplify the DNA template with AmpliTaq Gold™ 360 Master Mix

Set up the PCR reaction

1. Completely thaw the AmpliTaq Gold™ 360 Master Mix and store on ice.

   Note: Store reagents at 4°C after first use.
2. Vortex the tubes for 2 to 3 seconds, then centrifuge briefly (2 to 3 seconds) with a benchtop microcentrifuge to collect contents at the bottom of the tubes.

3. Prepare the reaction mix:

   IMPORTANT! Change pipette tips after each transfer to avoid contamination of reagents, specimen, or amplicons.

Component	Quantity (1 well)	Quantity (96 well plate)[1]
AmpliTaq Gold™ 360 Master Mix	5 µL	528 µL
UltraPure™ DNase/RNase-Free Distilled Water	1 µL	106 µL
Total volume	**6 µL**	**634 µL**

[1] Includes 10% additional volume.

   Note: Store on ice until ready for use.

4. Vortex the tubes for 2 to 3 seconds, then centrifuge briefly (2 to 3 seconds) with a benchtop microcentrifuge.

5. Label a plate "PCR plate" and add the following, in order:

Component	Quantity
Reaction mix	6 µL
DNA template (10ng)	1 µL
Pooled PCR primers (0.8 µM each)	3 µL

   IMPORTANT! Change pipette tips after each transfer.

6. Seal the plate with MicroAmp™ Clear Adhesive Film.

7. Vortex the plate for 2 to 3 seconds, then centrifuge in a swinging bucket centrifuge to collect contents to the bottom of the wells (5 to 10 seconds) at 1,000 × *g*.

   Note: Bubbles may be present within the wells, but do not adversely affect the reaction.

Run the PCR

1. Place the plate in a thermal cycler and set the volume.
2. Run the PCR with the following settings:

Parameter	Stage/step					
	Incubate	Cycling (35 cycles)			Final extension	Hold
		Denature	Anneal [1]	Extend [2]		
Temperature	95°C	95°C	58°C	72°C	72°C	4°C
Time	10 minutes	30 seconds	30 seconds	45 seconds	7 minutes	Hold until ready to purify.

[1] If your primer annealing temperatures are not between 60°C and 65°C, annealing conditions may need optimization. See "Primer Designer™ Tool" on page 5 for more information.

[2] Extension times may need to be lengthened for sequences over 700 bp. For more information, refer to the *AmpliTaq Gold™ 360 DNA Polymerase Protocol*.

3. Place the plate on ice or store the plate at 4°C until ready for treatment with ExoSAP-IT™ *Express* PCR Product Cleanup.

   Note: Place plates at –25°C to –15°C for longer-term storage.

Treat the amplicons with ExoSAP-IT™ *Express* PCR Product Cleanup

1. Remove the 96-well plate from the thermal cycler, then centrifuge in a swinging bucket centrifuge for 10 seconds at 1,000 x g.
2. Place the plate and the tube of ExoSAP-IT™ *Express* PCR Product Cleanup on ice.
3. Remove the MicroAmp™ Clear Adhesive Film.
4. Transfer 5 µL of each PCR product to a new 96-well plate.
5. Add 2 µL of ExoSAP-IT™ *Express* PCR Product Cleanup to each well.

   IMPORTANT! Change pipette tips between wells.

6. Label the plate "+ExoSAP-IT."
7. Seal the +ExoSAP-IT plate with MicroAmp™ Clear Adhesive Film.
8. Vortex the plate for 2 to 3 seconds, then centrifuge (5 to 10 seconds) at 1,000 × *g*.

9. Incubate the +ExoSAP-IT plate:

Parameter	Stage/step	
	Digest	ExoSAP-IT™ Inactivation
Temperature	37°C	80°C
Time	4 minutes	1 minute

10. Store the treated plate on ice for immediate use or at –20°C for longer term storage.

Run sequencing reactions using the BigDye™ Terminator v3.1 Cycle Sequencing Kit

Set up the sequencing reactions

IMPORTANT! Protect dye terminators from light. Cover the reaction mix and sequencing plates with aluminum foil before use.

1. Completely thaw the contents of the BigDye™ Terminator v3.1 Cycle Sequencing Kit and your primers, then store on ice.

2. Vortex the tubes for 2 to 3 seconds, then centrifuge briefly (2 to 3 seconds) with a benchtop microcentrifuge to collect contents at the bottom of the tubes.

3. Label microcentrifuge tubes "forward" and "reverse", then add the following components to each tube:

   IMPORTANT! Change pipette tips after each transfer.

Component	Quantity			
	Forward reaction mix		Reverse reaction mix	
	1 reaction	96-well plate [1]	1 reaction	96-well plate[1]
BigDye™ Terminator v3.1 Ready Reaction Mix	2 µL	211 µL	2 µL	211 µL
5x Sequencing Buffer	1 µL	106 µL	1 µL	106 µL
Deionized water (RNase/DNase-free)	3 µL	317 µL	3 µL	317 µL
M13 forward primer (3.2 µM)	1 µL	106 µL	—	—

Component	Quantity			
	Forward reaction mix		Reverse reaction mix	
	1 reaction	96-well plate [1]	1 reaction	96-well plate[1]
M13 reverse primer (3.2 µM)	—	—	1 µL	106 µL
Total volume	**7 µL**	**740 µL**	**7 µL**	**740 µL**

[1] Includes 10% additional volume.

Note: Store on ice and protect from light.

4. Vortex the tubes for 2 to 3 seconds, then centrifuge briefly (2 to 3 seconds) with a benchtop microcentrifuge.

5. Label a new 96-well reaction plate "sequencing".

6. Place the "PCR + ExoSAP-IT" plate on ice, then remove the MicroAmp™ Clear Adhesive Film.

7. For each reaction, add the following, in order:

Component	Quantity
Reaction mix	7 µL
Purified PCR product (ExoSAP-IT™ *Express* PCR Product Cleanup)	3 µL

IMPORTANT! Change pipette tips after each transfer.

Note: Use an 8-tip multi-channel P10 pipette, if available, for amplicon transfer.

8. Seal the plate with MicroAmp™ Clear Adhesive Film.

9. Vortex the plate for 2 to 3 seconds, then centrifuge in a swinging bucket centrifuge to collect contents to the bottom of the wells (5 to 10 seconds) at 1,000 × *g*.

Note: Bubbles may be present within the wells, but do not adversely affect the reaction.

Run the sequencing reactions

1. Place the prepared sequencing plate into the thermal cycler, set the reaction volume, then run with the following conditions:

Parameter	Stage/step				
	Incubate	Cycling (25 cycles)			Hold
		Denature	Anneal [1]	Extend	
Temperature	96°C	96°C	50°C	60°C	4°C
Time	1 minute	10 seconds	5 seconds	4 minutes [2]	Hold until ready to purify.

[1] If your primer annealing temperatures are not between 60°C and 65°C, annealing conditions may need optimization. See "Primer Designer™ Tool" on page 5 for more information.

[2] Shorter amplicons (<500bp) can be run with shorter extension times (for example 2 minutes).

Note: Cycle sequencing will complete in 2 to 2.5 hours.

2. Place the plate on ice or store at 4°C until ready to purify the reactions.

Purify the sequencing reactions

Salts, unincorporated dye terminators, and dNTPs in sequencing reactions obscure data in the early part of the sequence and can interfere with basecalling.

The following methods are recommended for clean-up of cycle sequencing reactions:

- "Purify sequencing reactions with BigDye XTerminator™" on page 10
- "Purify the sequencing reactions with Centri-Sep™ plates" on page 11
- "Purify the sequencing reactions with ethanol/EDTA precipitation" on page 13

Purify sequencing reactions with BigDye XTerminator™

The following protocol takes approximately 40 minutes.

Note: Use disposable reagent reservoirs and an 8-channel P200 pipette, if available, to facilitate the clean-up process.

Note: If you use a 3730 DNA Analyzer, either MicroAmp™ Clear Adhesive Film or standard heat sealing techniques can be used.
This protocol describes plate sealing with MicroAmp™ Clear Adhesive Film.

1. Remove the BigDye XTerminator™ bead solution from 4°C storage and place on ice.

2. Vortex the bottle of BigDye XTerminator™ beads for 8 to 10 seconds before mixing with the SAM solution.

IMPORTANT! For effective BigDye XTerminator™ clean up, it is essential to keep the materials well mixed. Keep reagents on ice between pipetting steps.

3. Prepare the SAM/BigDye XTerminator™ bead working solution:

Component	Volume per 10 µL reaction	Volume per 96-well plate
SAM solution	45 µL	4.75 mL
BigDye XTerminator™ bead solution	10 µL	1.06 mL
Total volume	**55 µL**	**5.81 mL**

4. Remove the MicroAmp™ Clear Adhesive Film from the sequencing plate.

5. Dispense 55 µL/well of the SAM/BigDye XTerminator™ bead working solution to each sample.

   IMPORTANT! To mix thoroughly, pipette the solution up and down 3-4 times before each transfer. Re-mix solution after each dispense step.

6. Seal the plate using MicroAmp™ Clear Adhesive Film.

7. Vortex the 96-well plate for 20 minutes at 1,800 rpm (for the Digital Vortex-Genie™ 2).

8. In a swinging bucket centrifuge, centrifuge the plate at 1,000 × *g* for 2 minutes.

   Note: To store for up to 10 days, seal the plate with MicroAmp™ Clear Adhesive Film, and store at 4°C for capillary electrophoresis (CE) preparation or at –20°C until use. BDX plates can be stored at room temperature for up to 48 hours inclusive of time on the CE instrument.

Purify the sequencing reactions with Centri-Sep™ plates

The following protocol takes approximately 45 minutes (~25 minutes for purification and ~20 minutes for drying).

IMPORTANT! Do NOT skip the drying step in this procedure. Running samples that have not been dried will affect sequencing results.

Note: Individual Centri-Sep™ Spin columns can be used if few sequencing reactions need to be purified. Centri-Sep™ Spin columns must be hydrated for approximately 2 hours before use. Refer to the *DNA Sequencing by Capillary Electrophoresis Chemistry Guide* (Pub. no. 4305080) for more information.

1. Prepare 2.2% SDS (sodium dodecyl sulfate) in standard deionized water.

   Note: Store 2.2% SDS at room temperature. The SDS will precipitate at 4°C or below.

2. Briefly centrifuge the sequencing plate in a swinging bucket centrifuge (5 to 10 seconds) at 1,000 x g.

3. Remove the MicroAmp™ Clear Adhesive Film.

4. Prepare the SDS heat treatment:

Component	Volume
Sequencing reaction	10 µL
UltraPure™ DNase/RNase-Free Distilled Water	10 µL
2.2% SDS	2 µL
Total volume	**22 µL**

5. Vortex the plate for 2 to 3 seconds, then centrifuge (5 to 10 seconds) at 1,000 × *g*.

6. Perform the SDS heat treatment.

Parameter	Stage/step		
	Denature	Incubate	Hold
Temperature	98°C	25°C	4°C
Time	5 min	10 min	Hold

7. Prepare the Centri-Sep™ 96-well plate:

   Note: The Centri-Sep™ 96-well plates come pre-hydrated. The initial centrifugation step removes the hydration solution.

   a. Allow the plate to equilibrate to room temperature.

   b. Place the Centri-Sep™ 96-well plate in an empty 96-well plate.

   c. Centrifuge for 2 minutes at 1,500 x g to remove the hydration solution from the plate.

   d. Discard the plate with flow-through hydration solution.

   e. Place a new MicroAmp™ Optical 96-Well Reaction Plate beneath the prepared Centri-Sep™ 96-well plate to collect purified BigDye™ sequencing reaction product.

8. Briefly centrifuge the SDS heat-treated extension product plate in a swinging bucket centrifuge (5 to 10 seconds) at 1,000 x g and remove the MicroAmp™ Clear Adhesive Film.

9. Dispense 20 µL SDS heat-treated extension product to the corresponding Centri-Sep™ well. Dispense slowly into the center of the well (e.g. electronic pipette setting 4). Do not touch the sides of the well or the gel material.

10. Place a new 96-well collection plate beneath the Centri-Sep™ plate. Using a swinging bucket centrifuge, centrifuge the Centri-Sep™ plate containing the SDS heat treated sample for 2 minutes at 1,500 x g to collect purified sample.

11. Dry the sample in a vacuum centrifuge without heat or in low heat for 10 to 15 minutes or until dry.

12. Go to "Resuspend purified sequencing reactions" on page 14.

    Note: To store, seal the plate with MicroAmp™ Clear Adhesive Film, and store at 4°C for CE preparation or –20°C until use.

Purify the sequencing reactions with ethanol/EDTA precipitation

The following protocol takes approximately 90 minutes.

Note: This method produces a clean signal, but it can cause subtle loss of small molecular weight fragments.

IMPORTANT! Absolute ethanol absorbs water from the atmosphere, which gradually decreases its concentration and can affect sequencing results. Store appropriately and replace frequently.

1. Prepare a 125 mM EDTA solution from 0.5 M EDTA, pH 8.0.
2. Prepare 70% ethanol using absolute ethanol.

   Note: Replace every 2 weeks.

   IMPORTANT! Do NOT pre-mix 125 mM EDTA solution and absolute ethanol. This can cause precipitation of the EDTA.

3. Briefly centrifuge the sequencing plate in a swinging bucket centrifuge (5 to 10 seconds) at 1,000 x g.
4. Remove the MicroAmp™ Clear Adhesive Film from the plate.
5. Add the following in order:

Component	Volume
sequencing reaction (starting volume)	10 µL
125 mM EDTA solution	2.5 µL
absolute ethanol	30 µL
Total volume	**42.5 µL/well**

   IMPORTANT! Dispense the EDTA solution directly into the sample in each well before adding ethanol. If droplets are visible on the wall of the well, briefly centrifuge the plate to ensure that the EDTA mixes with the sequencing reactions.

6. Seal the plate with MicroAmp™ Clear Adhesive Film.
7. Vortex the plate for 2 to 3 seconds, then centrifuge (5 to 10 seconds) at 1,000 × *g*.
8. Incubate the plate at room temperature for 15 minutes.

   IMPORTANT! Timing of this step is critical.

9. Centrifuge the plate in a swinging bucket centrifuge at 1,870 x g (4°C) for 45 minutes.

   IMPORTANT! Proceed to the next step immediately. If there is a delay between steps, centrifuge the plate for an extra 2 minutes and proceed to the next step immediately.

10. Slowly remove the MicroAmp™ Clear Adhesive Film to prevent disruption of the pellet. Place 4 layers of absorbent paper into the centrifuge and carefully invert the plate onto the paper without dislodging the pellet. Centrifuge at 185 × g for 1 minute.

    Do not tip out liquid first. Do not tap plate to help with liquid removal.

11. Add 30 µL of 70% ethanol to each well.

12. Seal the plate with MicroAmp™ Clear Adhesive Film, then centrifuge at 1,870 × *g* (4°C) for 15 minutes.

    IMPORTANT! Proceed to the next step immediately. If there is a delay between steps, centrifuge the plate for an extra 2 minutes and proceed to next step immediately.

13. Slowly remove the MicroAmp™ Clear Adhesive Film to prevent disruption of the pellet. Place 4 layers of absorbent paper into the centrifuge and carefully invert the plate onto the paper towel without dislodging the pellet. Centrifuge at 185 × g for 1 minute.

    Note: Do not tip out liquid first. Do not tap plate to help with liquid removal.

14. Allow the plate to air dry, face up and protected from light, for 5 to 10 minutes at room temperature.

15. Go to "Resuspend purified sequencing reactions" on page 14.

    Note: To store, seal the plate with MicroAmp™ Clear Adhesive Film, and store, protected from light, at 4°C for CE preparation or –20°C until use.

Resuspend purified sequencing reactions

Resuspend samples purified with the Ethanol/EDTA and Centri-Sep™ methods.

Note: It is not necessary to resuspend samples purified with the BigDye XTerminator™ Purification Kit.

1. Remove the MicroAmp™ Clear Adhesive Film.

2. Resuspend dried samples in 10 µL of Hi-Di™ Formamide, then cover with MicroAmp™ Clear Adhesive Film.

   Note: Do not heat samples to resuspend.

3. Vortex thoroughly (5 to 10 seconds), then centrifuge in a swinging bucket centrifuge (5 to 10 seconds) at 1,000 x g.

   Note: Run samples as soon as possible after resuspension.

Run capillary electrophoresis

1. Remove the MicroAmp™ Clear Adhesive Film and replace with a 96-well plate septa.

   IMPORTANT! Plates sealed with heat seal film can be placed directly into the 3730/ 3730xl instruments. All other instruments require 96-well plate septa.

2. Load plates into the genetic analyzer.

3. Select the capillary length, number of capillaries and polymer type.

   Note: There is no default run module for POP-6™ when using the BigDye™ Terminator v3.1 Cycle Sequencing Kit on a 3500/3500xL Genetic Analyzer. Refer to the instrument user guide for creating run modules.

4. Select or create an appropriate run module according to your specific instrument user guide.

   IMPORTANT! Select a run module with a BDx prefix if you purified your sequencing reactions with BigDye XTerminator™. If your instrument does not contain BDx run modules, download them. Refer to the *BigDye XTerminator™ Purification Kit User Bulletin* (Pub. no. 4483510).

5. Select the injection time. Refer to your specific instrument user guide for information on using default settings or changing injection times.

6. Start the run.

Related documentation

Document	Publication number	Description
BigDye™ Terminator v3.1 Cycle Sequencing Kit User Guide	4337035	Describes the BigDye™ Terminator v3.1 Cycle Sequencing Kit hardware and software and provides information on preparing, maintaining, and troubleshooting the system.
Troubleshooting Sanger sequencing data	MAN0014435	This document provides guidance for the review of your data and troubleshooting tips for improving sequencing data quality.
DNA Sequencing by Capillary Electrophoresis Chemistry Guide	4305080	This chemistry guide is designed to familiarize you with Applied Biosystems™ genetic analyzers for automated DNA sequencing by capillary electrophoresis, to provide useful tips for ensuring that you obtain high-quality data, and to help troubleshoot common problems.

Document	Publication number	Description
BigDye XTerminator™ Purification Kit User Bulletin	4483510	This user bulletin provides: • A list of BigDye XTerminator™ Purification Kit run modules • Instructions for downloading and running the BDX Updater Utility to install the run modules • Instructions for running the BDX Updater Utility after you recalibrate the autosampler
BigDye XTerminator™ Purification Kit Quick Reference Card	4383427	This quick reference card provides instructions for BigDye XTerminator™ purification. In particular, it includes information on compatible plate vortexers and heat seal information for 3730 users.
Using an SDS/Heat Treatment with Spin Columns or 96-Well Spin Plates to Remove Unincorporated Dye Terminators	4330951	This protocol provides instructions for adding an SDS/heat treatment to the spin column and spin plate purification methods. This SDS/heat treatment effectively eliminates unincorporated dye terminators from your cycle sequencing reactions.

Customer and technical support

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

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

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

Limited product warranty

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

Manufacturer: Multiple Life Technologies Corporation manufacturing sites are responsible for manufacturing the products associated with the workflow covered in this guide.

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

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

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Revision history: Pub. No. MAN0017063

Revision	Date	Description
A.0	13 June 2017	New document.

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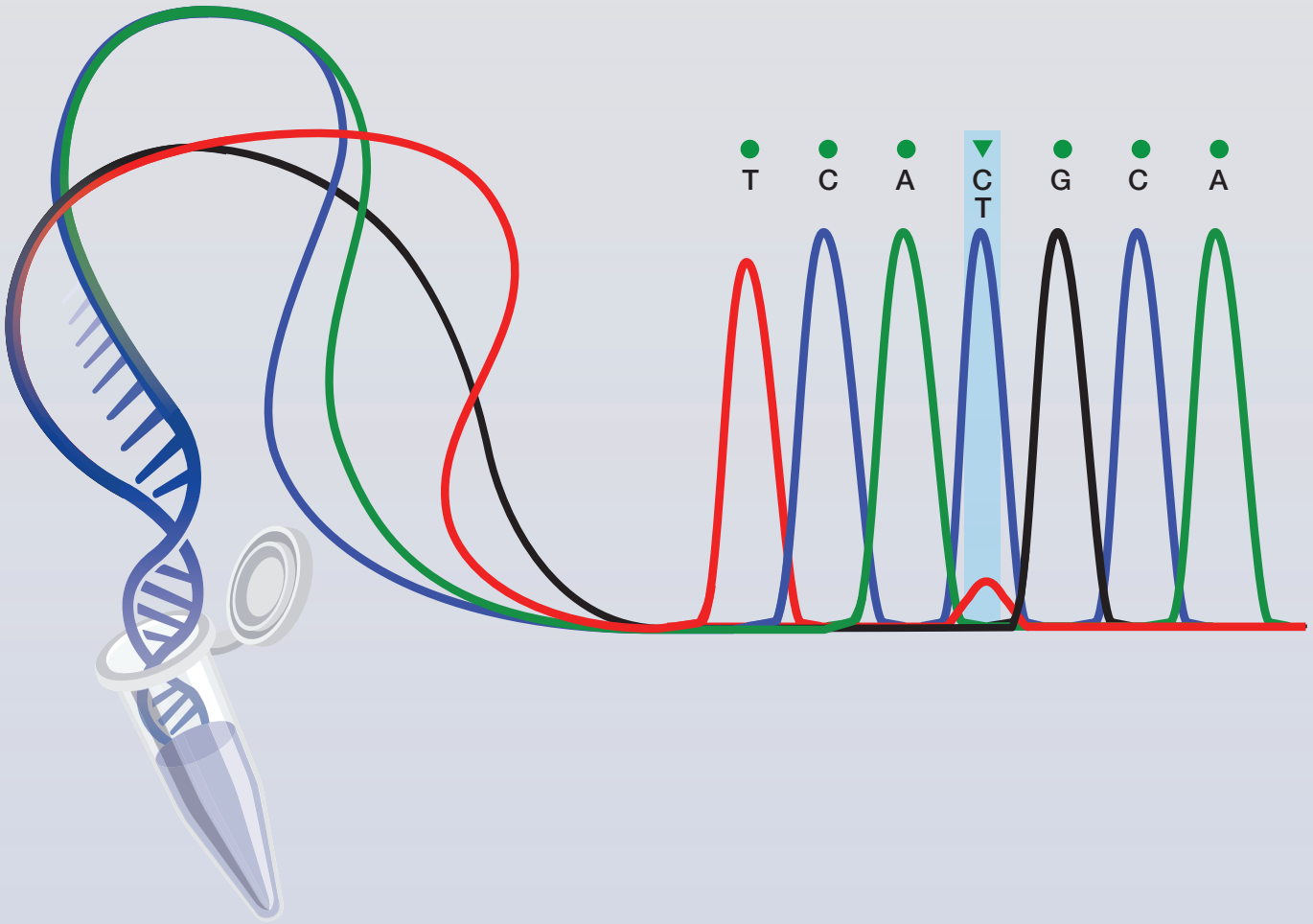


Sanger sequencing

Generate high-quality data with our proven workflow

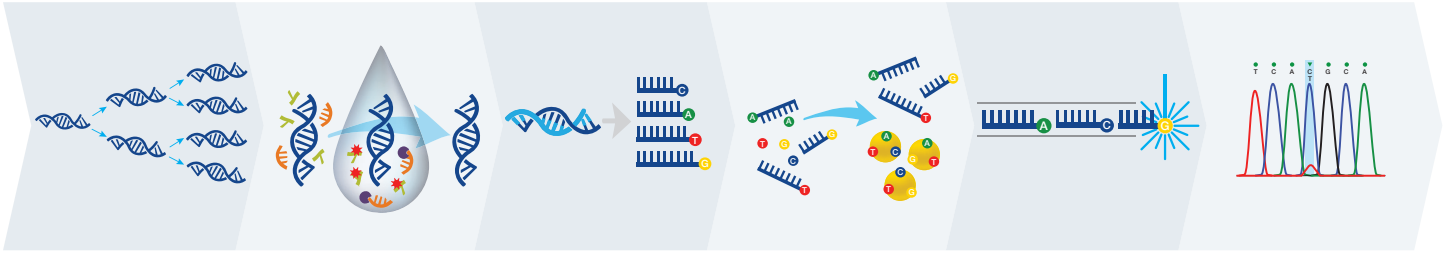
ThermoFisher SCIENTIFIC

Comprehensive solutions for your Sanger sequencing workflow

Proven through decades of results, Sanger sequencing is the gold-standard technology to:

- Study diseases with clearly defined phenotypes
- Sequence 1–2 genes or up to 96 targets
- Sequence 1–96 samples at a time without barcoding
- Confirm next-generation sequencing (NGS) variants with up to 99.99% accuracy
- Get longer read lengths (up to 1,000 bp)

The Sanger sequencing workflow



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Capillary electrophoresis **Page 7**

Data analysis **Page 8**

We commit to quality to help ensure results you can rely on

The term “gold standard” represents quality and dependability. Sanger sequencing is the gold-standard DNA sequencing method that powered the Human Genome Project and continues to generate highly accurate, reliable sequencing. We know that reliable results are important to you. That’s why we have dedicated teams and individuals focusing on quality across our supply chain so that you can continue to rely on the trusted gold-standard sequencing technology.

Learn more at **thermofisher.com/abquality**

“A gold-standard product is a product that is consistent and meets or exceeds customer expectations every time.”

—Justin, Manufacturing Supervisor

PCR amplification

Primer Designer tool for PCR and Sanger sequencing

Use our online Invitrogen™ Primer Designer™ Tool to search for the right PCR or Sanger sequencing primer pair from a database of ~650,000 predesigned primer pairs for resequencing the human exome and human mitochondrial genome. Choose from different amplicon lengths to accommodate various research applications and biological sample types.

- Our primers are free of known single-nucleotide polymorphisms (SNPs) and primer-dimers, highly target-specific, and used under universal PCR conditions
- Full primer coverage for Ion AmpliSeq™ Exome Panel and Ion AmpliSeq™ Cancer Hotspot Panel v.2 Sanger confirmation workflow
- Flexible primer configurations to meet your research needs: primers can be ordered unmodified, M13-tailed, HPLC-purified, or desalted
- All the primers have been checked by mass spectrometry and have passed stringent bioinformatics metrics; lab bench verification tests have shown >95% success rate

Access the tool at **thermofisher.com/primerdesigner**

Platinum II *Taq* Hot-Start DNA Polymerase

Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase helps you get to sequencing reactions faster. A unique combination of innovative buffer, high-performance *Taq* DNA polymerase, and superior Invitrogen™ Platinum™ hot-start technology helps enable exceptional PCR results, even in the toughest applications.

- Universal primer annealing at 60°C enables co-cycling of all assays, reducing tedious optimization steps
- An engineered *Taq* polymerase enables 4x faster DNA synthesis, inhibitor resistance, and robust amplification
- Platinum hot-start technology offers superior specificity, sensitivity, and yields, and allows for room temperature reaction setup
- 2X master mix formats help reduce pipetting errors with fewer pipetting steps

More formats and information are available at
thermofisher.com/platinumiitaq

Ordering information

Product	Quantity	Cat. No.
Platinum II Hot-Start PCR Master Mix (2X)	50 rxn	14000012
	200 rxn	14000013
	1,000 rxn	14000014

PCR cleanup

ExoSAP-IT *Express* reagent: fastest PCR cleanup method

The Applied Biosystems™ ExoSAP-IT™ *Express* reagent offers rapid turnaround times and improved efficiency of resource use while delivering the same superior cleanup as the original Applied Biosystems™ ExoSAP-IT™ reagent. The novel technology allows for a significant reduction in sample cleanup time with minimal steps, providing the simplest workflow (Figure 1).

- 5-minute protocol
- One-tube, one-step PCR cleanup
- 100% recovery of PCR products

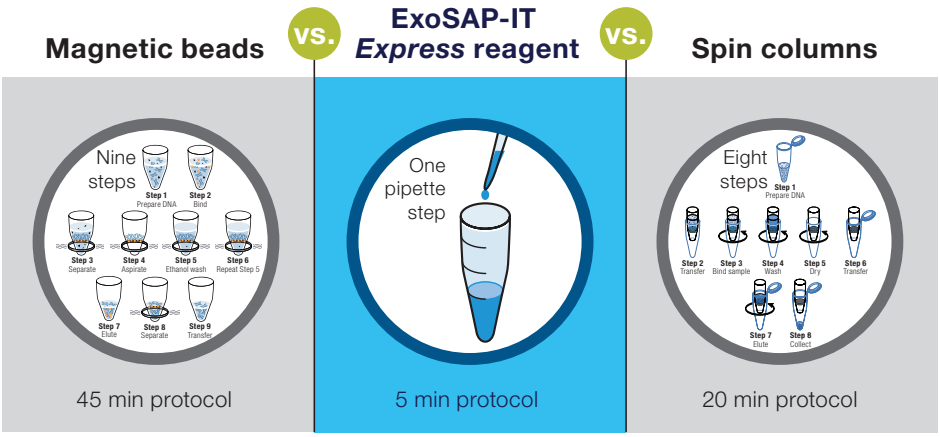


Figure 1. Use of ExoSAP-IT *Express* reagent eliminates spin columns, magnetic beads, centrifugation, filtration, and gel purification. With a 5-minute protocol, ExoSAP-IT *Express* reagent is the fastest and easiest method for PCR cleanup, minimizing pipetting errors and contamination.

Protocol

Treat 5 µL of PCR product with 2 µL of ExoSAP-IT *Express* reagent. The treatment is carried out at 37°C for 4 minutes, followed by an incubation at 80°C for 1 minute to irreversibly inactivate both enzymes. Once enzyme inactivation is complete, your PCR products are ready for downstream applications such as sequencing (Sanger/NGS), fragment analysis, SNP analysis, *in vitro* transcription, or single-base extension (Figure 2).

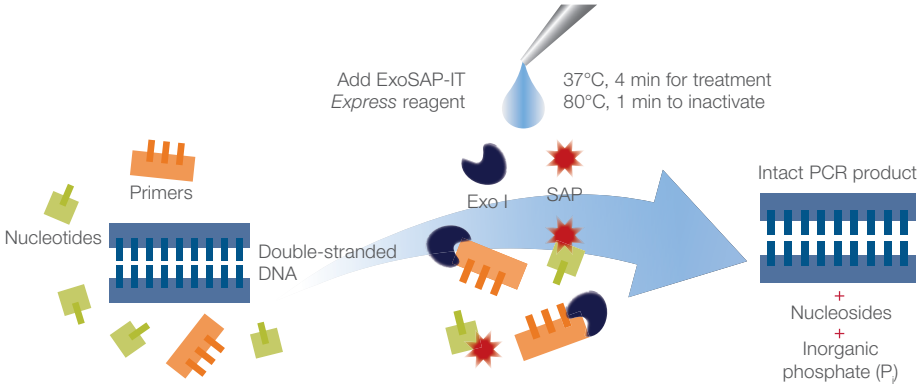


Figure 2. Enzymatic PCR cleanup using ExoSAP-IT *Express* reagent.

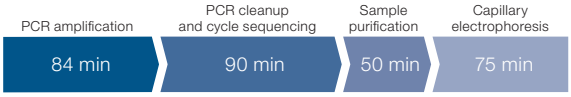
Ordering information

Product	Quantity	Cat. No.
ExoSAP-IT *Express* PCR Product Cleanup Reagent	100 rxn	75001.200.UL
	500 rxn	75001.1.ML
	2,000 rxn	75001.4X.1.ML
	5,000 rxn	75001.10.ML

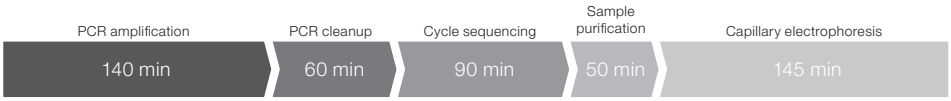
Cycle sequencing

BigDye Direct Cycle Sequencing Kit

The Applied Biosystems™ BigDye™ Direct Cycle Sequencing Kit provides a streamlined workflow by eliminating the PCR cleanup step, and improves resolution of sequencing data at the 5′ end. Moreover, the BigDye direct PCR and sequencing workflow requires use of only one plate, without having to transfer between steps. This helps reduce hands-on time and improves accuracy by reducing the possibility of pipetting errors.



BigDye Direct Cycle Sequencing Kit workflow, run with Applied Biosystems™ POP-7™ Polymer, takes 4 steps in approximately 5 process hr.



A traditional cycle sequencing workflow, run with Applied Biosystems™ POP-6™ Polymer, takes 5 steps in approximately 8 process hr.

Figure 3. The BigDye Direct kit delivers significant efficiency compared to standard sequencing. Traditional sequencing workflows can require more than 8 hours of process time and 5 steps to complete. In contrast, the BigDye Direct workflow typically requires only 5 hours and 4 steps, producing sequence reads up to 40% faster and with less hands-on time.

Ordering information

Product	Quantity	Cat. No.
BigDye Direct Cycle Sequencing Kit	24 rxn	4458689
	100 rxn	4458687
	1,000 rxn	4458688

BigDye Terminator v3.1 Cycle Sequencing Kit

The Applied Biosystems™ BigDye™ Terminator v3.1 Cycle Sequencing Kit has robust, highly flexible chemistry for *de novo* sequencing, resequencing, and finishing with PCR product, plasmid, fosmid, and BAC templates.

Ordering information

Product	Quantity	Cat. No.
BigDye Terminator v3.1 Cycle Sequencing Kit	24 rxn	4337454
	100 rxn	4337455
	1,000 rxn	4337456
	5,000 rxn	4337457
	25,000 rxn	4337458

BigDye Terminator v1.1 Cycle Sequencing Kit

The Applied Biosystems™ BigDye™ Terminator v1.1 Cycle Sequencing Kit is designed for specialty applications that require optimal basecalling adjacent to the primer, and for sequencing short PCR products with rapid electrophoresis.

Ordering information

Product	Quantity	Cat. No.
BigDye Terminator v1.1 Cycle Sequencing Kit	24 rxn	4337449
	100 rxn	4337450
	1,000 rxn	4337451
	5,000 rxn	4337452

Sequencing cleanup

BigDye XTerminator Purification Kit

Correctly cleaning up your sequencing reactions is an integral part of the Sanger sequencing workflow. If the sequencing reaction cleanup step is skipped or not performed properly, the residual dye in the reaction can compete with the labeled amplicons for entry into the capillary and can cause reduced signal intensity, which can interfere with the instrument's ability to make clear base calls. As a result, the data generated will be of poor quality.

The Applied Biosystems™ BigDye™ XTerminator Purification Kit provides a fast, simple purification method for removing unincorporated Applied Biosystems™ BigDye™ terminators and salts from DNA sequencing reactions; it also eliminates dye blobs in your reaction. Cleanup is complete in under 40 minutes and typically requires less than 10 minutes of hands-on labor.

Protocol

Dispense 55 µL of the SAM/BigDye XTerminator bead working solution to each sample well. Vortex for 20 minutes at 1,800 rpm, followed by centrifugation at 1,000 x *g* for 2 minutes.

Ordering information

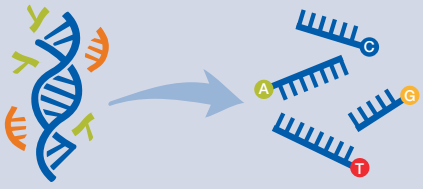
Product	Quantity	Cat. No.
BigDye XTerminator Purification Kit	100 preps	4376486
	1,000 preps	4376487
	2,500 preps	4376484
	40,000 preps	4376485

Simplify your sample prep workflow with the Sanger Sequencing Kit

The Applied Biosystems™ Sanger Sequencing Kit offers a convenient and affordable solution for preparing sequencing reactions. It provides all the reagents needed for 200 reactions of PCR cleanup, cycle sequencing, and sequencing product cleanup. The kit includes:

- ExoSAP-IT *Express* PCR Product Cleanup Reagent
- BigDye Terminator v3.1 Cycle Sequencing Kit
- BigDye XTerminator Purification Kit

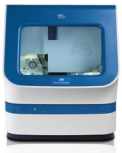
From PCR to purified sequencing reactions



Ordering information

Product	Quantity	Cat. No.
Sanger Sequencing Kit	200 rxn	A38073

Capillary electrophoresis

	Applied Biosystems™ SeqStudio™ Genetic Analyzer	Applied Biosystems™ 3500 Series Genetic Analyzers	Applied Biosystems™ 3730 Series Genetic Analyzers
	Easy-to-use, flexible system 	Meets the needs of verified and process-controlled environments 	Maximum throughput, scalability, and flexibility UPGRADED 
Number of capillaries	4	8 (3500), 24 (3500xL)	48- and 96-well plate compatible
Number of dyes	6	6	6
Capillary array length (cm)	28	36, 50	36, 50
RFID	Yes	Yes	No
Polymer type	POP-1, integrated into click-in cartridge	POP-6, POP-7, POP-4	POP-6, POP-7, conformational analysis polymer
Sample capacity	12 standard 8-strip tubes 1 standard 96-well plate	2 sample plates (96- or 384-well)	16 sample plates (96- or 384-well)
Integrated plate stacker	No	No	Yes
Applications	Sequencing and fragment analysis in same run	Sequencing, fragment analysis	Sequencing, fragment analysis
Minimum run time	30 minutes	30 minutes	20 minutes
Sequencing read length (bp)	At least 600	At least 650	At least 900
Maximum sequencing throughput (base pair reads/day)	67K	138K (3500), 403K (3500xL)	1.38M (3730), 2.76M (3730*xl*)
Maximum fragment throughput (samples/day)	192	384 (3500), 1,152 (3500xL)	3,456 (3730), 6,912 (3730*xl*)

SeqStudio Cartridge

The Applied Biosystems™ SeqStudio™ Cartridge is an easy-to-use reagent cartridge that includes capillaries, POP-1 universal polymer, buffer, and pump. The POP-1 universal polymer allows for flexibility to perform Sanger sequencing and fragment analysis on the SeqStudio Genetic Analyzer System with one cartridge. Just load your samples, click in the cartridge, and go.

On-instrument consumables

Using the right Applied Biosystems™ polymers can help reduce your re-run rate and enables high-quality, reproducible data.

Different polymer chemistries for different needs:

POP-4™ Polymer

- Primarily used for human identification applications, other fragment analysis applications, and for sequencing short DNA fragments (<500 bp)

POP-6™ Polymer

- Excellent resolution of nucleotides close to sequencing primer. Primarily used in conjunction with the BigDye Terminator v1.1 Cycle Sequencing Kit, for sequencing short PCR products

POP-7™ Polymer

- Mostly used for sequencing of read lengths up to ~1,000 bp in conjunction with the BigDye Terminator v3.1 Cycle Sequencing Kit. Also used for fragment analysis applications

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Data analysis

We offer a wide range of Applied Biosystems™ software solutions for viewing and interpreting your Sanger sequencing results.

Minor Variant Finder Software

The improved sensitivity achieved using Applied Biosystems™ Minor Variant Finder Software makes Sanger sequencing the ideal choice for oncologists and pathologists to call low-frequency somatic variants (5% or below) where the number of relevant targets is often limited. The software requires no change to your current Sanger sequencing workflow.

Features of MVF Software enable you to:

- Call minor variants at detection levels as low as 5%
- Sequence a moderate number of targets at low cost
- Confirm NGS findings in alignment view and Venn diagram

Sanger analysis modules—free cloud-based tools

Applied Biosystems™ Sanger analysis modules are innovative cloud-based secondary data analysis tools that bring together multiple data sets in one convenient place. This free solution makes it easier to view, store, and analyze Sanger sequencing data.

- **NGS confirmation**—confirm your NGS variants from one central location
- **Variant reporting in absolute genomic coordinates**—eliminate the need to calculate from references
- **Automated database search**—automatically report genomic annotations for SNPs
- **.vcf output for downstream analysis**—search multiple databases with the Ion Reporter™ annotation workflow

Additional software

Applied Biosystems™ Sequencing Analysis Software
Enables user to basecall, trim, display, edit, and print data from our entire line of capillary DNA sequencing instruments for data analysis and quality control.

Applied Biosystems™ SeqScape™ Software
Resequencing package designed for mutation detection and analysis, SNP discovery and verification, pathogen subtyping, allele identification, and sequence confirmation. It provides library functions for comparison to a known group of sequences, as well as functionalities to assist with 21 CFR Part 11 compliance.

Applied Biosystems™ Variant Reporter™ Software
Designed for reference-based and non–reference-based analysis such as mutation detection and analysis, SNP discovery and verification, and sequence confirmation. The software can call SNPs, insertions, deletions, and heterozygous insertions/deletions.

Applied Biosystems™ Sequence Scanner Software:
Free sequencing viewer software enables you to view, edit, print, and export sequence data from Applied Biosystems™ genetic analyzers. The software generates graphically expressive reports on results.

Learn more at **thermofisher.com/sangersoftware**

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

ThermoFisher SCIENTIFIC

7500 Real-Time PCR Systems Spectral Calibration Kit II

Catalog Number 4351151

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

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

Contents and storage

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

Related documentation

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

Limited product warranty

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

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

1 December 2015

ThermoFisher SCIENTIFIC

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Certificate of Analysis

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

Product No. **4351151**
Lot No. **2311565**

TEST	SPECIFICATION	RESULT

Material Test

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

Component	Part Number	Lot Number			
Cy3 Dye	4349412	2311260	562 ± 5 nm	**560 nm**	**Pass**
Cy5 Dye	4349413	2311268	659 ± 8 nm	**659 nm**	**Pass**
Texas Red® Dye	4349414	2311265	608 ± 5 nm	**608 nm**	**Pass**

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

ISO13485 REGISTERED

Singapore

FARAH DINA
14 DEC 2023
Quality Assurance

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To obtain a Certificate of Analysis on-line go to www.appliedbiosystems.com or email us at cofarequest@appliedbiosystems.com

Doc p/n: 100030349 Rev B

DNA-TECHNOLOGY

Nucleic Acid Extraction Kits

"Plus" – larger volume of NA solution

"Rapid" – quick method

"Genetics" – human DNA extraction from whole blood

"DBS" – dried blood spots

Suitable for extraction of AVRI pathogens NA, including SARS-CoV-2

Quick NA extraction: from 15-30 minutes

Manual dosing

Automated dosing

Extraction time depends on biomaterial, number of samples, and equipment being used.

Advertising and information material. For detailed information about the Nucleic Acid Extraction Kit, please refer to the instructions for use

	Sample preparation reagent kit product lines	Sample preparation reagent kits	Line features
	PREP-NA	PREP-NA PREP-NA PLUS PREP-NA-ULTRA PREP-NA-FET PREP-NA-S	Lysis and further NA precipitation
Higher purification degree	PREP-GS	PREP-GS PREP-GS PLUS PREP-GS Genetics	Lysis and further NA sorption
Quick extraction (express method)	PREP-RAPID	PREP-RAPID PREP-RAPID Genetics	Thermocoagulation of impurities
	PREP-OPTIMA	PREP-OPTIMA PREP-OPTIMA MAX	Alkaline cell lysis
Automation	PREP-MB		Lysis with further sorption on paramagnetic nanoparticles
	▸ Automated extraction	PREP-MB-NA-S PREP-MB DWP	PREP-MB DWP and PREP-MB-DBS DWP are compatible with KingFisher Flex (Thermo Fisher Scientific), Auto-Pure 96 (Allsheng)
	▸ Manual or automatic dispensing	PREP-MB MAX PREP-MB RAPID PREP-MB-DBS DWP	
Extraction from whole blood		PREP-RADIP Genetics PREP-GS Genetics PREP-OPTIMA MAX PREP-MB MAX	Suitable for genetic assays
Extraction from cell cultures		PREP-CM PREP-MB RAPID PREP-OPTIMA PREP-OPTIMA MAX	Ability to extract from blood cultures – PREP-CM
Extraction from dried blood spots		PREP-CITO DBS PREP-MB-DBS DWP	Suitable for genetic assays

PREP-NA

Extraction of DNA and RNA (human, microbial and viral) by precipitation

Method: lysis and further NA precipitation

PREP-NA

Extraction time: from 30 min*, from 50 min

Obtained NA sample volume: 50 µl

* for nasopharyngeal and oropharyngeal swabs when using shortened extraction method for PCR detection of AVRI pathogens, including SARS-CoV-2. Details of the method are in the annex to the amplification part of the instruction for SARS-CoV-2 kit.

PREP-NA PLUS

Extraction time: from 30 min*, from 50 min

Obtained NA sample volume: 300 µl

* for nasopharyngeal and oropharyngeal swabs when using shortened extraction method for AVRI Complex. Details of the method are in the annex to the amplification part of the instruction for AVRI Complex kit.

PREP-NA-ULTRA

Viral NA extraction from blood plasma with preconcentration

Extraction time: from 50 min

PREP-NA-FET

Fetal DNA extraction from mother's blood

Extraction time: from 2 hours

PREP-NA-S

Extraction of AVRI pathogens NA, including SARS-CoV-2

Extraction time: from 25 min

BIOMATERIAL	PREP-NA	PREP-NA-PLUS	PREP-NA-ULTRA	PREP-NA-FET	PREP-NA-S
Blood plasma					
Urine					
Feces					
Nasopharyngeal/oropharyngeal smears and swabs					
Scrapes from posterior pharynx					
Phlegm					
Saliva					
Urogenital scrapes					
Prostate fluid					
Ejaculate					
Cerebrospinal fluid					
Milk					

PREP-GS

Extraction of DNA (human and microbial) by sorption with extra purification

Method: lysis and further DNA sorption

PREP-GS

Extraction time: from 40 min
Obtained DNA solution volume: 100 µl

PREP-GS PLUS

Extraction time: from 40 min
Obtained DNA solution volume: 300 µl

PREP-GS Genetics

Extraction time: from 40 min

BIOMATERIAL	PREP-GS	PREP-GS PLUS	PREP-GS Genetics
Whole blood			
Blood plasma			
Urine			
Scrapes from posterior pharynx			
Phlegm			
Saliva			
Urogenital scrapes			
Prostate fluid			
Ejaculate			
Cerebrospinal fluid			
Milk			
Native tissues			

PREP-RAPID

Express method of DNA extraction. Sample transportation and extraction in one tube

Method: thermocoagulation of impurities

PREP-RAPID

Microbial DNA extraction
Extraction time: from 15 min

PREP-RAPID Genetics

Human DNA extraction
Extraction time: from 20 min

BIOMATERIAL	PREP-RAPID	PREP-RAPID Genetics
Whole blood		
Urine		
Scrapes from posterior pharynx		
Saliva		
Urogenital scrapes ♀*		
Prostate fluid		
Cerebrospinal fluid		

* We do not recommend to use PREP-RAPID for DNA extraction from men's urogenital scrapes

PREP-MB

Extraction of DNA and RNA using paramagnetic nanoparticles

Method: lysis and release of NA under the action of guanidine thiocyanate with subsequent sorption on paramagnetic nanoparticles and washing from impurities

PREP-MB RAPID

DNA extraction

Extraction time: from 40 min

PREP-MB-NA-S

DNA and RNA extraction

Extraction time: from 40 min

PREP-MB MAX

DNA extraction

Extraction time: from 60 min

Obtained DNA solution volume: 50-300 µl

BIOMATERIAL	PREP-MB RAPID	PREP-MB MAX	PREP-MB-NA-S
Whole blood			
Urine			
Feces			
Nasopharyngeal/oropharyngeal smears and swabs			
Rectal scrapes			
Urogenital scrapes			
Ejaculate			
Cerebrospinal fluid			
Milk			
Amniotic fluid			
Ascitic fluid			
Cell culture			

PREP-MB

Extraction of DNA and RNA using paramagnetic nanoparticles
on KingFisher (Thermo Fisher Scientific) and Auto-Pure (Allsheng) instruments

Method: lysis and release of NA under the action of guanidine thiocyanate with subsequent sorption on paramagnetic nanoparticles and washing from impurities

PREP-MB DWP

Extraction of DNA and RNA
of AVRI pathogens, including SARS-CoV-2

Total time of preparation for NA extraction and NA extraction from 96 samples: from 40 min

Extraction time: from 20 min

Obtained DNA solution volume: 50-300 µl

Compatible instruments: KingFisher Flex
(Thermo Fisher Scientific), Auto-Pure 96 (Allsheng)

PREP-MB-DBS DWP

DNA extraction from dried blood spots

Extraction time: from 60 min

Compatible instruments: KingFisher Flex
(Thermo Fisher Scientific), Auto-Pure 96 (Allsheng)

BIOMATERIAL	PREP-MB DWP	PREP-MB-DBS DWP
Nasopharyngeal, oropharyngeal smears, swabs		
Dried blood spots		

Example of working with a large amount of samples using **PREP-MB DWP** reagent kit

DeepWell preparation:
20 min

DTstream
(DNA-Technology)

RNA extraction:
20 min

KingFisher Flex (Thermo Fisher Scientific)
Auto-Pure 96 (Allsheng)

PREP-OPTIMA

Extraction of DNA (human, microbial and viral).
Universal DNA extraction kit

Method: alkaline cell lysis occurring during thermal incubation

PREP-OPTIMA

Extraction time: from 25 min
Obtained DNA solution volume: 100-450 µl

PREP-OPTIMA MAX

Extraction time: from 25 min
Obtained DNA solution volume: 100-450 µl

BIOMATERIAL	PREP-OPTIMA	PREP-OPTIMA MAX
Whole blood		
Urine		
Feces		
Nasopharyngeal, oropharyngeal smears		
Phlegm		
Rectal scrapes		
Buccal epithelium		
Urogenital scrapes		
Ejaculate		
Milk		
Amniotic fluid		
Synovial fluid		
Native tissues		
Fungal culture		
Bacterial culture		
Cell culture		

PREP-CM

Bacterial and fungal DNA extraction from microbial cultures

Method: alkaline cell lysis occurring during thermal incubation

PREP-CM

Extraction time: from 40 min
Obtained DNA solution volume: 400 µl

BIOMATERIAL	PREP-CM
Fungal culture	
Bacterial culture	
Cell culture	
Blood culture	

PREP-CITO DBS

Human DNA extraction from dried blood spots

Method: alkaline cell lysis occurring during thermal incubation. Removal of possible impurities and stripping of blood from the carrier takes place in the pre-washing stage

PREP-CITO DBS

Extraction time: from 40 min

DNA yield: 30-140 ng when extracted from 10 µl of blood dried on three filter paper discs

Amount of obtained DNA depends on the amount of leukocytes in sample

BIOMATERIAL	PREP-CITO DBS
Dried blood spots	

PREP-L

Lysozyme pretreatment of biomaterial before DNA extraction

Method: enzymatic destruction of peptidoglycans that make up the cell walls of microorganisms, by lysozyme

Pretreatment time:
from 30 min at t= 37 °C
from 60 min at t=18-25 °C

Biomaterial for pretreatment:

- Feces
- Meconium
- Bacterial culture from this biomaterial

Used together with PREP-MB MAX and PREP-NA PLUS NA extraction kits

PREP-FU

Biomaterial pretreatment to obtain lymphocytes from whole blood

Pretreatment time: 1 hour

Biomaterial for pretreatment: whole blood

PREP-PK

Biomaterial pretreatment by proteinase K before nucleic acid extraction

Method: proteolysis by proteinase K and elimination of inhibitory effects

Pretreatment time:

formalin-fixed, paraffin-embedded tissues: DNA — from 150 min,
RNA — from 60 min.

native tissues – 60 min;
cervical scrapes – 90 min.

Biomaterial for pretreatment:

- formalin-fixed, paraffin-embedded tissues (FFPE);
- native tissues;
- cervical scrapes taken into transport-fixating medium for liquid-based cytology

Used together with PREP-NA PLUS nucleic acid extraction kit

PREP-PK reagent kit is not intended for RNA extraction from biomaterial fixated in BD SurePath transport medium.

STOR-F

Transport and storage of human biomaterial

Method: saline solution with the addition of a preservative that prevents the growth of microorganisms

Suitable for further DNA and RNA extraction, including SARS-CoV-2 RNA

Compatible biomaterial:
scrapes/smears of epithelial cells from urogenital tract, oropharynx, nasopharynx, rectum, eye conjunctiva, skin

Transport and storage of biomaterial:
at t = 2 °C – 8 °C for no longer than 7 days
at t = 18 °C – 25 °C for no longer than 48 hours

STOR-M

Transport and storage of human biomaterial, including those containing mucus

Method: saline solution with mucolytic.
Preservative prevents non-specific microorganisms from reproduction, mucolytic affects disulfide bonds of mucopolysaccharides to thin mucus.

Suitable for further DNA extraction

Compatible biomaterial:

scrapes/smears of epithelial cells from urogenital tract, oropharynx, nasopharynx, rectum, eye conjunctiva, skin, including those containing mucus

Transport and storage of biomaterial:

at t = 2 °C – 8 °C for no longer than 3 months

at t = 18 °C – 25 °C for no longer than 28 days

BIOMATERIAL	NA extraction reagent kits	DNA		RNA	
		human	microbial	human	microbial
Whole blood	PREP-GS Genetics	■			
	PREP-RAPID Genetics	■			
	PREP-MB MAX	■	■		
	PREP-OPTIMA MAX	■	■		
Blood plasma	PREP-NA	■	■	■	■
	PREP-NA PLUS	■	■	■	■
	PREP-NA-ULTRA		■		■
	PREP-NA-FET	■			
	PREP-GS		■		
	PREP-GS PLUS		■		
Urine	PREP-NA	■	■	■	■
	PREP-NA PLUS	■	■	■	■
	PREP-RAPID		■		
	PREP-MB MAX	■	■		
	PREP-GS		■		
	PREP-GS PLUS		■		
	PREP-OPTIMA	■	■		
	PREP-OPTIMA MAX	■	■		
Feces	PREP-NA	■	■	■	■
	PREP-MB MAX	■	■		
	PREP-OPTIMA	■	■		
	PREP-OPTIMA MAX	■	■		
Ejaculate	PREP-NA	■	■	■	■
	PREP-NA PLUS	■	■	■	■
	PREP-MB MAX	■	■		
	PREP-GS		■		
	PREP-GS PLUS		■		
	PREP-OPTIMA	■	■		
	PREP-OPTIMA MAX	■	■		

BIOMATERIAL	NA extraction reagent kits	DNA		RNA	
		human	microbial	human	microbial
Nasopharyngeal, oropharyngeal smears	PREP-NA				
	PREP-NA-S				
	PREP-MB RAPID				
	PREP-MB-NA-S				
	PREP-MB MAX				
	PREP-GS				
	PREP-GS PLUS				
	PREP-OPTIMA				
	PREP-OPTIMA MAX				
Scrapes from posterior pharynx	PREP-RAPID				
	PREP-NA				
	PREP-NA PLUS				
Saliva	PREP-RAPID				
	PREP-NA				
	PREP-GS				
	PREP-GS PLUS				
Rectal scrapes	PREP-MB RAPID				
	PREP-MB MAX				
	PREP-OPTIMA				
	PREP-OPTIMA MAX				
Urogenital scrapes	PREP-RAPID				
	PREP-NA				
	PREP-NA PLUS				
	PREP-MB MAX				
	PREP-GS				
	PREP-GS PLUS				
	PREP-OPTIMA				
	PREP-OPTIMA MAX				

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DNA-TECHNOLOGY

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DNA-TECHNOLOGY

670-2 2022-07-05

CE IVD

For professional use only

PREP-GS DNA Extraction Kit

PREP-GS PLUS DNA Extraction Kit

PREP-GS Genetics DNA Extraction Kit

INSTRUCTION FOR USE

EC REP

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REF

P-003/1EU,

P-003/2EU,

P-023/4EU

VER

670-2.2022.07.05

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1. INTENDED USE

The **PREP-GS DNA Extraction Kit** and **PREP-GS PLUS DNA Extraction Kit** are intended for DNA extraction from biological materials (see Table 1) for further analysis by polymerase chain reaction (PCR). In the **PREP-GS PLUS DNA Extraction Kit** the total volume of purified DNA is larger (300 µL) comparing to standard **PREP-GS DNA Extraction Kit** (100 µL) for more PCR tests. The **PREP-GS Genetics DNA Extraction Kit** is intended for DNA extraction from whole peripheral blood for further DNA genetic testing by PCR.

Table 1. Biological material for DNA/RNA extraction by **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit**

Extraction Kit	**PREP-GS DNA Extraction Kit**	**PREP-GS PLUS DNA Extraction Kit**	**PREP-GS Genetics DNA Extraction Kit**
Biological material	Phlegm, saliva, urine, ejaculate, prostate fluid, cerebrospinal fluid, milk serum, minced tissue, epithelial scrapes from posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault, biological samples with PCR inhibitors		Peripheral blood

This medical device is an auxiliary agent in clinical laboratory diagnostics.

The application of the kits does not depend on population and demographic aspects. There are no contradictions for use of the **PREP-GS DNA Extraction Kit, PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit.**

The **PREP-GS DNA Extraction Kit, PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kits only as directed in this instruction for use.

2. METHOD

The **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** are based on nucleic acids release under the action of a chaotropic agent, followed by precipitation and purification of nucleic acids from impurities.

3. CONTENT

The detailed description of content is represented in Tables 2-4.

Table 2. The **PREP-GS DNA Extraction Kit** content, for P-003/1EU

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	15 mL	1 vial
Sorbent	Brown suspension	2.0 mL (1.0 mL in each tube)	2 tubes
Washout solution №1	Colorless transparent liquid	20 mL	1 vial
Washout solution №2	Colorless transparent liquid	20 mL	1 vial
Washout solution №3	Colorless transparent liquid	20 mL	1 vial
Elution buffer	Colorless transparent liquid	10 mL	1 vial

Table 3. The **PREP-GS PLUS DNA Extraction Kit** content, for P-003/2EU

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	7.5 mL	1 vial
Sorbent	Brown suspension	1.0 mL	1 tube
Washout solution №1	Colorless transparent liquid	10 mL	1 vial
Washout solution №2	Colorless transparent liquid	10 mL	1 vial
Washout solution №3	Colorless transparent liquid	10 mL	1 vial
Elution buffer	Colorless transparent liquid	15 mL	1 vial

Table 4. The **PREP-GS Genetics DNA Extraction Kit** content, for P-023/4EU

Reagent	Description	Total volume	Amount
Lysis buffer	Light blue slightly foaming liquid	7.2 mL	1 vial
Sorbent	Brown suspension	960 µL	1 tube
Washout solution №1	Colorless transparent liquid	19.2 mL	1 vial
Washout solution №2	Colorless transparent liquid	9.6 mL	1 vial
Washout solution №3	Colorless transparent liquid	9.6 mL	1 vial
Elution buffer	Colorless transparent liquid	14.4 mL	1 vial

All components are ready to use and do not require additional preparation for operation.

The kits are intended for single use and designed for 100 analyzed samples (including negative controls) for **PREP-GS DNA Extraction Kit**, 50 analyzed samples (including negative controls) for **PREP-GS PLUS DNA Extraction Kit** and 48 analyzed samples (including negative controls) for **PREP-GS Genetics DNA Extraction Kit.**

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs and sterile containers to collect clinical material;
- Sterile tubes containing transport media: “DNA-Technology” made **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or physiological saline solution or sterile PBS for the transportation of the sample;
- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

  Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF 16000 x g);
- Solid-state thermostat (temperature range 65-98 °C);

- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- RNase and DNase free non-filtered pipette tips for aspirator with trap flask;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

When extracting DNA from phlegm (method 1):

- 10% trisodium phosphate x 12H2O;
- 1.0M HCl solution;
- 5.0% chloramines solution;
- distilled water.

When extracting DNA from phlegm (method 2):

- mucolysin.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** must be stored at temperatures from 2 °C to 8 °C over the storage period. The excessive temperature can be detrimental to product performance.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions.

It is allowed to transport the kit in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 8 °C inside the thermobox.

Shelf-life of the kit following the first opening of the primary container:

- sorbent, washout solution №2, washout solution №3 and elution buffer should be stored at temperatures from 2 °C to 8 °C during the storage period;
- lysis buffer and washout solution №1 should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit stored in under undue regime should not be used.

An expired the **PREP PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or

discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **PREP-GS DNA Extraction Kit**, **PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit** is designed to extract DNA from a wide variety of biological sample types, such as saliva, phlegm, milk, urine, ejaculate, prostate fluid, cerebrospinal fluid, scrapes of epithelial cells from the posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault, etc. for **PREP-GS DNA Extraction Kit** and **PREP-GS PLUS DNA Extraction Kits** and peripheral whole blood for **PREP-GS Genetics DNA Extraction Kit**.

Sample collection

Blood sampling

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant. After taking the material, it is necessary to mix the blood with anticoagulant turning the tube 2 – 3 times.

It is not allowed to use heparin as an anticoagulant.

Phlegm sampling

Sample taking is made in amount no less than 1.0 mL into single-use graduated sterile flacks with wide neck and screwing caps with volume no less than 50 mL.

After sample collection, flask is tightly screwed and marked.

Epithelial scrapes sampling

Procedural limitations for genitourinary smears sampling - local application of medicines, vaginal ultrasound less than 24 hours before the procedure.

Sampling procedure is carried out using special sterile disposable instruments – urogenital swabs, cytobrushes or tampons, depending on the source of clinical material in accordance with established procedures.

In case of pregnancy the use of cytobrushes for genitourinary smears sampling is contraindicated.

The taking of the scrapes is carried out:

- in plastic 1.5 mL tubes with 500 µL of a sterile physiological solution;
- in tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR.

Remove mucus with sterile cotton swab before taking scrape from cervical channel.

Order of taking:

1. Open the tube.
2. Scrape epithelial cells from the corresponding biotope (posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault, etc.) with a sterile swab.
3. Put the swab into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution.
4. Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab.
5. Close the tube tightly and mark it.

Urine sampling

Take the portion (approximately 50 mL) of the first-void urine to sterile container and close it tightly.

Saliva, cerebrospinal fluid, synovial fluid sampling

Collect the saliva, cerebrospinal fluid, synovial fluid (approximately 500 µL) to the sterile container and close it tightly.

Ejaculate, prostate fluid sampling

Put 100 µL of the liquid sample into the 1.5 mL tube with transport medium (or alternatively with 500 µL of sterile buffered saline).

Milk sampling

Collect the sample into the sterile container and close it tightly.

Milk collection period must not exceed 24 hours. Keep at temperatures from 2 °C to 8 °C during the collection period.

Transportation and storage of the samples

Samples may be transported and stored at temperatures from 2 °C to 8 °C for no more than 24 h. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from minus 18 °C to minus 22 °C for one month.

In case of usage transport media, biological material samples are transported and stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Sample preparation

Preparation of the phlegm:

Method 1:

1. Put approximately 500 µL of biological sample into sterile 1.5 mL tube and close it tightly.
2. Add to the sample an equal volume of 10% triple-substituted sodium phosphate x12H2O and mix intensively.
3. Incubate the mixture at 37 °C for 18–24 hours, then neutralize with 1M HCl (down to pH 6.8–7.4).
4. Centrifuge the tube at RCF(g) 100 for 20 minutes.
5. Take out the supernatant into the 5.0% solution of chloramine for disinfection.
6. Add 500 µL of distilled water to precipitate, mix by pipetting and put to the new 1.5 mL tube.
7. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
8. Remove the supernatant, leaving approximately 100 µL (precipitate+liquid fraction) in the tube.

Method 2:

1. Add mucolysin to the sampling container in the 5:1 ratio (5 parts of mucolysin to 1 part of phlegm), referring to container calibrations.
2. Close the lid of the container, mix the content and incubate for 20–30 minutes at room temperature, shake the container every 2-3 minutes.

The samples are ready for DNA extraction.

Storage of processed phlegm in a container is accepted at temperatures from 2 °C to 8 °C for one day or at temperatures not above minus 16 °C for along time (in case of repeated DNA extraction necessity).

Preparation of the epithelial scrapes:

1. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
2. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction) in the tube.

The samples are ready for DNA extraction.

Preparation of the urine:

1. Transfer 1.0 mL of the sample to the 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant completely.
4. Add 1.0 mL of sterile buffered saline to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction) in the tube.

The samples are ready for DNA extraction.

Preparation of the saliva, cerebrospinal fluid, synovial fluid:

1. Transfer 500 µL of the sample to the 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction).
4. Add 500 µL of sterile buffered saline to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction).

The samples are ready for DNA extraction.

Preparation of the ejaculate, prostate fluid:

1. Vortex the tubes with samples for 5-10 seconds.

2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.

3. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction) in the tube.

The samples are ready for DNA extraction.

Preparation of the milk:

1 Mix thoroughly and put 1.0 mL of the sample into the 1.5 mL tube.

The samples are ready for DNA extraction.

8. PROCEDURE

DNA extraction from biological material

Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control in volumes as indicated.

Assay procedure:

The lysis buffer and washout solution №1 can form the precipitate. Dissolve it at 50 °C for 15-20 minutes prior to use.

8.1 Mark the required number of 1.5 mL tubes for each test sample and negative control (C-).

Example: to test 5 samples, mark 5 tubes for samples and 1 tube for "C-". The resulting number of tubes is 6.

For pre-processed samples with obtaining pellet and supernatant (phlegm method 1, saliva, cerebrospinal fluid, urine, ejaculate, prostatic fluid and epithelial scrapes) tubes with 50 µL of material prepared for testing must be marked.

8.2 Prepare the mixture of lysis buffer and sorbent. Add into the one tube:

- 150 x (N+1) µL of lysis buffer,
- 20 x (N+1) µL of preliminarily resuspended sorbent,

N is a quantity of the samples to be tested taking to account "C-".

8.3 Add 170 µL of prepared mixture to marked tubes. Close the tubes.

Always open the tube that you are working with, and close it after handling. It is not allowed to work simultaneously with several tubes with open caps.

8.4 Add 50 µL of prepared sample (**PREP-GS**, **PREP-GS PLUS** kits) or 100 µL of peripheral blood (**PREP-GS Genetics** kit) into the marked tubes. Do not add samples to the "C-" tube and tubes with pre-processed samples with obtaining pellet and supernatant (phlegm method 1, saliva, cerebrospinal fluid, urine, ejaculate, prostatic fluid and epithelial scrapes) (see Table 5).

8.5 Add 50 µL (**PREP-GS**, **PREP-GS PLUS** kits) or 100 µL (**PREP-GS Genetics** kit) of specimen transport medium or sterile buffered saline to "C-" tube (see Table 5).

8.6 Close the tubes tightly and vortex them for 3–5 seconds.

8.7 Incubate the tubes at 50 °C for 20 minutes (**PREP-GS**, **PREP-GS PLUS** kits) or 10 minutes (**PREP-GS Genetics** kit) (see Table 5).

8.8 Centrifuge the tubes at RCF(g) 16000 for 1 minute.

8.9 Remove the supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

8.10 Add 200 µL (**PREP-GS**, **PREP-GS PLUS** kits) or 400 µL (**PREP-GS Genetics** kit) of washout solution №1, close tubes tightly and vortex them for 3–5 seconds (see Table 5).

8.11 Centrifuge the tubes at RCF(g) 16000 for 1 minute.

8.12 Remove the supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

8.13 Add 200 µL of washout solution №2, close tubes tightly and vortex them for 3–5 seconds.

8.14 Centrifuge the tubes at RCF(g) 16000 for 1 minute.

8.15 Remove the supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

8.16 Add 200 µL of washout solution №3, close tubes tightly and vortex them for 3–5 seconds.

8.17 Centrifuge the tubes at RCF(g) 16000 for 1 minute.

8.18 Remove the supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

8.19 Open the tubes and dry precipitate by incubation at 50 °C for 5 minutes.

8.20 Add to precipitate 100 µL (**PREP-GS** kit) or 300 µL (**PREP-GS PLUS** or **PREP-GS Genetics** kits) of elution buffer, close the tubes tightly and vortex them for 5-10 seconds.

8.21 Incubate tubes at 50 °C for 5 minutes (see Table 5).

8.22 Centrifuge the tubes at RCF(g) 16000 for 1 minute. Transfer the supernatant into the new tube if sample is to be stored for more than 7 days.

Supernatant containing extracted DNA is ready for adding to PCR-mix.

The obtained DNA sample can be stored at temperatures from 2 °C to 8 °C for no longer than 7 days. Before using the DNA sample for PCR steps, incubate tubes at 50 °C for 5 minutes and then centrifuge the tubes at RCF(g) 16000 for 1 minute. DNA preparation can be stored at temperatures from minus 18 °C to minus 22 °C for no longer than 6 months for **PREP-GS**, **PREP-GS PLUS** kits and no longer than 1 year for **PREP-GS Genetics** kit (see Table 5).

Table 5.

<table>
<tr><td>Extraction kit</td><td colspan="2">PREP-GS</td><td>PREP-GS PLUS</td><td>PREP-GS Genetics</td></tr>
<tr><td>Volume of analyzed sample and negative control sample required for DNA extraction procedure</td><td colspan="2">50 µL</td><td colspan="2">100 µL</td></tr>
<tr><td>Time of incubation in lysis buffer</td><td colspan="2">20 min</td><td colspan="2">10 min</td></tr>
<tr><td>Volume of washout solution №1 required for 1 sample extraction</td><td colspan="2">200 µL</td><td colspan="2">400 µL</td></tr>
<tr><td>Volume of elution buffer required for 1 sample extraction</td><td>100 µL</td><td colspan="3">300 µL</td></tr>
<tr><td>Storage period of purified DNA at temperatures from minus 18 °C to minus 22 °C</td><td colspan="2">up to 6 months</td><td colspan="2">up to 1 year</td></tr>
</table>

9. QUALITY CONTROL

“DNA-Technology Research&Production”, LLC declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for In vitro Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our official representative in EU by quality issues of **PREP-GS DNA Extraction Kit, PREP-GS PLUS DNA Extraction Kit** and **PREP-GS Genetics DNA Extraction Kit**.

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10. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution	VER	Version
EC REP	Authorized representative in the European Community	NON STERILE	Non-sterile

REF P-003/1EU,
P-003/2EU,
P-023/4EU

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