

SeCore™ Sequencing Kits

Instructions for Use

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Revision 05

In Vitro Diagnostic use and distribution in the European Union

For In Vitro Diagnostic Use

The customer is responsible for validation of assays and compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

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Product Information

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

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.

Product description

Purpose of the kit

Use the SeCore™ Sequencing Kits to directly identify DNA sequence from target HLA genes. To use the SeCore™ Sequencing Kits, you will:

1. Amplify locus-specific DNA by cycling an amplification mix, FastStart® *Taq* DNA Polymerase, and sample genomic DNA.
2. Treat the resulting product with ExoSAP-IT® reagent prior to sequencing to degrade the unincorporated primers and hydrolyze free nucleotides.
3. Determine the nucleotide chronology and resulting HLA-subtype by multicolor, fluorescence-based, BigDye® sequencing.
4. Purify the final reactions by an ethanol precipitation prior to loading.
5. Load formamide-denatured samples and detect results on an automated sequencing instrument.

Class I SeCore™ Sequencing Kit contents and storage conditions

Upon receipt of the SeCore™ Sequencing Kit, immediately store the components at –30°C to –10°C in a non-frost-free freezer.

Components are packaged in either 0.5mL or 2.0mL Sarstedt Screw Cap Micro Tubes (72.730.711, 72.609.711) with Sarstedt Screw Caps (65.716.725). Additionally, 500 test pack sizes contain components packaged in either 8.0mL or 15.0mL Nalgene HDPE Narrow Mouth Bottles (342002-9025, 342002-9050).

For vial volumes, refer to the label on each vial.

Kit	Pre-PCR Components		Post-PCR Components		
Class I	Amp Mix (locus-specific primers)	FastStart® Taq DNA Polymerase (5 U/μL)	ExoSap-IT® Reagent	Seq Mix (sequencing primers, dye terminators, sequencing enzyme)	Sequencing PPT Buffer
A Locus	25 Test Kit 1 vial	1 vial	1 vial or bottle	10 vials or bottles: <ul style="list-style-type: none"> •Exon1-Fwd •Exon1-Rev •Exon2-Fwd •Exon2-Rev •Exon3-Fwd •Exon3-Rev •Exon4-Fwd •Exon4-Rev •Exon5-Fwd •Exon5-Rev 	1 vial or bottle
	500 Test Kit 2 bottles				
B Locus	25 Test Kit 1 vial	1 vial	1 vial or bottle	9 vials or bottles: <ul style="list-style-type: none"> •Exon1-Fwd •Exon2-Fwd •Exon2-Rev •Exon3-Fwd •Exon3-Rev •Exon4-Fwd •Exon4-Rev •Exon5-Fwd •Exon5-Rev 	1 vial or bottle
	500 Test Kit 2 bottles				
C Locus	25 Test Kit 1 vial	1 vial	1 vial or bottle	14 vials or bottles: <ul style="list-style-type: none"> •Exon1-Fwd •Exon1-Rev •Exon2-Fwd •Exon2-Rev •Exon3-Fwd •Exon3-Rev •Exon4-Fwd •Exon4-Rev •Exon5-Fwd •Exon5-Rev •Exon6-Fwd •Exon6-Rev •Exon7-Fwd •Exon7-Rev 	1 vial or bottle
	500 Test Kit 2 bottles				

Class II SeCore™ Sequencing Kit contents and storage conditions

Upon receipt of the SeCore™ Sequencing Kit, immediately store the components at –30°C to –10°C in a non-frost-free freezer.

Components are packaged in either 0.5mL or 2.0mL Sarstedt Screw Cap Micro Tubes (72.730.711, 72.609.711) with Sarstedt Screw Caps (65.716.725). Additionally, 500 test pack sizes contain components packaged in either 8.0 mL or 15.0 mL Nalgene HDPE Narrow Mouth Bottles (342002-9025, 342002-9050).

† The DRB1 Locus kit will be identified as the “DRB1 Locus Single Amplification Kit” throughout this document.

*Eleven Group specific Amp Mixes included in the SeCore™ DRB Group Sequencing Kit are:
 DRB1*01, DRB1*03/11/13/14, DRB1*04, DRB1*07, DRB1*08/12, DRB1*09, DRB1*10, DRB1*15/16, DRB3, DRB4, DRB5

Kit	Pre-PCR Components		Post-PCR Components		
Class II	Amp Mix (locus-specific primers)	FastStart® Taq DNA Polymerase (5 U/μL)	ExoSap-IT® Reagent	Seq Mix (sequencing primers, dye terminators, sequencing enzyme)	Sequencing PPT Buffer
DPB1 Locus	25 Test Kit 2 vials total: • 1 Exon 2 • 1 Exons 3/4	1 vial	1 vial or bottle	8 vials or bottles: • Exon2-Fwd • Exon4 Fwd • Exon2-Rev • Exon4-Rev • Exon3-Fwd • Codon 8- Fwd • Exon3-Rev • Codon 85- Rev	1 vial or bottle
	500 Test Kit 4 bottles total: • 2 Exon 2 • 2 Exons 3/4				
DQB1 Locus	25 Test Kit 2 vials total: • 1 Exon 2 • 1 Exon 3	1 vial	1 vial or bottle	4 vials or bottles: • Exon2-Fwd • Exon2- Rev • Exon3-Fwd • Exon3- Rev	1 vial or bottle
	500 Test Kit 4 bottles total: • 2 Exon 2 • 2 Exon 3				
DRB1 Locus [†]	25 Test Kit 1 vial	1 vial	1 vial or bottle	3 vials: • Exon2-Fwd • Exon2-Rev • TG Codon 86-Rev	1 vial or bottle
	500 Test Kit 1 bottle				

DRB1 Locus Exon 2 &3 Sequencing Kit	25 Test Kit 2 vials total: • 1 Exon 2 • 1 Exon 3	1 vial	1 vial or bottle	5 vials: • Exon2-Fwd • Exon2-Rev • Exon3-Fwd • Exon3-Rev • TG Codon 86-Rev	1 vial or bottle
	500 Test Kit 2 bottles total: • 1 Exon 2 • 1 Exon 3				
DRB Group (DRB1, DRB3, 4 & 5 Locus)	11 vials *	1 vial	1 vial	3 vials: • Group Exon 2-Fwd • Group Exon 2-Rev • TG Codon 86 Rev	1 vial

For vial volumes, refer to the label on each vial.

Materials and equipment required but not included

IMPORTANT! The customer is responsible for validating all materials and equipment before use, and for compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

Item	Description						
96-well thermal cycler with heated lid	<ul style="list-style-type: none"> • Gene Amp 2700 • Gene Amp 9600 • Gene Amp 9700 • Veriti® <p>Note: SeCore™ Sequencing Kits have been tested with the above thermal cyclers.</p>						
Automated DNA sequencer	<p>SeCore™ Sequencing Kits have been validated on the following DNA sequencers:</p> <ul style="list-style-type: none"> • Applied Biosystems 3100 • Applied Biosystems 3730 and 3730 xL DNA Analyzers • Applied Biosystems 3500 xL or 3500xL Dx 						
Sequencer software	<table border="1"> <thead> <tr> <th data-bbox="524 919 906 968">Instrument</th> <th data-bbox="906 919 1468 968">Software</th> </tr> </thead> <tbody> <tr> <td data-bbox="524 968 906 1115">3100 and 3730 Genetic Analyzer</td> <td data-bbox="906 968 1468 1115"> <ul style="list-style-type: none"> • Data Collection Software v1.1 or higher (Win NT) • Sequencing Analysis Software v3.7 or higher (Win NT) </td> </tr> <tr> <td data-bbox="524 1115 906 1230">3500 Genetic Analyzer</td> <td data-bbox="906 1115 1468 1230"> <ul style="list-style-type: none"> • 3500 Data Collection Software v1.0.0 • Sequencing Analysis Software v3.7 or higher (Win NT) </td> </tr> </tbody> </table>	Instrument	Software	3100 and 3730 Genetic Analyzer	<ul style="list-style-type: none"> • Data Collection Software v1.1 or higher (Win NT) • Sequencing Analysis Software v3.7 or higher (Win NT) 	3500 Genetic Analyzer	<ul style="list-style-type: none"> • 3500 Data Collection Software v1.0.0 • Sequencing Analysis Software v3.7 or higher (Win NT)
Instrument	Software						
3100 and 3730 Genetic Analyzer	<ul style="list-style-type: none"> • Data Collection Software v1.1 or higher (Win NT) • Sequencing Analysis Software v3.7 or higher (Win NT) 						
3500 Genetic Analyzer	<ul style="list-style-type: none"> • 3500 Data Collection Software v1.0.0 • Sequencing Analysis Software v3.7 or higher (Win NT) 						
HLA Sequencing Analysis Software	uTYPE™ SBT software , One Lambda, Inc. Cat. no. 539992 (CE)						
uTYPE™ Allele Library Update	One Lambda, Inc. Cat. no. 53999100						

Item	Source			
Automated DNA sequencer accessories	The following accessories have been tested with SeCore™ kits:			
	Instrument	Item	One Lambda, Inc. Cat. No.	
	3100 Genetic Analyzer	36 cm, 3100 Capillary Array	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	36 cm, 3730 Capillary Array	4331247 or 4331244	
		50 cm, 3730 Capillary Array	4331250 or 4331246	
		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 Capillar 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

IMPORTANT! The customer is responsible for validating all general purpose supplies before use, and for compliance with regulatory requirements that pertain to their procedures and uses.

Item	Description										
Plates	96-well PCR microtiter plate, standard or optical-grade polypropylene, 0.2 mL, half- or semi-skirted design, with or without barcode. Cat. no. N8010560.										
Strip tubes ,strip caps, silicone mat	<table border="1"> <thead> <tr> <th>Description</th> <th>One Lambda, Inc. Cat. no.</th> </tr> </thead> <tbody> <tr> <td>MicroAmp® 0.2-mL Reaction Tubes</td> <td>N8011533</td> </tr> <tr> <td>MicroAmp® 8-Tube Strip, 0.2-mL</td> <td>N8010580</td> </tr> <tr> <td>MicroAmp® 8-Cap Strip</td> <td>N8010535</td> </tr> <tr> <td>MicroAmp® 96-Well Full Plate Cover</td> <td>N8010550</td> </tr> </tbody> </table>	Description	One Lambda, Inc. Cat. no.	MicroAmp® 0.2-mL Reaction Tubes	N8011533	MicroAmp® 8-Tube Strip, 0.2-mL	N8010580	MicroAmp® 8-Cap Strip	N8010535	MicroAmp® 96-Well Full Plate Cover	N8010550
	Description	One Lambda, Inc. Cat. no.									
	MicroAmp® 0.2-mL Reaction Tubes	N8011533									
	MicroAmp® 8-Tube Strip, 0.2-mL	N8010580									
	MicroAmp® 8-Cap Strip	N8010535									
MicroAmp® 96-Well Full Plate Cover	N8010550										
Table top centrifuge with plate adapters for 96-well plates	The centrifuge must reach a force of 2500 x g										
Pipettes and tips	<ul style="list-style-type: none"> • 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										
Agarose gel electrophoresis apparatus	—										
Ice or cooling system	For 0.2-mL tubes to keep samples cold before cycle sequencing										
Water	Sterile, molecular biology grade										
Absolute Ethanol (100%)	Molecular-biology grade										
Molecular Biology grade Agarose	Cat. no. 75000500										
PCR Marker suitable to cover range of 300–1300 bp	Cat. no. 74601250 (recommended)										
Loading dye	Without Bromophenol Blue or Xylene Cyanol										

Methods

Before starting

Obtain specimens and prepare DNA samples

1. Collect blood samples in ACD or EDTA- anticoagulated tubes.

Note: Do not use heparinized samples.

IMPORTANT! Perform DNA extraction according to DNA extraction kit manufacturer instructions. Phenol, Ethanol and SDS may cause inhibition of the PCR reaction.

2. Prepare purified DNA with an A_{260}/A_{280} between 1.7 and 1.9.
3. Prepare a working solution of the purified DNA at 15–30 ng/ μ L by diluting the DNA in molecular-grade water. Discard DNA aliquoted in water after use.

Note: Change pipette tips in between pipetting each DNA sample and each different mix or reagent to prevent cross-contamination. The same pipette 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. If there is any question that this may have occurred, change the pipette tip to prevent contamination.

Calculate the sample number

Class I

Locus	Amp Mix Sets	Notes
A, B and C	1	<p>Amplification set up will depend on the number of sequencing mixes that are going to be tested.</p> <p>If eight sequencing mixes or less will be tested:</p> <ul style="list-style-type: none"> Set up one reaction for each sample plus one negative control per group of samples being amplified. <p>If more than eight sequencing mixes will be tested:</p> <ul style="list-style-type: none"> Set up two reactions for each sample plus one negative control per group of samples being amplified.

Class II

Locus	Amp Mix Sets	Notes
DRB1 Single Amplification Kit	1	Set up one reaction for each sample plus one negative control per group of samples being amplified.
DRB1 Locus Exon 2 & 3 Sequencing Kit	2	Set up two reactions for each sample plus one negative control per group of samples being amplified.
DRB Group (Group Specific Set)	11	<ul style="list-style-type: none"> Designed to amplify the 11 major DRB1/3/4/5 groups separately for better resolution and ease of analysis. Perform all 11 amplification reactions with samples of unknown typing. Set up one negative control for each set of Amp Mix.
DPB	2	<ul style="list-style-type: none"> Perform both amplification reactions for samples of unknown typing. Set up one negative control for each set of Amp Mix.
DQB1 Locus	2	Set up two reactions for each sample plus one negative control per group of samples being amplified.

Note: For the DRB Group only: When a low resolution HLA typing of the sample is known, you may choose the necessary groups to amplify for high resolution DRB1 sequencing.

Prepare a master mix

The Amp Mix of the SeCore™ Sequencing Kits does not contain the FastStart® DNA Polymerase. To prepare the master mix for genomic DNA amplification, you will combine the locus specific Amp Mix with FastStart® *Taq* DNA Polymerase in a clean tube.

Note: For 25 test kits, prepare a master mix for a minimum of 5 samples to aid in the accuracy of pipetting small volumes of enzyme. Kit reagent volumes are sufficient for 5 sets of such reactions.

Note: For 500 test kits, prepare a master mix for a minimum of 20 samples to limit the amount of inadvertent enzyme loss during pipetting. Kit reagent volumes are sufficient for 25 sets of such reactions.

IMPORTANT! Due to the viscous nature of FastStart *Taq* Polymerase, care should be taken to remove excess *Taq* that remains on the pipette tip before dispensing the enzyme into the master mix. *Taq* should be pipetted from the top of the vial and the user should avoid submerging the tip into the enzyme. Failure to remove excess *Taq* will result in a shortage of the enzyme.

1. Calculate the volumes of Amp Mix and FastStart® *Taq* DNA Polymerase you will need for a master mix based on the following table. For each amplification reaction, combine:

Component	Class I per rxn	Class II per rxn	Class I 5 rxn	Class II 5 rxn	Class I 20 rxn	Class II 20 rxn
Amp Mix	19.8 µL	22.8 µL	99 µL	114 µL	396 µL	456 µL
FastStart® <i>Taq</i> Polymerase	0.2 µL	0.2 µL	1.0 µL	1.0 µL	4.0 µL	4.0 µL
Total master mix volume per reaction	20 µL	23 µL	100 µL	115 µL	400 µL	460 µL

2. Add the components to a clean tube, pulse-vortex 2 to 3 times to mix thoroughly, then centrifuge briefly to bring the contents to the bottom of the tube.

Amplify DNA

1. Combine the following for each reaction:

Component	Class I reactions	Class II reactions
Purified sample DNA (15–30 ng/μL) <i>or</i> Molecular-biology-grade water (for negative control)	5 μL	2 μL
PCR master mix (containing Amp Mix and FastStart® <i>Taq</i> Polymerase)	20 μL	23 μL
Total reaction volume	25 μL	25 μL

2. Cover the plate or tubes, then centrifuge briefly to bring the contents to the bottom of the wells or tubes.
3. Place the tubes in the thermal cycler. Run the PCR according to the following conditions (the total PCR time is approximately 1.5 hours):

Initial Step	Each of 35 Cycles			Final Extension	Final Step
	Melt	Anneal	Extend		
Soak	CYCLE			HOLD	Soak
95°C 4 minutes	95°C 20 seconds	63°C 20 seconds	72°C 40 seconds	72°C 5 minutes	4°C ∞*

*Remove in time to proceed to the next step

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

Note: Immediately perform agarose gel electrophoresis and purify the PCR amplicons (described in the next sections) *or* store the PCR products at 4°C. Always purify the PCR amplicons (Exo-SAP IT®) within 18 hours.

Confirm the presence of PCR products

Confirm the presence of PCR products by 2.0% agarose gel electrophoresis.

1. Briefly centrifuge the tubes or plate before removing the caps from the tubes or reaction plate containing the PCR products.
2. Load the agarose gel with 5 μ L of amplified DNA plus loading dye.
3. Run the gel according to your laboratory protocol. Run the gel for a sufficient amount of time to cause the bands to separate.

Note: Amplicons of the correct size should be >35 ng per band when 5 μ L are loaded in each well.

Expected products for each locus specific amplification

Kit	Product Size (bp)	Internal Control Size (bp)
A Locus	~1200 and ~990	None
B Locus Single Amp	~1400 and ~950	None
C Locus	~1375 and ~1600	None
DRB1 Locus Single Amplification Kit	~300	None
DRB1 Locus Exon 2 & 3 Sequencing Kit	Exon 2: ~500-850 Exon 3: ~450	None
DRB Group	~300	~600
DQB1 Locus	~350 and ~375	None
DPB1 Locus	~300 and ~1000	None

Notes:

- **DRB1 Locus Single Amplification Kit:** You may observe a weak band larger than the 300-bp band for some DRB1 Locus Single Amplification Kit amplification products. The sequencing result is not affected by the presence of the weak band.
- **DRB Group Kit:** Set up sequencing reactions for the positive 300 bp reactions only. For all other kits, set up sequencing reactions for all wells containing PCR product.

Purify PCR products

Treat the PCR amplicons with ExoSAP-IT® reagent to degrade unincorporated primers and hydrolyze dNTPs.

IMPORTANT! Follow the *Taq* guidelines listed above for pipetting viscous enzymes when adding ExoSAP-IT®

1. Briefly centrifuge the tubes or plate before removing the caps from the tubes or reaction plate containing the PCR products.
2. If you did not perform gel electrophoresis, (see **Confirm the presence of PCR products** on page 16), remove and discard 5 µL from each amplified DNA product.
3. Add 4 µL of ExoSAP-IT® reagent to the amplification tubes.
 - For the DRB group specific kits, add ExoSAP-IT® reagent only to the tubes with positive 300-bp products.
 - For all other kits, add the ExoSAP-IT® reagent to all tubes or wells containing PCR products.
4. Cover the tubes and centrifuge ~5 seconds to bring contents to the bottom of the tube.
5. Vortex the reaction mixtures thoroughly (~10 seconds).
 IMPORTANT! Mix ExoSAP-IT® reagent well with the PCR products. This is critical to the success of the purification of PCR amplicons.
6. Centrifuge briefly to pull the contents to the bottom of the tubes or wells.
7. Place the tubes or plate in the thermal cycler and run the following profile:

1 Cycle		
CYCLE		Soak
37°C 20 minutes	80°C 20 minutes	4°C ∞*

*Remove in time to proceed to the next step

8. Place the tubes or plate in the thermal cycler, close the lid, then start the run.
 Note: Store ExoSAP-IT® reagent-treated amplicons at -20°C for up to two weeks.

Perform cycle sequencing

Determine sequencing reactions

Possible sequencing reactions for each kit

A, Locus	B Locus	C Locus
Exon1 forward	Exon1 forward	Exon1 forward
Exon1 reverse	—	Exon1 reverse
Exon2 forward	Exon2 forward	Exon2 forward
Exon2 reverse	Exon2 reverse	Exon2 reverse
Exon3 forward	Exon3 forward	Exon3 forward
Exon3 reverse	Exon3 reverse	Exon3 reverse
Exon4 forward	Exon4 forward	Exon4 forward
Exon4 reverse	Exon4 reverse	Exon4 reverse
Exon5 forward	Exon5 forward	Exon5 forward
Exon5 reverse	Exon5 reverse	Exon5 reverse
—	—	Exon6 forward
—	—	Exon6 reverse
—	—	Exon7 forward
—	—	Exon7 reverse

DRB1 Locus Single Amplification	DRB1 Locus Exon 2 & 3	DRB Group	DQB1 Locus	DPB1 Locus
Exon2 forward	Exon2 forward	Exon2 forward	Exon2 forward	Exon2 forward
Exon2 reverse	Exon2 reverse	Exon2 reverse	Exon2 reverse	Exon2 reverse
Codon 86 reverse	Exon3 forward	Codon 86 reverse	Exon3 forward	Exon3 forward
—	Exon3 reverse	—	Exon3 reverse	Exon3 reverse
—	Codon 86 reverse	—	—	Exon4 forward
—	—	—	—	Exon4 reverse
—	—	—	—	Codon 8 forward
—	—	—	—	Codon 85 reverse

Specific instructions for each locus

A, B Single Amp, C Locus Kits

Set up forward and reverse sequencing reactions for exons 2, 3 and 4 for all samples. Sequencing reactions for additional exons can be set up concurrent to exons 2, 3 and 4 or within 2 weeks of amplification date if purified reactions are stored at -20°C.

DRB1 Locus (Single Amplification and Exon 2 & 3) Kits

Set up all reactions as listed in the preceding table.

DRB Group

For each...	Set up...
Positive DRB group specific PCR reaction	2 sequencing reactions

Note: The minimum number of sequencing reactions is 2 when a sample has only one positive amplification. The maximum number of sequencing reactions is 8, when a sample has two positive amplifications of DRB1 locus and two positive amplifications of DRB3/4/5 loci.

Optional: Set up the Codon 86 reaction (see page 19).

DPB1 Locus

Set up the following forward and reverse sequencing reactions for exons 2, 3, and 4:

Sequencing mix	Amplicon
Exon 2	Exon 2
Exon 3	Exon 3/4
Exon 4	Exon 3/4

Note: You will have 6 sequencing reactions for each sample.

DQB1 Locus Kit

Set up forward and reverse sequencing reactions for exons 2 and 3 for both of the positive amplification reactions.

Sequencing mix	Amplicon
Exon 2	Exon 2
Exon 3	Exon 3

Note: You will have 4 sequencing reactions for each sample.

Before starting sequencing reactions

Note: Verify that the appropriate sequencing mix and the treated amplicon is placed in the correct tube or well.

- The sample layout will depend on instrument configuration, i.e. the number of capillaries.
- Refer to uTYPE™ Sequencing Analysis software instructions for use for sample naming recommendations.

Suggested Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1F	S1R										
B	S2F	S2R										
C												
D												
E												
F												
G												
H	↓	↓	↓	↓								

Note: S1F and S2F stand for forward reactions of sample 1 and 2, whereas S1R and S2R stands for reverse reactions of sample 1 and 2.

SeCore™ DR Codon 86 sequencing

Ambiguous DRB1 typing results

If DRB1 typing results are ambiguous, examine the Codon 86 associated nucleotides. Two consecutive positions with a KK sequence (K= G/T) indicate a Codon 86 related ambiguity. These can arise when using the DRB1 Locus Single Amplification, DRB1 Locus Exon 2 & 3 Sequencing Kits or the DRB1 amplicons from the DRB Group Sequencing Kit:

To resolve the ambiguity, prepare a new sequencing reaction using the TG Codon 86 Rev Seq Mix.

DRB1 gene groups

DRB1 genes can be divided into two groups based on the sequence at Codon 86:

- The group with the sequence GTG.

or

- The group with GGT at this position.

Using the target specific, GTG, sequencing primer (TG Codon 86 Rev Seq Mix) allows for separate sequencing of one of the two sample alleles, to resolve the Codon 86 associated ambiguity. The result will be the anti-sense sequence of one allele of the sample allele pair.

DR Codon 86 sequencing can be set up with or after DR sequencing.

Procedure for Codon 86 sequencing

1. Set up the sequencing reaction using the ExoSAP-IT®-treated amplicons of the DRB1 Locus Single Amplification and DRB1 Locus Exon 2 & 3 Sequencing Kits and DRB Group Sequencing Kit. See **Prepare** sequencing reactions on page 21.
2. Perform the procedures in **Purify sequencing extension products** on page 22 and **Perform capillary electrophoresis** on page 23.
3. Analyze the data together with the data obtained using the DRB1 Locus Single Amplification and DRB1 Locus Exon 2 & 3 Kits or DRB Group Kit.

SeCore™ DPB1 Codons 8 and 85 sequencing

Ambiguous DPB1 typing results

If DPB1 typing results are ambiguous, examine the sequence at Codon 8 and Codon 85. Distinguish ambiguous results by using the nucleotide sequence at Codon 8, Codon 85, or by using both codons. Use the Codon 8 CTT forward sequencing primer (Z39) and Codon 85 GAG reverse sequencing primer (Z38) to identify the sequence at these locations. Set up DP Codon 8 and Codon 85 sequencing with or after DP sequencing.

Procedure for Codon 8 and 85 sequencing

1. Set up the sequencing reaction using the ExoSAP-IT®-treated exon 2 amplicon. See **Prepare** sequencing reactions on page 21.
2. Perform the procedures in **Purify sequencing extension products** on page 24 and **Perform capillary electrophoresis** on page 25.
3. Analyze the data together with the data obtained using the SeCore™ DPB1 Sequencing Kit.

Prepare sequencing reactions

Note: Keep sequencing reaction mixtures as cold as possible by using a cold block or ice.

1. Briefly centrifuge the purified PCR products before removing the caps from the tubes or reaction plate.
2. *For Class II reactions only*, add 40 μ L of ultra pure water to ExoSAP-IT[®] reagent-treated amplicons 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 μ L 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. Add 8 μ L of each locus specific sequencing mix to the appropriate tube or well.
6. 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.
7. Place the tubes or plate in the thermal cycler and run the profile listed in the following table (the total reaction time is approximately 1.5 hours):

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

8. 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 precipitate the sequencing reaction products within 24 hours (see page 24).

Purify sequencing extension products

After cycle sequencing, remove excess terminators by ethanol precipitation.

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 $2000 \times g$ or greater.
7. Remove the cover, cover the plate with a paper towel, and invert. Centrifuge with the paper towel briefly (10–60 seconds at $500 \times g$) 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 $2000 \times g$ or greater.
10. Remove the supernatant by an inverted spin as described in step 7 of this procedure.

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
3100	Run Module	RapidSeq36_POP6
	Injection Time	10 seconds
	Run Time	1800 seconds
3730/3730xL	Run Module	StdSeq36
	Injection Time	5 seconds
	Run Time	1800 seconds
3500xL	Run Module	StdSeq50_POP6*
	Injection Time	Default
	Run Time	3780 seconds

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

Note: The first time Big Dye®1.1 Terminators are used, the user must run matrix standards. Follow the package instructions for the BigDye® 1.1 Terminator Matrix Standard (One Lambda, Inc. Cat. no. 4336824).

Parameters and settings for other instrument configurations may require optimization.

Analyze data

Use uTYPE™ HLA sequence analysis software

1. Use Sequencing Analysis software to process collected raw data and create sequence files.
2. Use uTYPE™ SBT HLA sequencing analysis software to process the sequence files and create an HLA typing report. Refer to software Instructions for Use for typing instructions.

Note: We strongly recommend that you implement the most recent IMGT allele database for sequence data analysis. Note: For users of the DRB1 Locus Exon 2 & 3 Sequencing Kit, the appropriate changes need to be made to the Exon Trim Options settings within the uTYPE™ HLA sequence analysis software. With the **Preferences** menu, click the **Exon Trim Options** tab and change the DRB1 exon 2 and 3 trim values from “Default” to “Full Exon”.

Troubleshooting

Observation	Possible Causes	Recommended Action
Absence of PCR product or weak PCR product	FastStart® <i>Taq</i> was not added to the amplification mix or not mixed properly when added	Repeat amplification paying attention to the addition and mixing of <i>Taq</i> with the amplification mix. Use a tube that is the appropriate size for the amount of mix.
	Thermal cycler failure	<ul style="list-style-type: none"> • Check the cycler run history. • Contact One Lambda, Inc. .
	The DNA concentration is not optimal	Re-quantitate the DNA and adjust the concentration to 15–30 ng/μL.
	Poor quality or degraded DNA	<ul style="list-style-type: none"> • Run DNA on a 1% agarose gel to evaluate the quality. Intact genomic DNA should be ~3000 bp. • Re-amplify the sample using 0.3μl of <i>Taq</i> per test.
	The visibility of the product bands on the agarose gel are masked by the dye in the loading buffer	Repeat gel electrophoresis using a different gel loading buffer.
Presence of extra amplification products (with the exception of the DRB1 product)	The incorrect thermal cycling program was used	Repeat the amplification and confirm that the correct program was used.
	Contaminated amplification mix, DNA, or work area	De-contaminate the work area or re-extract DNA. Repeat the amplification.
Weak Signal	Sequencing reactions were not vortexed thoroughly after adding the Sequencing PPT Buffer and ethanol	Repeat the sequencing reactions. Vortex thoroughly (minimum of 60 seconds) after adding ethanol. Be sure all reactions are mixing.
	Precipitate is present in PPT buffer	Vortex until particles are dissolved and buffer is clear.
Random sequence failures	Poor sequencing reaction due to error in pipetting	Be sure that the ExoSAP-IT® reagent-treated PCR product and the correct sequencing mix are added and combined.
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%

Observation	Possible Causes	Recommended Action
Excessive background (baseline noise)	ExoSAP-IT [®] reagent was not added to the amplicons prior to sequencing	Add ExoSAP-IT [®] reagent to the PCR product.
	ExoSAP-IT [®] reagent was not thoroughly combined with the PCR product <i>or</i> ExoSAP-IT [®] reagent was over-vortexed	Be sure to vortex thoroughly after adding ExoSAP-IT [®] reagent.
	ExoSAP-IT [®] reagent was over-vortexed	Be sure to vortex briefly, but thoroughly after adding ExoSAP-IT [®] reagent.
	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 [®] reagent-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.
	Insufficient or 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.
Excessive Dye Blobs	PPT Buffer was not added to the sequencing reactions prior to adding ethanol	Repeat the sequencing reaction. Add the sequencing PPT buffer before adding ethanol.
	Failure to 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	<ul style="list-style-type: none"> • Be sure that both the ExoSAP-IT[®] reagent-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.
	Failure to 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 and confirm that 80% ethanol is being used for the wash step.

Appendix A: Limitations and Cautions

Precautions

- Before implementing the SeCore™ HLA Sequencing Kit in your laboratory, perform quality assurance and quality control for sequencing based typing methods using known molecularly typed samples. Such samples can be obtained from the International Workshop Reference Cell Panel and the UCLA DNA Reference Panel.
- Perform DNA extraction according to DNA extraction kit manufacturer instructions. Phenol, ethanol, and SDS may cause inhibition of the PCR reaction.
- Every attempt has been made to validate all amplification primers used in SeCore™ HLA Sequencing Kits with DNA samples typed by molecular methods. Due to the limited access to such reference materials, some rare alleles may not have been tested with these kits.
- A weak band larger than the 300-bp band may be seen for some DRB1 Locus Single Amplification Kit amplification products. The sequencing result is not affected by the presence of the doublet.
- The possible ambiguities of all loci can be found at www.ebi.ac.uk/imgt/hla/ambig.html.
- You must perform HLA typing using the SeCore™ HLA Sequencing kits in the presence of an HLA Lab Director, Technical Supervisor and/or general Supervisor following accepted laboratory accreditation standards (ASHI and EFI). We must emphasize that these products are for professional use only.
- We recommend that you verify all homozygous results by another method. Do not use the SeCore™ HLA Sequencing Kit results as the sole basis for making clinical decisions.
- The A locus Exon 4-Reverse sequencing primer contains a mismatch towards A*32 and A*74 samples. Additionally, this mismatch applies to the A*02:65 allele. Samples containing these alleles may exhibit poor heterozygous peak balance in the Exon 4-Reverse sequence data. The Exon 4-Forward sequence data will be unaffected.
- In rare cases, alleles with unknown variants or newly-defined sequence at amplification primer binding sites may affect amplification efficiency and result in incorrect typing results.

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 biohazardous materials may require special handling, and disposal limitations may apply.
-

Biological hazard safety

WARNING! – 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, Bloodborne 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 facilities
- 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

Limited Product Warranty

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

Symbols and Explanation

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
 <i>ISO 7000 Reg No. 3082</i>	In Vitro Diagnostic Medical Device
 <i>ISO 7000 Reg No. 3082</i>	Manufacturer
 <i>ISO 7000 Reg No. 0518</i>	Contains sufficient for <n> tests
 <i>ISO 7000 Reg No. 1641</i>	Consult Instructions for Use
 <i>ISO 7000 Reg No. 0632</i>	Temperature Limitation (Range)
 <i>ISO 7000 Reg No 2607</i>	Use By
 <i>ISO 7000 Reg No. 2493</i>	Catalog Number
 <i>ISO 7000 Reg No 2492</i>	Batch Code
 <i>ISO 7000 Reg No 2497</i>	Date of Manufacture
	Authorized Representative in the European Community
 <i>ISO 7000 Reg No. 0434A</i>	Caution See Instructions for Use

Batch field on the label is for traceability of manufacturing event

REF

22000D	SeCore™ A Locus Sequencing Kit *
22110D	SeCore™ B Locus Sequencing Kit, Single Amplification *
22200D	SeCore™ C Locus Sequencing Kit
22300D	SeCore™ DRB1 Locus Sequencing Kit *
A15573	SeCore™ DRB1 Locus Exon 2 & 3 Sequencing Kit *
22410D	SeCore™ DQB1 Locus Sequencing Kit
22510D	SeCore™ DPB1 Locus Sequencing Kit
5300025	SeCore™ A Locus Sequencing Kit *
5311025D	SeCore™ B Locus Sequencing Kit, Single Amplification System *
5320025	SeCore™ C Locus Sequencing Kit
5330025	SeCore™ DRB1 Locus Sequencing Kit *
A15571	SeCore™ DRB1 Locus Exon 2 & 3 Sequencing Kit *
5331025	SeCore™ DRB1, DRB3, DRB4, DRB5 Locus Sequencing Kit Group Specific Set *
5341025D	SeCore™ DQB1 Locus 2AMP
5351025	SeCore™ DPB1 Locus Sequencing Kit



*0197 Applies to Annex II List B products only



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