

510(k) Summary

1. General Information 21 CFR 807.92(a)(1)

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2. Name of Device and Classification – 21 CFR 807.92(a)(2)

Name: SeCore[®] HLA Sequencing System and uTYPE[®] Dx v1.0 HLA
Sequence Analysis Software

Classification: Unclassified

Product Code: MZI, Test, Qualitative, for HLA, Non-Diagnostic

3. Predicate Device – 21 CFR 807.92(a)(3)

Manufacturer	Product Name	510(k) No.
Life Technologies Corporation (previously known as Invitrogen Corporation)	SSP UniTray [®] HLA System	BK000019 BK020068
Life Technologies Corporation (previously known as Invitrogen Corporation)	UniMatch [®] Plus Software	BK030003 BK070046

4. Device Description – 21 CFR 807.92(a)(4)

The SeCore[®] HLA Sequencing System uses the sequence based typing method (SBT) to provide DNA sequencing for typing of HLA Class I (A, B, C) and Class II (DP, DQ, DR) Loci using genomic DNA. This technology uses polymerase chain reaction (PCR), where denatured single strand DNA is hybridized to oligonucleotide primers. In an automated procedure, new strands of DNA are synthesized from the end of the primer by heat-resistant enzyme *Taq* polymerase from a pool of deoxyribonucleotide triphosphates (dNTP's).

The effectiveness of the PCR process is checked by passing a portion of the reaction through an agarose gel in order to separate the reaction products. The success of the PCR reaction can be gauged by the presence or absence of bands of appropriate size when the gel is visualized under UV light. The remainder of the reaction is treated with an enzyme cocktail consisting of exonuclease I and shrimp alkaline phosphatase, which degrades unincorporated primers and dephosphorylates unused deoxyribonucleotide triphosphates (dNTPs). BigDye[®] Terminator Sanger Sequencing Chemistry is then used to generate DNA sequence data. It includes dye terminators, which are dye-labeled dNTPs that carry two dyes, one a donor, the other an acceptor. The donor dye efficiently collects laser light and transfers it to one of four different acceptor dyes. The resulting labeled DNA fragments are separated by capillary electrophoresis using the 3500 Dx / 3500xL Dx Genetic Analyzer CS2 and are scanned, producing a primary sequencing data sample file (.ab1), which is used by the uTYPE[®] Dx v1.0 HLA Sequence Analysis Software, to match the sequencing results to known HLA allele sequences.

Each locus specific SeCore[®] HLA Sequencing System Kit includes:

- Pre-PCR reagents:
 - PCR Amplification mix including locus specific amplification primers
 - FastStart[®] Taq DNA polymerase enzyme for PCR amplification
- Post-PCR reagents:
 - ExoSAP-IT[®] reagent
 - Sequencing Mix (containing locus specific sequencing primers, dye terminators, and sequencing enzyme)
 - Precipitation (PPT) buffer

5. Intended Use/Indications for Use – 21 CFR 807.92(a)(5)

The SeCore[®] HLA Sequencing System is intended for the identification and definition of Class I and II Human Leukocyte Antigens (HLA) and are for use in HLA typing. The SeCore[®] HLA Sequencing System provides human histocompatibility information of HLA Class I (A, B, and C) and Class II (DPB1, DQB1 and DR) loci using genomic DNA isolated from whole blood specimens.

HLA typing using the SeCore[®] HLA Sequencing Kits must be performed in the presence of an HLA Lab Director, Technical Supervisor and/or general Supervisor following accepted laboratory accreditation standards (ASHI). These products are for professional use only. This test must not be used as the sole basis for making clinical decisions.

The uTYPE[®] Dx v1.0 HLA Sequence Analysis Software is intended to interpret and match sequencing data generated on the Applied Biosystems 3500 Dx /3500 xL Dx Genetic Analyzer CS2 with the 3500 Dx Series Data Collection Software using FDA-cleared HLA sequencing assay kits to known HLA type sequences.

6. Performance Data – 21 CFR 807.92(b) and Non-Clinical Test Summaries – 21 CFR 807.92(b)(1):

Precision:

Site to Site reproducibility

Site-to-site reproducibility testing of the SeCore[®] HLA Sequencing System was performed at three (3) external sites using the 3500xL Dx Genetic Analyzer CS2 instrument. Testing was performed using one (1) lot of each SeCore[®] Sequencing Kit and representative GSSP Kits, using four (4) previously characterized DNA samples tested in triplicate for a total of twenty-eight (28) samples. Two (2) operators at each site tested the samples six (6) times over six (6) non-consecutive days using one 24-capillary instrument.

The total number of genotyping events for the 3 external sites combined was 432 for loci A, B, C, DRB1, DQB1 and DPB1 and 1080 events for locus DR group. The genotyping results were all concordant with the known genotype (100%) and the ambiguous results for each sample were shown to be reproducible, indicating that the same ambiguous results are obtained per site, operator, run, and replicate for each sample¹.

Lot-to-lot reproducibility

Two studies were conducted for lot-to-lot reproducibility of the SeCore[®] HLA Sequencing Kit and the 3500xL Dx Genetic Analyzer CS2 instrument consumables:

- Study 1 tested 3 SeCore[®] Sequencing Kit lots with 1 instrument consumable lot.
- Study 2 tested 3 instrument consumables lots and 1 SeCore[®] Sequencing Kit lot.

¹ Genotyping was considered concordant if at least one pair of alleles matched between the SeCore[®] Sequencing Kit result and the known DNA genotype.

For each study, five (5) replicates each of three (3) previously characterized DNA samples were tested over five (5) non-consecutive days using one 24-cap instrument for the B and DRB1 locus. The total number of genotyping events for each study was 225 for locus B and locus DRB1.

The genotyping results were all concordant with the known genotype (100%) and the ambiguous results for each sample are reproducible, meaning that the same ambiguous results are obtained per lot, run, and replicate for each sample².

Based on the analytical performance data, it is recommended that users use the following as system acceptance criteria:

- SeCore kit signal intensity ≥ 300 RFU
- GSSP kit signal intensity ≥ 100 RFU
- Both kits: Noise-to-signal intensity $\leq 8\%$

Linearity/assay reportable range

Not applicable, this assay is not quantitative.

Stability and Shipping Studies

SeCore[®] HLA Sequencing kits are stable through the labeled expiry date of 18 months when stored at -20°C. The SeCore[®] HLA Group Specific Sequencing Primer kits are stable through the labeled expiry date of 30 months when stored at -20°C. Testing has been verified through accelerated, real time, open pack, and transport stability studies in accordance with EN13640:2002.

Detection Limit:

The SeCore[®] HLA Sequencing System has been validated for use with 15-30ng/uL of genomic DNA collected from whole blood.

Interference Testing

² Genotyping was considered concordant if at least one pair of alleles matched between the SeCore[®] Sequencing Kit result and the known DNA genotype.

An evaluation was performed using the substances in Table 1 in accordance with CLSI document EP7-A23 using one lot of representative Class I and Class II SeCore[®] Sequencing and GSSP kits. As part of the study, the interfering substance was added directly to the purified DNA.

Table 1. Concentration of substances where assay inhibition was evident

Substance	Highest concentration without inhibition
SDS (w/v)	0.0050%
100% EtOH	200 mmol/L
Phenol (v/v)	0.0125%
Sucrose	100,000 µmol/L
EDTA	100 µmol/L
ACD (w/v)	0.1%
500x Cholesterol	50x
Bilirubin, conj.	10.7 µmol/L
Hemoglobin	0.0156 g/L
Hemolyzed Blood (w/v)	0.001%

Design Verification Studies

The following assay verification studies confirm the specifications as outlined in the instructions for use and as utilized in the manufacturing of the SeCore[®] HLA Sequencing System:

- Reagent guardbanding
- Amplification and Sequencing Primer concentration
- Group Specific Sequencing Primer (GSSP) concentration
- Thermal cycling parameters: Amplification, purification, cycle sequencing.
- Ethanol purification time and centrifugation speed
- Sequencer Parameters

7. Clinical Study Summary – 21 CFR 807.92(b)(2)

Clinical Studies

An evaluation of the performance of the Life Technologies SeCore[®], uTYPE[®] Dx and 3500xL Dx System, comprising SeCore[®] Sequencing Kits tested using the 3500 Dx / 3500xL Dx Genetic Analyzer CS2 and 3500 Dx Series Data Collection Software with uTYPE[®] Dx HLA Sequence Analysis Software, compared to the predicate device SSP UniTray[®] with UniMatch[®] Plus Interpretation Software, was conducted at three sites in the United States.

The study objective was to determine if the concordance rate was at least 0.95. The corresponding statistical hypotheses for each locus of the 5 loci are $H_0: r \leq 0.95$ and $H_a: r > 0.95$. A one-sided type I error rate of $\alpha = 0.05$ and a statistical power of 80% at a true concordance rate of 0.98 was used for the calculated sample size. Based on Fisher's exact test, a sample size of 260 (split between the three sites) was needed. While a sample size of 260 was calculated to ensure at least 80% power, the total sample size was increased to 299 (total across all sites). This was to ensure adequate power (approximately 85%) for the statistical analyses.

From three testing sites, 299 samples were tested to evaluate the equivalence between the SeCore® Sequencing Analysis System with uTYPE® Dx v1.0 HLA Sequence Analysis Software and the predicate device, SSP UniTray® with UniMatch® Plus interpretation software. The concordance rates for Class I and Class II loci (A, B, C, DRB1, DRB3/4/5, DQB1, DPB1) range between 97.3% and 100%, and the one-sided 95% lower confidence limits for the concordance rates all exceed 95%³.

³ A SeCore® Sequencing Kit result was considered concordant with a uTYPE® Dx Software result if at least one pair of alleles matched between the results.