LIFECODES HLA Null Allele SSO TYPING KIT
For Research Use Only. Not for use in Diagnostic Procedures

TABLE OF CONTENTS

Definition of Symbols................................. 1
Reagents by Catalog Number....................... 2
Summary and Explanation........................... 2
Principles of the Procedure......................... 2
Reagents............................................. 3
  A. Identification................................ 3
  B. Warnings and Cautions....................... 3
  C. Storage Instructions......................... 3
  D. Purification or Treatment for Use......... 3
  E. Instability Indications..................... 3
Instrument Requirements.......................... 3
Specimen Collection and Preparation............. 3
Procedure........................................ 4
  A. Materials Provided........................ 4
  B. Materials required, but not Provided.... 4
  C. Additional materials to be provided by the
     user ......................................... 4
Directions for Use................................ 4
  A. Purify Genomic DNA......................... 4
  B. Amplification................................ 4
  C. Hybridization............................... 5
  D. Analyze sample using the Luminex
     Instrument................................. 6
Results............................................ 6
Quality Control.................................... 7
Limitation of the Procedure....................... 7
Troubleshooting.................................. 8
Expected Values.................................. 8
References........................................ 8
Limited License................................... 8
Trademarks Used.................................. 8
Appendix A....................................... 9
  Gel Electrophoresis............................ 9
  Gel Interpretation............................. 9

DEFINITION OF SYMBOLS (Product Labels and Supplemental Documents)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch Code</td>
<td>LOC</td>
</tr>
<tr>
<td>Catalog Number</td>
<td>REF</td>
</tr>
<tr>
<td>Temperature limitations</td>
<td>°C</td>
</tr>
<tr>
<td>Upper limit of temperature</td>
<td>°C</td>
</tr>
<tr>
<td>Use By Date</td>
<td></td>
</tr>
<tr>
<td>Keep away from light</td>
<td></td>
</tr>
<tr>
<td>Sufficient for N Tests</td>
<td></td>
</tr>
<tr>
<td>Do Not Freeze</td>
<td></td>
</tr>
<tr>
<td>Caution – See instructions for Use</td>
<td></td>
</tr>
<tr>
<td>Consult instructions for use</td>
<td></td>
</tr>
<tr>
<td>Manufacturer</td>
<td></td>
</tr>
<tr>
<td>Danger</td>
<td></td>
</tr>
</tbody>
</table>
**SOMMAIRE ET EXPLICATION**

La typologie HLA basée sur la PCR est une procédure courante en laboratoire. La PCR amplifie le DNA et est utilisé comme moyen d’enrichir pour une région choisie du DNA. Pour le procédé HLA, un sous-ensemble de LA est utilisé pour déterminer les propriétés de l’amplifié DNA. Différents types d’assays, tels que SSP (1), direct SSOP (2), RFLP (3) et reverse SSOP dot blot techniques (4), ont été utilisés en typologie HLA. Comme SSOP et reverse dot blot methods, LIFECODES HLA-SSO Typing kits utilise sequence-specific oligonucleotides (SSOs) pour identifier les HLA alleles présents dans un PCR amplifié sample. Il est le set de SSOs employé, non les méthodes qui déterminent la capacité à distinguer les alleles variés. L’instrument Luminex qui permet de distinguer entre des populations de microsphères. Donc, un mélange de plusieurs SSOs peut être analysées par le Luminex Instrument. Up to 100 different populations of Luminex Microspheres can be mixed together and analyzed by the Luminex Instrument because each population of microspheres can be distinguished by its unique fluorescence signature or color. A different SSO probe can be attached to each color microsphere. Therefore, a mixture of several probes can be distinguished from each other by virtue of their association with particular color microspheres. The Luminex Instrument is also able to quantify the relative amounts of labeled PCR product hybridizing to each Luminex Microsphere. Therefore, the relative signal obtained with the SSO probes in the LIFECODES assay, as with other SSOP methods, can be used to assign the probes as having positive or negative reactivity with the amplified DNA sample (see Results section). This in turn provides the information needed to determine the HLA phenotype of the sample.
Null 1 is used as a complementary assay to increase the resolution of LIFECODES HLA-A, HLA-B, HLA-C assays, solving the allelic ambiguity between A*24:02/24:09N, B*51:01/51:11N, and C*04:01/04:09N. Null 2 is used as a complementary assay to increase the resolution of LIFECODES HLA-DRB3,4,5 assay, solving the allelic ambiguity between DRB4*01:03/DRB4*01:03:01:02N, and DRB5*01:02/DRB5*01:08N.

Merging of the information from the Null 1 or 2 assay with the corresponding locus-specific LIFECODES SSO Typing kit assay results to generate a suggested typing of a sample can be conducted through manual analysis or software analysis. For manual analysis, the output of the Null 1 or 2 assays identifies the presence or absence of specific null alleles. The result from the corresponding locus-specific LIFECODES SSO typing kits may carry ambiguities involving these null alleles (e.g., A*24:02/24:09N, B*51:01/51:11N, C*04:01/04:09N, DRB4*01:03/DRB4*01:03:01:02N, or DRB5*01:02/DRB5*01:08N). If the null allele is identified with the Null product, all allele combinations that do not include the null allele can be eliminated from the results obtained from the corresponding locus-specific LIFECODES SSO typing kit. If the null allele is absent based on the Null product result, all allele combinations that include the Null allele can be eliminated from the results obtained from the corresponding locus-specific LIFECODES SSO typing kit. In the product insert and in the software, this process of combining the results of the two products is referred to as merging the results.

**REAGENTS**

A. **Identification**
   
   See tables in Reagents by Catalog Number section for complete listing of catalog numbers.

B. **Warnings or Cautions**
   
   1. For Research use only. Not for use in diagnostic procedures.
   2. Separate pipettes should be designated for Pre-PCR manipulations as well as for Post-PCR manipulations.
   3. **Biohazard:** All biological and blood samples should be treated as potentially infectious. Use Universal Precautions when handling.
   4. Dilution Solution, Probe mixes, TAA Polymerase and R-Phycoerythrin Conjugated Streptavidin contain hazardous compounds. Avoid contact with skin and eyes and dispose of all materials after use according to local regulations. See Material Safety Data Sheets for additional information.

C. **Storage Instructions**
   
   1. Refer to kit component packaging label for proper storage temperatures.
   2. Probe mixes and R-Phycoerythrin Conjugated Streptavidin are light sensitive, **KEEP FROM LIGHT; DO NOT FREEZE.**
   3. Do not use components past their expiration date.

D. **Purification or Treatment Required for Use**
   
   See “Specimen Collection and Preparation.”

E. **Instability Indications**
   
   1. If salts have precipitated out of solution during shipping or storage, resolubilize completely prior to use by vortexing at room temperature (18 to 30°C).
   2. Do not use R-Phycoerythrin Conjugated Streptavidin that has been frozen during shipment or storage.

**INSTRUMENT REQUIREMENTS**

1. Luminex Instrument and XY Platform (Product Number 888300, 888302)

The following Thermal Cyclers are recommended: 96-Well GeneAmp® PCR System 9700 set to MAX mode (Base Cat # N8050200, Gold Block Cat #4314878) , Veriti™ 96-Well Thermal Cycler set to 9700 MAX mode (Cat #4375786). Refer to Table 2 for maximum ramp speeds.

**SPECIMEN COLLECTION AND PREPARATION**

A. Human DNA can be purified from Whole blood, Buffy coats and Buccal swabs using a method that meets the criteria below. DNA extracted from blood preserved in EDTA and ACD (Acid Citrate Dextrose) have been tested and shown to perform in this assay.

B. DNA extracted from blood preserved in heparin cannot be used in this assay. Other preservatives have not been tested.

C. The isolated DNA should be in 10 mM TRIS, pH 8.0-9.0, or in nuclease free water. If a chelating agent such as EDTA is present the final concentration of the chelating agent should not exceed 0.5 mM.

D. The presence of alcohol, detergents or salts may adversely affect DNA amplification.

E. Final DNA concentration should be 10 to 200 ng/µL.

F. Absorbance measurements of the DNA sample at 260 and 280nm should give a ratio of 1.65 to 2.0.

G. DNA can be used immediately after isolation or stored at –20°C for up to 1 year. Repeated freeze/thawing should be avoided since this can result in DNA degradation.
**PROCEDURE**

A. **Materials Provided** (See tables in Reagents by Catalog Number section for specific information)
   - Appropriate Master Mix (MX)
   - Appropriate Probe Mix (BM)
   - Dilution Solution (DS)
   - Threshold Table(s), Probe Hit Chart(s)
   - LIFECODES Taq Polymerase, General Purpose Reagent for Laboratory Use (LIFECODES Cat. No 628076) 2 Vials

B. **Materials, Required, but Not Provided**
   - Luminex Sheath Fluid (1x  LIFECODES Cat. No. 628005)
   - Nuclease-free water (LIFECODES Cat. No. 757003; 20mL)
   - PCR tubes and caps - Corning® Thermowell Tube Strips (Costar® Cat. No. 6542, LIFECODES Cat. No. 888640) or Corning® Thermowell PCR 96 well plates (Cat. No. CLS6551) or Themoscientific AB Gene® Superplate 96-well PCR plate (Cat. No. AB-2100)
   - Costar® plate (Costar® Cat. No. 6509, LIFECODES Cat. No. 888630)
   - Thermowell Clear Polyethylene Tape (Costar® No. 6524)
   - R-Phycoerythrin Conjugated Streptavidin (SA-PE), 1mg/mL (LIFECODES Cat No. 628511)
   - Luminex Calibration Kits (Luminex 100/200 Calibration Kit, Luminex 100/200 Performance Verification Kit, LIFECODES Cat. Nos. 628018 and 628019 respectively)

C. **Additional materials to be provided by the user**
   - Vortex Mixer
   - Silicone compression Mat. Axygen Scientific #CM-FLAT or equivalent
   - Bath Sonicator
   - Microcentrifuge
   - Barrier filter tips
   - Pipettors, Multichannel pipettors and tips (1-20µL, 20-200µL, 1000µL)
   - Spreadsheet analysis software
   - Heat Block
   - 70% Isopropanol or 20% Bleach
   - Retainer tray – Applied Biosystems #403081 (for use with the 9700 thermal cycler only)

**DIRECTIONS FOR USE**

**NOTES:**
- Probe mixes and SA-PE are light sensitive: keep away from light and do not freeze.
- Warm beads at 55º - 60ºC for at least 5-10 minutes to thoroughly solubilize components in probe mixture.
- Sonicate briefly (~15 sec), then vortex probe mix for about 15 seconds to thoroughly suspend the beads.
- Take extreme caution in the aliquoting process, using calibrated pipettes. Failure to do so may result in reagent loss and sample failure.
- All temperatures must be precisely maintained. Fluctuations as little as +/- 0.5°C can affect results.
- At the hybridization stage, samples should not remain in the diluted state at 56°C for more than 5 minutes (see Results section). It is recommended to assay the amplified samples as soon as possible. If the samples cannot be run on the Luminex Instrument the same day, the amplified product can be stored up to 3 days at 2-8ºC prior to use. For longer storage, store at −20ºC up to one week until ready to assay. The amplified product can only be frozen and thawed once. Repeated freezing and thawing will result in degradation of amplified samples and will yield poor results if assayed.

A. Purify genomic DNA, using method of choice; final concentration should be 10 to 200 ng/µL. Adjust, if necessary, with nuclease free water. Keep all samples at similar concentrations.

B. DNA amplification (PCR)
   1. Allow the Master Mix to warm to room temperature (18 to 30°C).
   2. Gently vortex for approximately 10 seconds. This will ensure the salts are in solution. Spin briefly (5 – 10 seconds) in microcentrifuge to bring contents to the bottom of the tube.
   3. Using Table 1 below, prepare the components for amplification for n+1 reactions using the indicated amount of each component per reaction (except for DNA). Bring to a final volume of 20µL per reaction with nuclease free water. Gently vortex.
   4. Pipette the appropriate amount of Genomic DNA (40 to 120ng) into the PCR tubes.
   5. Aliquot the amplification mix into the PCR tubes containing the genomic DNA. (The total volume of amplification mix and genomic DNA should equal 20µL for each sample reaction.)
   6. Cap tubes tightly to prevent evaporation during PCR.
7. Place samples in the thermal cycler and run program, see Table 2 and 3.

**Table 1. Reaction Components for Amplification**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount per PCR sample reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIFECODES Master Mix</td>
<td>6µL</td>
</tr>
<tr>
<td>Genomic DNA 10-200ng/µL</td>
<td>Total of ~80ng</td>
</tr>
<tr>
<td>LIFECODES Taq Polymerase</td>
<td>0.2µL (1U)</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>To 20µL final volume</td>
</tr>
</tbody>
</table>

**Table 2. Thermal Cycler Conditions for Amplification**

<table>
<thead>
<tr>
<th>Thermal Cycler</th>
<th>Mode (Ramp speed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneAmp® PCR System 9700</td>
<td>MAX mode (3.9°C/sec)</td>
</tr>
<tr>
<td>Veriti™ 96-Well Thermal Cycler</td>
<td>9700 MAX mode (3.9°C/sec)</td>
</tr>
</tbody>
</table>

**Table 3. Thermal Cycler Conditions for Amplification**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature and Incubation Time</th>
<th># of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95° C for 3 min</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>95° C for 15 sec</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>60° C for 30 sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72° C for 30 sec</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>95° C for 10 sec</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>63° C for 30 sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72° C for 30 sec</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>72° C for 2 min</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>4° C forever</td>
<td>1</td>
</tr>
</tbody>
</table>

**Note:** To be sure of sample amplification, refer to Product Gel Electrophoresis (Appendix A).

C. Hybridization

- Be sure hybridization buffer components of the LIFECODES probe mix are solubilized and that the beads are thoroughly suspended.
- Turn on the Luminex Instrument and XY Platform to allow for 30 minute warm-up.
  1. Warm probe mix in a 55° to 60°C heat block for at least 5 to 10 minutes to thoroughly solubilize components in probe mixture.
  2. Sonicate briefly (~15 sec), then vortex probe mix for about 15 seconds to thoroughly suspend the beads.
  3. Combine 15 µL of the appropriate probe mix with 5 µL of locus specific PCR product into each well of a thermal cycler 96 well plate (Costar® No. 6509). When aliquoting probe mix to more than 10 wells, gently vortex probe mix after each set of ten. Seal plate with polyethylene tape (Costar® No. 6524).
  4. Place silicone compression mat on top of plate prior to hybridization.
  5. Hybridize samples under the following incubation conditions:

**Table 4. Thermal Cycler Conditions for Hybridization**

- 97°C for 2 minutes
- 47°C for 10 minutes
- 56°C for 8 minutes
- 56°C HOLD

- Ensure that the detection laser on the Luminex Instrument is turned on at least 30 minutes before the hybridization ends.

6. While the samples are hybridizing, prepare a 1:200 SA-PE mixture / Dilution Solution. Combine 0.85 µL 1mg/mL SA-PE and 170µL Dilution Solution (DS) per sample. It is recommended to make enough Dilution Solution Mixture for n+1 samples to account for pipetting loss. (See Table 5)
7. Keep Dilution Solution/ SA-PE mixture in the dark, at room temperature; SA-PE is light sensitive! The Dilution Solution may be warmed at 45ºC for 5 minutes and vortexed upon arrival to ensure all components are in solution. Dilution solution must be at room temperature (18 to 30ºC) before making the mixture. Prepare prior to use and discard any remaining portion.

Table 5. Dilution Solution Preparation Volumes

<table>
<thead>
<tr>
<th># of Samples</th>
<th>Dilution Solution (DS)</th>
<th>SA-PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>170µL</td>
<td>0.85µL</td>
</tr>
<tr>
<td>5</td>
<td>850µL</td>
<td>4.25µL</td>
</tr>
<tr>
<td>10</td>
<td>1700µL</td>
<td>8.5µL</td>
</tr>
<tr>
<td>20</td>
<td>3400µL</td>
<td>17µL</td>
</tr>
<tr>
<td>50</td>
<td>8500µL</td>
<td>42.5µL</td>
</tr>
</tbody>
</table>

Note: DO NOT CANCEL HYBRIDIZATION PROGRAM BEFORE REMOVING THE TRAY FROM THE THERMAL CYCLER!

8. At the 56ºC hold, while the tray is on the thermal cycler, dilute each sample with 170µL of the prepared Dilution Solution/ SA-PE mixture. It is critical to dilute all samples within 5 minutes (following the 8 minute 56ºC HOLD step).

9. Remove the sample tray from the thermal cycler and place in the Luminex Instrument.

D. Analyze sample using the Luminex Instrument*

For best results, assay the samples immediately using the Luminex Instrument.
1. Turn on the Luminex Instrument between 30 minutes and 4 hours before assaying the samples.
2. Prior to analyzing the samples on the Luminex Instrument, set up a Batch Run by which the samples will be analyzed.
   a) Select Create a New Batch from the File menu.
      - For example, if analyzing for Null Class I, add Batch for Null Class I
      - The Batch Template is provided on website and is named, in this case, Null1 xxxxxx (lot#).
      - Please note that the template versions are lot number specific and correspond to the probe mix lot numbers.
      - Follow the stepwise instructions that appear on the screen for creating batches.
      - When naming the batch, do not include commas in the name because information after a comma will be lost upon exportation of the data.
      - For further instructions on creating batches and multibatches, refer to the Luminex User’s Manual
   b) Click the eject icon to eject the plate holder. Place the 96 well thermal cycler plate containing the samples in the XYP heater block present on the plate holder.
   c) Click the Retract icon. The samples are now ready to be analyzed. A prime step should be performed before starting the run.
   d) After the samples have been run through the instrument, a sanitization step with 70% Isopropanol or 20% household bleach should be performed followed by two wash steps. The instrument can be turned off at this point if it is not going to be used for the remainder of the day.
3. After a batch is complete, the data is exported as a comma separated values (csv) file. These files are named ‘OUTPUT.CSV’ and saved in a folder with the Batch Name. This data is then available for making typing assignments as described below.

*Refer to Luminex User’s Manual for instrument operation, including daily startup, calibration, maintenance, and shutdown procedures.

RESULTS

Sample typing can be done as follows:
The generated CSV files can be opened and the data processed with common spreadsheet programs such as Microsoft Excel, Lotus 123, Corel Quattro Pro, or similar software. Analysis is comprised of the following steps:
1) Verify that the Number of Events for each SSO in each sample is at least 60. This information is found in the DataType: Count section of the CSV file.
2) Determine that the values for the Consensus probes for each sample are above their minimum Median Fluorescent Intensity or MFI. The minimum thresholds are lot specific and can be found in the Threshold Table.

Caution:
• To obtain reliable results, there must be sufficient data gathered by the Luminex Instrument.
• Collect at least 60 events for each SSO.

3) Subtract the Background Control value for each probe from the sample values producing the background corrected data set. Background Control values are found in the Threshold Table and are lot specific. Background values are average MFI values for each bead to compensate for background noise due to bead variation.
4) For each sample, divide the background-corrected data for each probe by the background-corrected value for the corresponding consensus probe producing the normalized data set.
5) For each probe, record the normalized value on the Threshold Table Worksheet.
6) Once all values have been assigned, the probe hit pattern (i.e., the combination of all positive and negative assignments for a given sample) can be compared with the Probe Hit Chart (LC1024) provided on website.

**Caution:**
- There is a separate threshold table for each locus.
- These threshold tables are Lot-specific; be certain that the Lot # on the threshold tables matches the Lot # in the typing kit.
- If a normalized value for a particular probe falls above the maximum threshold for a negative assignment and below the minimum value for a positive assignment, the sample should be considered as indeterminate for this probe. The sample should be typed, first assuming the value to be negative and then again assuming the value to be positive.
- See EXPECTED VALUES section for further information on threshold values.

**QUALITY CONTROL**
It is recommended that one negative and positive control be run with each test, such as a water blank and a previously typed sample respectively. Consensus SSO probes, listed on the Threshold Table, hybridize to their respective locus specific alleles. Values obtained with the Consensus SSOs from positive controls should exceed the threshold value for the SSO as set forth in the Threshold Table Worksheet.

The LIFECODES Probe Mix(es) contain one or more consensus SSO probes identified in the typing kit worksheets. These consensus probes hybridize to all alleles and act as internal controls to verify amplification and to confirm that hybridizations occurred. If the minimum value is not obtained for these SSOs, the sample may not produce the correct typing and the sample test should be repeated.

The assay should be run as recommended in the package insert as well as performed with any other quality control procedures that are in accordance with local, state, federal and/or accreditation agencies requirements.

**LIMITATIONS OF THE PROCEDURE**
The PCR conditions and assay conditions described require precisely controlled conditions. Deviations from these parameters may lead to product failure.

All instruments must be calibrated according to manufacturer’s recommendations and operated within manufacturer’s prescribed parameters.

1) Beads must be pre-warmed and well suspended prior to use. This ensures that the hybridization buffer components are in solution.
2) 47°C and 56°C incubations require a high degree of accuracy (+/- 0.5°C). A thermal cycler should be employed. Temperature should be verified, within wells of the 96 well thermal cycler plate, using a thermocouple (e.g., Bio-Rad, Model VPT-0300 or equivalent). The temperature within wells and among wells should not vary more than +/- 0.5°C.
3) Time at 56°C is critical and should not exceed a total of 13 minutes. This includes the 8-minute incubation plus no more than 5 minutes to dilute all the samples with Dilution Solution/ SA-PE mixture.
4) **Once diluted, sample analysis must be complete within 1 hour (protect from light).**
5) Do not mix components from other kits and lots.

Due to the complex nature of HLA typing, qualified personnel should review data interpretation and typing assignments.
TROUBLESHOOTING

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>POSSIBLE CAUSE</th>
<th>SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Bead Count</td>
<td>Probe Mix not well suspended</td>
<td>Prewarm, sonicate and vortex probe mix and repeat assay.</td>
</tr>
<tr>
<td></td>
<td>Instrument not functioning properly</td>
<td>Calibrate Instrument. (Refer to Luminex IS User’s manual.)</td>
</tr>
<tr>
<td></td>
<td>Sample flow path blocked</td>
<td>Remove and sonicate needle. Perform backflush. Call Immucor Transplant Diagnostics, Inc. if problem persists. (888) 329-0255</td>
</tr>
<tr>
<td>CON Threshold Failure</td>
<td>Sample failed to amplify or amplified poorly*</td>
<td>Check DNA concentration and purity.</td>
</tr>
<tr>
<td></td>
<td>Low DNA</td>
<td>Heat Master Mix at 37°C for 5 minutes, vortex gently and spin down briefly.</td>
</tr>
<tr>
<td></td>
<td>Salts in Master Mix are out of solution</td>
<td>Replace R-Phycoerythrin Conjugated Streptavidin.</td>
</tr>
<tr>
<td></td>
<td>Poor Taq Polymerase</td>
<td>Use LIFECODES Taq Polymerase (GPR) Catalog # 628076.</td>
</tr>
<tr>
<td></td>
<td>Amplification conditions not within specific parameters</td>
<td>Run Thermal profile on Thermal cycler to verify parameters are within specified parameters.</td>
</tr>
<tr>
<td></td>
<td>Low Median Fluorescent Intensity Value (MFI)</td>
<td>Warm dilution solution at 45°C for 5 minutes before use and vortex. Store at room temperature. Replace R-Phycoerythrin Conjugated Streptavidin.</td>
</tr>
<tr>
<td>Multiple SSO failures or sample fails to yield a HLA typing result</td>
<td>Allele specific amplification</td>
<td>Run Thermal profile on Thermal cycler to verify parameters are within specified parameters.</td>
</tr>
<tr>
<td></td>
<td>Amplification conditions not within specific parameters</td>
<td>Run Thermal profile on Thermal cycler to verify parameters are within specified parameters.</td>
</tr>
<tr>
<td></td>
<td>DNA sample contaminated</td>
<td>Re-isolate DNA from Blood sample.</td>
</tr>
<tr>
<td></td>
<td>DNA partially degraded</td>
<td>Re-isolate DNA from Blood sample.</td>
</tr>
<tr>
<td></td>
<td>Evaporation during hybridization step</td>
<td>If not using an entire plate, leave one row empty on each side of samples to be assayed to allow plate to be sealed tightly.</td>
</tr>
</tbody>
</table>

* PCR amplification can be verified by gel electrophoresis (See Appendix A).

EXPECTED VALUES

Each Locus has one CON probe and two SSO probes. If a sample contains one of the loci being assayed, the consensus probe and at least one of the SSO probes for that locus should be positive. Probe values typically can be resolved as positive and negative. In some rare instances, a value may fall between the positive and negative cutoff values and therefore is considered to be indeterminate. If a sample contains indeterminate values for a particular SSO probe, the sample should be typed with the probe as a negative and again with the probe as a positive. If two SSO probes for a locus are indeterminate, the sample cannot be typed and should be re-assayed.

- As noted in the Limitations of the Procedure section, it is critical to precisely follow the protocol. Any deviations can lead to sample typing failure.

REFERENCES


LIMITED LICENSE

Taq polymerase is manufactured for Immucor Transplant Diagnostics by Promega Corp. It is licensed to Promega under U.S. Patent Nos. 5,338,671 and 5,587,287 and their corresponding foreign patents. The purchase of this product includes a limited, non-transferable license under U.S. patent 5,981,180 or its foreign counterparts, owned by Luminex Corporation, to perform multiplex analysis of specimens for HLA typing.

Manufacturer: Immucor Transplant Diagnostics, Inc., 550 West Avenue, Stamford, CT 06902 USA.
Phone: 203-328-9500, 888-329-0255 Fax: 203-328-9599

European Technical Service: Phone: +32/3 385 4791

This document last revised and issued: Rev: 4; 25 July 2016

TRADEMARKS USED

AB Gene® AB Gene House
Costar® Corning Incorporated
Microseal™ Bio-Rad Laboratories, Inc.
IDNA™ Agarose Lonza Group, Ltd.
GelStar™ Lonza Group, Ltd.
Luminex® Gene Amp® Luminex Corporation
Lumino® Roche Molecular System
Veri™ Applied Biosystem
LIFECODES® Immucor Inc.
APPENDIX A

Gel Electrophoresis

The PCR reactions performed in the LIFECODES HLA-SSO Typing Kits are designed to produce both double and single stranded products, which are the predominant products that hybridizes to the SSOs. For quality assurance or to troubleshoot an experiment it might be necessary to perform gel electrophoresis to examine the PCR reaction for the presence of amplified DNA.

Materials Required (as listed or equivalent)

- Electrophoresis Grade Agarose (Lonza Group, Ltd. IDNA® Agarose No. 50170)
- Electrophoresis apparatus/power supply
- 1X Gel Buffer (40xTAE, Promega No. V4281)
- GelStar® Nucleic Acid Gel Stain (Lonza Group, Ltd. No. 50535)
- UV Transilluminator (ChromatoVUE, UVP Inc. Model TM36)
- Photographic imaging system

The relative migration of the single stranded product is dependent upon the gel concentration and buffer system employed. Approximate migrations for each amplification are listed below for samples run in a 2% Agarose gel in 1X TAE buffer.

Electrophoresis Conditions

1. Remove GelStar® Nucleic Acid Stain (Lonza Group, Ltd. No 50535) from freezer to thaw. Keep in dark.
2. The gel used for this procedure must be 2%, i.e. for a 200ml gel bed use 4 grams of agarose to 200mL 1X TAE (Dilute from 40X TAE). Add 10µL GelStar® Nucleic Acid Stain to the molten agarose. When pouring the gel be sure to leave ample room for DNA to run a significant distance (1 to 2 inches). USE CAUTION: GelStar® is a potential Carcinogen.

NOTE: It is possible to run gels with 20µL of 10mg/mL Ethidium Bromide in place of GelStar® Nucleic Acid Stain. Product band intensity will be less in gels containing Ethidium Bromide than in gels containing GelStar®. USE CAUTION: Ethidium Bromide is a known Carcinogen.

3. Keep gel in dark and allow to solidify.
4. Load a mixture of 2.5µL of each PCR product and 2.5µL 2X loading buffer with visible dye per sample, per amplification. Let gel run in the dark at approximately 160 volts for 45 minutes or until sample runs far enough to see separate bands for single and double stranded product (bromophenol blue band or other visible marker migrates 1 to 2 inches from wells).
5. Photograph using UV Transilluminator accompanied by a GelStar® Yellow Photographic Filter (Lonza Group, Ltd No 50536).

CAUTION: Wear protective equipment when handling GelStar® Nucleic Acid Stain or Ethidium Bromide and when photographing gel using UV Transilluminator.

6. Gel analysis

<table>
<thead>
<tr>
<th>Null Class1</th>
<th>Null Class2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double Strand(s) (bp)</td>
<td>~210, ~280</td>
</tr>
<tr>
<td>Single Strand(s) (bp)</td>
<td>~150,~180</td>
</tr>
</tbody>
</table>

Gel Interpretation

Amplification Non-amplification

Double Stranded DNA ---(Bright)
Single Stranded DNA ---(less bright)

Primer Band