SeCore™ SBT

Sequence Based Typing

Key Benefits

SeCore HLA typing kits combine the accuracy of bidirectional sequencing and the power and flexibility of our improved sequence analysis software to give you high resolution results.

- Bidirectional sequencing of Class I and Class II HLA alleles
- Identical cycling profiles for all loci reduces sample handling errors and improves efficiency
- Shorter amplification and sequencing time (90 minutes each) mean that the entire sequencing process can be completed in a single day
- Enhancements to uTYPE™ HLA Sequence Analysis Software for more efficient analysis, interpretation and reporting of results, including the option of displaying P and G groups[1] on the analysis screen and reports.
- Robust allele identification of all loci and balanced allele peaks for heterozygous identification
- A comprehensive set of Group Specific Sequencing Primers (GSSPs) for resolving ambiguities

[1]Feature available in uTYPE RUO and CE-IVD only.

Overview

SeCore SBT uses the Sanger sequencing method to sequence HLA Class I and Class II (A, B, C, DRB1, DRB3,4,5, DQB1 and DPB1) alleles from genomic DNA. High resolution, locus specific sequencing kits provide reagents for amplification, purification, and sequencing of HLA alleles. Denatured sequencing fragments are processed by capillary electrophoresis on a genetic analyzer. Output files generated by the genetic analyzer are imported into *uTYPE™ HLA Sequence Analysis Software*, which analyzes the output data against data from the IMGT/HLA database to determine molecular typing.

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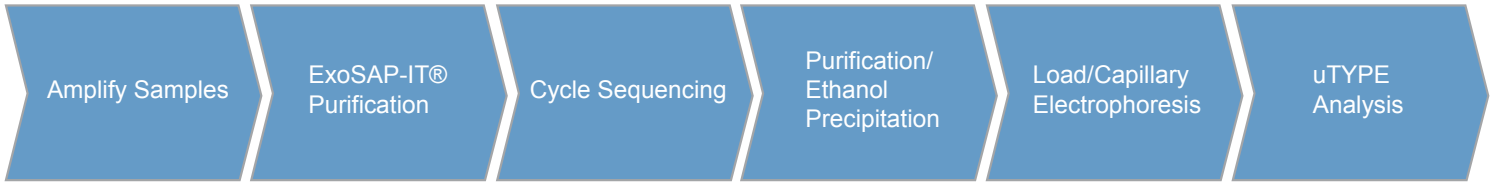
A Thermo Fisher Scientific Brand

SeCore SBT Principle

SeCore HLA Typing Kits offer bidirectional sequencing of Class I and Class II HLA alleles.



1. HLA locus specific PCR amplification is completed in 90 minutes using a master mix containing amplification mix, FastStart™ Taq DNA Polymerase, and sample genomic DNA. Exon coverage is detailed in Table 1, below.
2. Amplified product is cleaned-up with ExoSAP-IT® to eliminate unincorporated primers and dNTPs.
3. Cycle sequencing is achieved in 90 minutes using the BigDye Terminator v1.1 Cycle Sequencing Kit.
4. Sequencing fragments are purified by ethanol precipitation and denatured using Hi-Di™.
5. Denatured product is detected by capillary electrophoresis on a genetic analyzer.
6. Results are output from the genetic analyzer in the form of .ab1 files: uTYPE HLA Sequence Software is used to analyze the output against the IMGT/HLA database to determine molecular typing.

Table 1. SeCore SBT Exon Coverage

Locus	Exon Coverage	Amplifications	Tests
A	Bidirectional Sequencing of Exons 1,2,3,4,5	1	25 and 500
B	Bidirectional Sequencing of Exons 1*,2,3,4,5	1	25 and 500
C	Bidirectional Sequencing of Exons 1,2,3,4,5,6,7	1**	25 and 500
DRB1	Bidirectional Sequencing of Exon 2; and Codon 86	1	25 and 500
DRB1	Bidirectional Sequencing of Exons 2,3; and Codon 86	2	25 and 500
DRB1, DRB3,4,5 Group Specific Kit	Bidirectional Sequencing of Exon 2; and Codon 86	1	25
DQB1	Bidirectional Sequencing of Exons 2,3	2	25 and 500
DPB1	Bidirectional Sequencing of Exons 2,3,4; and Codons 8 and 85	2	25 and 500

*SeCore B Locus contains unidirectional sequencing primer for Exon 1 Forward (1F) only.
**May require a second amplification reaction if all 14 bi-directional sequencing primers are used.

Table 2. SeCore SBT Ordering Information (For In Vitro Diagnostic Use) CE 0197		
Description	**25 Test**	**500 Test**
SeCore A Locus	5300025	22000D
SeCore B Locus	5311025D	22110D
SeCore C Locus	5320025	22200D
SeCore DRB1 (Exon 2) Locus	5330025	22300D
SeCore DRB1 (Exon 2 &3) Locus	A15571	A15573
DRB1, DRB3,4,5 Group Specific Kit	5331025	
SeCore DQB1 Locus	5341025D	22410D
SeCore Locus DPB1	5351025	22510D

Table 3. SeCore SBT Ordering Information (For Research Use Only-not for use in diagnostic procedures)		
Description	**25 Test**	**500 Test**
SeCore A Locus	5300925	22009D
SeCore B Locus	5311925D	22119D
SeCore C Locus	5320925	22209D
SeCore DRB1 (Exon 2) Locus	5330925	22309D
SeCore DRB1 (Exon 2 &3) Locus	A15572	A15574
DRB1, DRB3,4,5 Group Specific Kit	5331925	
SeCore DQB1 Locus	5341925D	22419D
SeCore Locus DPB1	5351925	22519D

SeCore GSSP

The SeCore Group Specific Sequencing Primer (GSSP) kits target a DNA haplotype allowing the user to resolve cis/trans (phase) ambiguous allele pairs. Cis/trans ambiguous allele pairs arise from standard Sanger sequence based typing. The GSSP binds to only one of the two alleles present in the DNA sample aiding in the determination of the final HLA typing

Table 4. SeCore GSSP Ordering Information[1] (For In Vitro Diagnostic Use) CE	
Description	**IVD**
SeCore GSSP	A11256

Table 5. SeCore GSSP Ordering Information[1] (For Research Use Only-not for use in diagnostic procedures)	
Description	**RUO**
SeCore GSSP	A11255

[1]When ordering SeCore GSSPs, place the order with the appropriate Catalog ID listed above, and in the comments section of the order form, list the Z code corresponding with the GSSPs to be ordered. For a complete list of GSSP Z codes please reference the *SeCore GSSP Reference Table* at www.onelambda.com.

SeCore Express SBT

Express Kits are designed for HLA laboratories handling a minimum batch size of 96 samples of a single locus. Kits are available for HLA A[2], B[2], C[2], DRB1[3], and DQB1 Loci.

Table 6. SeCore Express SBT Ordering Information (For In Vitro Diagnostic Use) CE 0197

Description	1536 Test
SeCore Locus A, single amplification system[2]	A11310
SeCore Locus B, single amplification system[2]	A11311
SeCore Locus C, single amplification system[2]	A11312
SeCore Locus DRB1, single amplification system[3]	A11313
SeCore Locus DQB1, single amplification system	A11528

Table 7. SeCore Express SBT Ordering Information (For Research Use Only-not for use in diagnostic procedures)

Description	1536 Test
SeCore Locus A, single amplification system[2]	A11305
SeCore Locus B, single amplification system[2]	A11306
SeCore Locus C, single amplification system[2]	A11307
SeCore Locus DRB1, single amplification system[3]	A11308
SeCore Locus DQB1, single amplification system	A11527

Please Note: Express kits are designed for a minimum batch size of 96 samples.

[2]Only Exon 2 + 3 amplification and sequencing primers are available for the A,B, and C Express Kits.
[3]Only exon 2 amplification and sequencing primers are available for the DRB1 Express Kits.

Table 8. SeCore Workflow Timing[4]

Step	Hands-On Time	Hands-Off Time	Total Assay Time
Amplify DNA	15 minutes	90 minutes	105 minutes
ExoSAP-IT PCR Purification	5 minutes	40 minutes	45 minutes
Sequencing Reaction	20 minutes	60 minutes	80 minutes
Sequencing Purification	10 minutes	40 minutes	50 minutes
Capillary Electrophoresis ♦	10 minutes	♦	10 minutes + ♦
Total Time	60 minutes	230 minutes + ♦	290 minutes + ♦ (~4 hours 50 minutes)

[4]Estimated typical workflow based on time required to process 96 reactions
♦An additional 50 to 450 minutes should be added for Capillary Electrophoresis "Hands-off Time" depending on model of AB Genetic Analyzer used. See Table 9 below.

Table 9. Capillary Electrophoresis[5] "Hands-Off Time" by Instrument ♦

	3130xl (16 capillary)	3500xL Dx (24 capillary)	3730xl (96 capillary)
Time Per Injection	~50 minutes	~60 minutes	~50 minutes
Number of Injections Per 96 Reactions	6 injections	4 injections	1 injections
Total Time ♦	**300 minutes** (5 hours)	**240 minutes** (4 hours)	**50 minutes**

[5]Capillary Electrophoresis is based on POP-6™ polymer

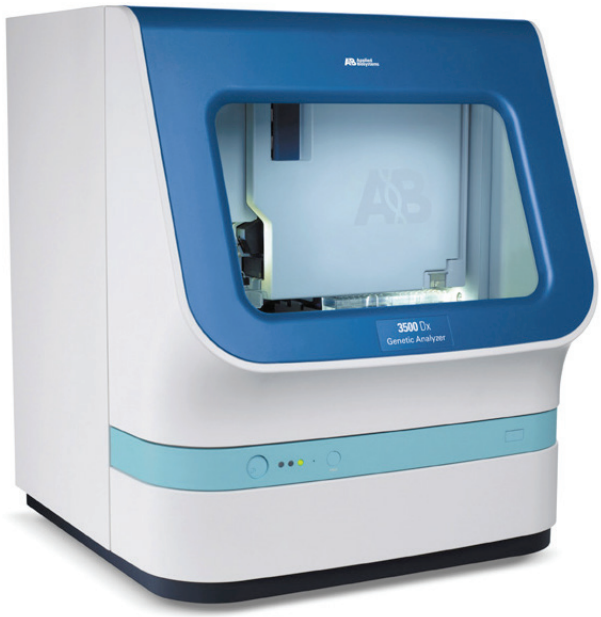
Instrumentation Required for the SeCore Dx Workflow (IVD)

Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Available from Thermo Fisher Scientific
Catalog ID: 4452300

The Veriti Dx 96-Well Thermal Cycler delivers the proven reliability you expect for In Vitro Diagnostic Use.

- Classified as US FDA Class I Medical Device for In Vitro Diagnostic Use
- Conforms to IVDD (98/79/EC) requirements and labeled in Europe with CE-IVD mark
- Manufactured to ISO 13485 and GMP requirements
- Features an easy-to-use graphical interface (6.5 inch VGA touch screen)
- Innovative VeriFlex™ Blocks provide convenient PCR optimization
- Standard and fast-enabled cycling to address your current and future PCR needs
- Reduced PCR reaction time when using faster ramp rates



3500xL Dx Genetic Analyzer CS2, 24 Capillary

Available from Thermo Fisher Scientific
Catalog ID: 4461447

The 3500xL Dx Genetic Analyzer CS2 is an In Vitro Diagnostic Use-labeled instrument, making it the first Sanger sequencing platform cleared for sequencing analysis of DNA.

- Reduced instrument hands-on time with simplified installation and removal of consumables and accessories
- Controlled and efficient run setup with intuitive user interface, preconfigured plate templates, and software-controlled functions (plate setup, data collection, and analysis)
- Maintenance calendar with programmable prompts and service history
- Security and electronic signature, RFID (radio frequency identification) tracking of consumables, built-in quality controls, and real-time data quality evaluation

Find out more at **onelambda.com**

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