SeCore™ GSSP Kits

Instructions for Use

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In Vitro Diagnostic use and distribution in the European Union

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Contents

Product Information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

Intended use

SeCore™ HLA Sequencing and GSSP kits are intended for the identification and definition of Class I and II Human Leukocyte Antigens (HLA). The SeCore™ HLA Sequencing and GSSP kits provides human histocompatibility information of HLA Class I (A, B and C) and Class II (DPB1, DQB1 and DRB) Loci using genomic DNA isolated from whole blood specimens.

Purpose of the kits

Use SeCore™ GSSP (Group Specific Sequencing Products) to resolve ambiguous HLA heterozygous combinations in conjunction with SeCore™ Sequencing Kits. The reagents are for post-amplification sequencing only. For instructions on how to set up the locus specific genomic DNA amplification, refer to the SeCore™ Sequencing Kits Instructions for Use.

To use the SeCore™ GSSP Kit, you will:

1. Utilize a group specific sequencing primer mix, Big Dye™ Terminator, and PPT Buffer for each SeCore™ GSSP tests.
2. Use the ExoSAP-IT®-treated amplicon, prepared from the SeCore™ Sequencing Kit, as the template for GSSP reactions.
3. Analyze the data generated from the GSSP with the original data from the corresponding SeCore™ Sequencing Kit to determine the sequence result.

SeCore™ GSSP Kit contents and storage

SeCore™ GSSP kits are supplied as individual loci and/or groups. Products are designated by a "Z" number.

If Catalog ID@ A11256 was purchased, each Z solution ordered will be packaged with an accompanying vial of BigDye™ Terminator buffer and a vial of PPT buffer. The volumes provided are adequate for testing 25 different amplification products per Z solution.

A11256 Packaging and Storage

Description	Quantity	Storage
Z# Sequencing Primer Mix	Product dependent	–30°C to –10°C in a **non-frost-free freezer**
Big Dye™ Terminator	Product dependent	–30°C to –10°C in a **non-frost-free freezer**
PPT Buffer	Product dependent	–30°C to –10°C in a **non-frost-free freezer**

If the SeCore™ GSSP Primer Set, Catalog ID# A10918, was purchased, the package will contain one or more Z solutions only, which is dependent on the custom primers ordered. The volumes provided are adequate for testing 100 different amplification products per Z solution.

A10918 Packaging and Storage

Description	Quantity	Storage
Z# Sequencing Primer Mix	Product dependent	–30°C to –10°C in a **non-frost-free freezer**

When purchasing the SeCore™ GSSP Primer Set, Catalog ID# A10918, the SeCore™ GSSP Escort Kit, Catalog ID# A10919 must be ordered in order to process. Each Escort kit contains 81 vials of Big Dye™ Terminator buffer and 81 vials of PPT buffer to accompany 81 Z solutions ordered via A10918. The volumes provided are adequate for testing 100 different amplification products per vial.

A10919 Packaging and Storage

Description	Quantity	Storage
Big Dye™ Terminator	Product dependent	–30°C to –10°C in a **non-frost-free freezer**
PPT Buffer	Product dependent	–30°C to –10°C in a **non-frost-free freezer**

Components are packaged in 0.5mL Sarstedt Screw Cap Micro Tubes (72.730.711) with Sarstedt Screw Caps (65.716.725).

Materials and equipment required but not included

<table>
<tr><th>Item</th><th>Source</th></tr>
<tr><td>96-well thermal cycler with heated lid</td><td>
<table>
<tr><th>Supplier</th><th>Model</th></tr>
<tr><td>One Lambda, Inc. (Applied Biosystems LLC)</td><td>• Gene Amp 2700
• Gene Amp 9600
• Gene Amp 9700
• Veriti®</td></tr>
<tr><td>MJ Research</td><td>PTC-225 DNA Engine Tetrad</td></tr>
</table>
Note: SeCore™ Sequencing Kits have been tested with the above thermal cyclers. You must validate different equipment.
</td></tr>
<tr><td>Automated DNA sequencer</td><td>SeCore™ Sequencing Kits have been validated on the following DNA sequencers:
• Applied Biosystems 3100
• Applied Biosystems 3730 DNA Analyzer
• Applied Biosystems 3500 xL or 3500xL Dx
Note: You must validate other DNA sequencers.</td></tr>
</table>

Reagents and accessories tested with SeCore™ Kits

Instrument	Product Name	Cat. no.	
3100 Genetic Analyzer	3100 Capillary Array, 36 cm	4315931	
	POP-6™ Polymer	4316357	
	310 Running Buffer, 10X	402824	
	Matrix Standards Kit, BigDye® Terminator v1.1	4336824	
	Hi-Di™ Formamide	4311320	
3730 DNA Analyzer	3730 Capillary Array, 36 cm	4331247 or 4331244	
	3730 Capillary Array, 50 cm	4331246 or 4331250	
	POP-6™ Polymer	4316357	
	3730 Running Buffer, 10X	4335613	
	Sequencing Standards, BigDye® Terminator v1.1	4336799	
	Hi-Di™ Formamide	4311320	
3500xL or 3500xL Dx Genetic Analyzer		3500xL	3500xL Dx
	50 cm, 3500 Capillary Array	4404685	4404684
	POP-6™ Polymer	4393712	4393711
	Cathode Buffer Container for 3500 Genetic Analyzer	4408256	4408258
	Anode Buffer Container for 3500 Genetic Analyzer	4393927	4393925
	Conditioning Reagent for 3500 Genetic Analyzer	4393718	4409543
	Sequencing Standards, BigDye® Terminator v1.1	4404314	4404314
	Hi-Di™ Formamide	4311320	4311320

General purpose supplies required but not included

Additional products available from Thermo Fisher Scientific

Item	Cat. no.
MicroAmp® 96-well Tray/Retainer Set	403081
MicroAmp® 0.2-mL Reaction tubes (8 tubes/strip)	N8011533
	N8010580
MicroAmp® 8-Cap Strip (8 caps/strip)	N8010535
MicroAmp® Optical 96-Well Reaction Plate	N8010560
MicroAmp® 96-Well Full Plate Cover	N8010550
Analysis Software: uTYPE® SBT software	539992

General purpose supplies

Item	Description	
Table top centrifuge with plate adapters for 96-well plates	The centrifuge must reach a force of 2500 x *g*	
Pipettes and tips	• 1–10 µL • 10–200 µL • 100–1000 µL	
Electronic dispensing pipettes	capable of dispensing 1–125 µL aliquots	
Multichannel (8 or 12 channels) pipettes	1–100 µL adjustable volume	
CoolSafe System to fit 0.2-mL tubes	Diversified Biotech Cat. No. CSAF-1000	
Absolute Ethanol	Molecular-biology grade	
Sequencer software	Instrument	Software
	3100 and 3730 Genetic Analyzer	• Data Collection Software v1.1 or higher (Win NT) • Sequencing Analysis Software v3.7 or higher (Win NT)
	3500 Genetic Analyzer	• 3500 DX Data Collection Software v1.0 • Sequencing Analysis Software v3.7 or higher (Win NT)

Methods

Before Starting

Follow these sample guidelines

Use locus-specific genomic DNA amplifications purified with ExoSAP-IT® from SeCore™ Sequencing Kits with this kit. You may store ExoSap-IT®-treated amplicons at –20ºC for up to two weeks. Follow the same procedure for A11256 and A10918/A10919.

Note: Change pipet tips in between the pipetting of each DNA sample and each different mix or reagent to prevent cross-contamination. The same pipet tip may be used to dispense the same mix or reagent into multiple tubes provided the tip does not come into contact with genomic DNA or PCR product. Change the pipet tip to prevent contamination if there is any question that this may have occurred.

Perform cycle sequencing

Prepare sequencing reactions

Note: Keep sequencing reaction mixtures as cold as possible by using the recommended CoolSafe system or ice.

1. Briefly centrifuge the purified PCR products before removing the caps from the tubes or reaction plate.
2. If not performed previously, as part of SeCore Kit Sequencing, *for Class II reactions only*, dilute ExoSAP-IT® reagent-treated amplicons with 40µl of molecular biology grade water and mix well.
3. Place a new set of tubes or plate on ice to hold the sequencing reactions.
4. For Class I and Class II reactions, add 2 µL of ExoSAP-IT® reagent-treated PCR amplicons (or class II amplicon dilutions from step 2 of this procedure) to the appropriate tube or well.
5. Mix equal volumes of the Sequencing Primer Mix and BigDye® Terminator.

   IMPORTANT! Volumes in the vials may not be equal. Measure the volumes before combining the components.

   Note: After mixing, store the primer/Big Dye™ Terminator vial at −20°C up to the expiration date on the kit label.
6. Add 8 µL of each Sequencing Primer/Big Dye™ Terminator mix to the appropriate tube or well.
7. Cover the tubes or plate, vortex briefly to mix, and centrifuge the tubes or plate to bring contents to the bottom of the tubes or plate.
8. Place the tubes or plate in the thermal cycler and run the profile listed in the following table:

Each of 25 Cycles			Final Step
Melt	Anneal	Extend	
CYCLE			Soak
95°C 20 seconds	50°C 15 seconds	60°C 60 seconds	4°C ∞*

*Remove in time to proceed to the next step

9. Start the run. When the thermal cycler block is >80 °C, place the tubes or plate in the thermal cycler, then close the lid.

   Notes

   - The total reaction time is approximately 1.5 hours.
   - Ethanol precipitates the sequencing reaction products within 24 hours (see page 12).

Purify sequencing extension products

After cycle sequencing, remove excess terminators by ethanol precipitation.

Procedure

1. Vortex PPT Buffer to dissolve particles, until solution is clear.
2. Add 2 µL of PPT Buffer to each sequencing reaction mixture.

   Note: Use of a single channel pipette or single channel repeater pipette is recommended for this step. Dispensing PPT Buffer into a reagent trough or boat to aid in multi-channel pipetting can result in significant reagent loss.
3. Centrifuge briefly to ensure all contents are mixed and at the bottom of the well.
4. Add 40 µL of 100% ethanol to each mixture.
5. Cover the plate and vortex well for a minimum of 60 seconds.

   Note: Make sure the contents of all tubes or wells are mixed thoroughly.
6. Centrifuge the plate in a centrifuge fitted with a plate-adaptor for 30 minutes at 2000 × *g* or greater.
7. Remove cover, cover with a paper towel, and invert. Centrifuge with the paper towel briefly (10–60 seconds at 500 × *g*) in the inverted position to remove as much liquid as possible.
8. Add 100 µL of 80% ethanol to the DNA pellets. *Do not* vortex the plate.

   Note: Prepare fresh 80% ethanol daily.
9. Centrifuge the plate for 5 minutes at 2,000 × g or greater.
10. Remove the supernatant by an inverted spin as described in step 7 of this procedure.

Note: Store DNA pellets at –20°C for a maximum of 7 days.

Perform capillary electrophoresis

Prepare Loading Samples

1. Add 15 µL of Hi-Di™ Formamide (One Lambda, Inc. Cat. no. 4311320) to each DNA pellet.
2. Cover the plate and briefly centrifuge to bring contents to the bottom of the plate wells.
3. Denature the samples at 95°C for 2 minutes in a thermal cycler.
4. Centrifuge briefly to remove any air bubbles in the samples.

   Note: If air bubbles get into the capillary, they will cause damage to the capillary.
5. Place the plate in the sequencer autosampler.
6. Use the following electrophoresis conditions

Instrument	Parameters	POP-6 Settings
ABI 3100	Run Module	RapidSeq36
	Injection Time	10 seconds
	Run Time	1800 seconds
ABI 3730/3730xL	Run Module	StdSeq36
	Injection Time	5 seconds
	Run Time	1800 seconds
ABI 3500xL	Run Module	StdSeq50_POP6*
	Injection Time	Default
	Run Time	3780 seconds

*For access to the RapidSeq50_POP6 run module, please contact your local One Lambda representative or HLA Technical Support.

Analyze data

Use uTYPE™ SBT HLA sequencing analysis software to process the sequence files and create an HLA typing report. Analyze the data generated from the GSSP kit together with the original data from the SeCore™ Sequencing Kit.

IMPORTANT! If your database is not current, order the latest version of the uTYPE™ Allele Library Update at: www.onelambda.com. Contact your One Lambda representative for more information.

Note: GSSP primers may detect polymorphic sites from untargeted alleles. Any peaks detected from untargeted alleles generally exhibit peak heights less than 33% of the peak height produced by the targeted allele.

Troubleshooting

Observation	Possible Causes	Recommended Action
Excessive background (baseline noise)	Poor or incorrect matrix	Repeat the spectral calibration and reinject the samples.
	Poor injection	Reinject the samples.
	The injection time was set too high	Reduce injection time and reinject the samples. (Samples of poor quality may have lower signal strengths but may still be analyzed and typed.)
	Poor sequencing reaction due to pipetting error	Be sure that both the ExoSAP-IT®-treated PCR product and the correct sequencing mix are added and combined.
	The incorrect mobility file was chosen- the peaks will be shifted or will be on top of each other	Choose the correct mobility file.
	Excess 100% ethanol was added	Resequence. Check to be sure that the pipette is set at the correct volume.
	Ethanol evaporation during precipitation	Prepare new sequencing reactions. Purify the sequencing extension products away from direct air flow (for example, exhaust from a centrifuge or other equipment), which may cause ethanol evaporation.
Weak Signal	Sequencing reactions were not vortexed thoroughly after adding PPT Buffer and ethanol	Repeat the sequencing reactions. Vortex thoroughly (minimum of 60 seconds) after the adding ethanol. Be sure all reactions are mixing.
	Injection time needs to be increased	Repeat sequencing reaction(s) and increase injection time.

Observation	Possible Causes	Recommended Action
Excessive Dye Blobs	The PPT Buffer was not added to the sequencing reactions prior to adding ethanol	Repeat the sequencing reaction. Add the PPT Buffer before adding ethanol.
	Did not wash the sequencing reactions with 80% ethanol	Repeat sequencing reactions without omitting the 80% ethanol wash step.
	Poor sequencing reaction due to error in pipetting or weak amplification product	• Be sure that both the ExoSAP-IT® treated PCR product and the correct sequencing mix are added and combined. • In the case of a weak amplification, confirm the intensity of the amplification product by running an agarose gel.
	Did not remove all of the remaining ethanol during the precipitation	Repeat the sequencing reactions. The 500 x *g* centrifuge step is very important in removing all remaining ethanol in the reaction tubes.
	The ethanol used in wash step was too dilute	Repeat the sequencing reactions. Use 80% ethanol for the wash step.
Excessive Signal Strength	The injection time is set too high	Reduce the injection time and reinject. (Samples of poor quality may have lower signal strengths but may still be analyzed and typed.)
Weak signal from short fragments Longer fragments show a stronger signal	Did not use fresh 80% ethanol	Prepare fresh 80% ethanol daily.
Strong signal from short fragments Longer fragments show a weaker signal	Over amplification of short fragments during purification	Repeat sequencing reactions. Reduce the amount of ethanol stepwise during the wash step down to a minimum concentration of 70%
Random sequence failures	Poor sequencing reaction due to error in pipetting	Be sure that the ExoSAP-IT® treated PCR product and the correct sequencing mix are added and combined.

Appendix A: Limitations and Cautions

Precautions

- To ensure the best performance of SeCore™ GSSP Kits, use the products with the materials, reagents, and equipment recommended on pages 9–9 of this document.
- GSSP primers may detect polymorphic sites from untargeted alleles. Any peaks detected from untargeted alleles generally exhibit peak heights less than 33% of the peak height produced by the targeted allele.
- We recommend that you verify all homozygous results by another method.

Appendix B: Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc.). To obtain SDSs, see the "Documentation and Support" section in this document.
- All testing should be performed in accordance with local, regional and national acceptable laboratory accreditation standards and/or regulations.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or bio hazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! – BIOHAZARD**.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Blood borne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/ WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Obtain SDSs

Safety Data Sheets (SDSs) are available from www.onelambda.com

For the SDSs of chemicals not distributed by One Lambda, Inc., contact the chemical manufacturer.

Obtain support

For the latest services and support information for all locations, go to: www.onelambda.com

At the website, you can:

- Access telephone and fax numbers to contact Technical Support and Sales
- Submit a question directly to Technical Support (1lambda-techsupport@thermofisher.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are provided with the product or are available upon request.

Limited Product Warranty

One Lambda, Inc. and/or its affiliate(s) warrant their products as set forth in the Conditions of Sale. If you have any questions, please contact One Lambda at (1lambda-techsupport@thermofisher.com)

IVD Symbols

EXPLANATION OF SYMBOLS (reference EN ISO 15223-1: Medical devices – Symbols to be used with medical device labels, labeling and information to be supplied)

Symbol	Description
IVD	In Vitro Diagnostic Medical Device
 *ISO 7000 Reg No. 3082*	Manufacturer
 *ISO 7000 Reg No.0518*	Number of Tests
 *ISO 7000 Reg No. 1641*	Consult Instructions for Use
 *ISO 7000 Reg No. 0632*	Temperature Limitation (range)
 *ISO 7000 Reg No. 0534*	Lower Temperature Limitation
 *ISO 7000 Reg No. 1641*	Upper Temperature Limitation
 *ISO 7000 Reg No 2607*	Use By
REF *ISO 7000 Reg No. 2493*	Catalog Number
LOT *ISO 7000 Reg No 2492*	Batch Code
 *ISO 7000 Reg No. 0659*	Warning: Product may contain bio hazardous material
 *ISO 7000 Reg No 2497*	Date of Manufacture
EC REP	Authorized Representative in the European Community

ISO 7000 Reg No. 0434A	Warning: Attention, see instructions for use

EC REP

European Representative:
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Schiffgraben 41,
30175 Hannover, Germany
Tel: (+49) 511-6262 8630
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CE IVD

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REF	Catalog #'s
A11256	SeCore™ GSSP Kit , 25 tests
A10918	SeCore ™ GSSP Primer Set
A10919	SeCore™ Primer Escort