# LABType™ SSO TYPING TESTS

REF

| Catalog ID | Product Name |
|---|---|
| RSSO1A* | LABType™ SSO Class I A Locus Typing Test |
| RSSO1B* | LABType™ SSO Class I B Locus Typing Test |
| RSSO1S4* | LABType™ SSO Class I Bw4 Supplement Typing Test |
| RSSO1S1* | LABType™ SSO Class I B7 Supplement Typing Test |
| RSSO1E47* | LABType™ SSO Class I Exon 4-7 Supplement Typing Test |
| RSSO1C | LABType™ SSO Class I C Locus Typing Test |
| RSSO2P | LABType™ SSO Class II DPA1/DPB1 Typing Test |
| RSSO2Q | LABType™ SSO Class II DQA1/DQB1 Typing Test |
| RSSO2B1* | LABType™ SSO Class II DRB1 Typing Test |
| RSSO2345* | LABType™ SSO Class II DRB3,4,5 Typing Test |
| RSSOH1A* | LABType™ HD Class I A Locus Typing Test |
| RSSOH1B* | LABType™ HD Class I B Locus Typing Test |
| RSSOH1C | LABType™ HD Class I C Locus Typing Test |
| RSSOH2B1* | LABType™ HD Class II DRB1 Typing Test |

| Catalog ID | Product Name |
|---|---|
| RSO1AT* | LABType™ SSO Class I A Locus Typing Test - 20 tests |
| RSO1BT* | LABType™ SSO Class I B Locus Typing Test - 20 tests |
| RSO1S4T* | LABType™ SSO Class I Bw4 Supplement Typing Test - 20 tests |
| RSO1S1T* | LABType™ SSO Class I B7 Supplement Typing Test - 20 tests |
| RSO1E47T* | LABType™ SSO Class I Exon 4-7 Supplement Typing Test - 20 tests |
| RSO1CT | LABType™ SSO Class I C Locus Typing Test - 20 tests |
| RSO2PT | LABType™ SSO Class II DPA1/DPB1 Typing Test - 20 tests |
| RSO2QT | LABType™ SSO Class II DQA1/DQB1 Typing Test - 20 tests |
| RSO2B1T* | LABType™ SSO Class II DRB1 Typing Test - 20 tests |
| RSO2345T* | LABType™ SSO Class II DRB3,4,5 Typing Test - 20 tests |
| RSOH1AT* | LABType™ HD Class I A Locus Typing Test - 20 tests |
| RSOH1BT* | LABType™ HD Class I B Locus Typing Test - 20 tests |
| RSOH1CT | LABType™ HD Class I C Locus Typing Test - 20 tests |
| RSOH2B1T* | LABType™ HD Class II DRB1 Typing Test - 20 tests |

IVD For In Vitro Diagnostic Use.

## INTENDED USE

DNA typing of HLA Class I or Class II alleles

## SUMMARY AND EXPLANATION

Historically, the established method for the determination of HLA antigens has been the lymphocytotoxicity test.[1] However, with the advent of PCR technologies, DNA based tissue typing techniques have become routine in the laboratory. For most DNA-based methodologies, the PCR process is used only as an amplification step to acquire the needed target DNA. The HLA typing process then requires a post-amplification step to discriminate between the different alleles (e.g., RFLP, SSOP, reverse dot blot). LABType™ SSO uses sequence-specific oligonucleotide probes (SSO) bound to fluorescently coded microspheres to identify alleles encoded by the sample DNA. The introduction of a step to amplify the target DNA by polymerase chain reaction (PCR), coupled with hybridization and detection in a single reaction mixture, makes this method suitable for both small and large-scale testing. In contrast to the lymphocytotoxicity reaction scale (1 = negative to 8 = positive), LABType™ test results are either positive or negative. This abolishes the need for complicated interpretation of results. In addition, single nucleotide changes can be discriminatory in PCR-SSO, while cross-reacting groups (CREGs) provide major challenges to serological typing.

## PRINCIPLES

LABType™ applies Luminex® technology to the reverse SSO DNA typing method. First, target DNA is PCR-amplified using a group-specific primer. The PCR product is biotinylated, which allows it to be detected using R-Phycoerythrin-conjugated Streptavidin (SAPE).

The PCR product is denatured and allowed to rehybridize to complementary DNA probes conjugated to fluorescently coded microspheres. A flow analyzer, either the LABScan™ 100 (Luminex® 100/200) or LABScan3D™ (Luminex® FLEXMAP 3D), identifies the fluorescent intensity of PE (phycoerythrin) on each microsphere. The assignment of the HLA typing is based on the reaction pattern compared to patterns associated with published HLA gene sequences.

## REAGENTS

### A. Identification

The LABType™ SSO DNA typing system provides sequence-specific oligonucleotide probes immobilized on microspheres for identification of HLA alleles in amplified genomic DNA samples through a controlled DNA-DNA hybridization reaction, followed by flow analysis using either the LABScan™ 100 or LABScan3D™ flow analyzer. The system components consist of:

- Pre-optimized and tested mixture of microspheres with probes covalently attached
- Hybridization reaction buffers to facilitate the binding of target DNA to the probe
- Wash Buffer to wash off unbound DNA
- SAPE buffer for diluting Stock SAPE solution
- DNA amplification reagents (pre-optimized HLA loci-specific primer mix): With every LABType product, the use of the locus-specific primer mix and bead mix is essential. These reagents are lot specific and are not interchangeable between lots.
- D-mix (specially formulated amplification buffer mix).

The microsphere mixture consists of a set of fluorescently labeled microspheres that bear unique sequence-specific oligonucleotide probes for HLA alleles. Each microsphere mixture includes negative and positive control microspheres for subtraction of non-specific background signals and normalization of raw data to adjust for possible variation in sample quantity and reaction efficiency. The microsphere mixtures are pre-optimized for particular PCR products obtained by DNA amplification using the specified HLA locus-specific primer mixes. The HLA locus-specific primer mixes are pre-optimized for amplification of specific HLA genes from 40 ng of purified genomic DNA in 20 µl volume when used in conjunction with D-mix, the prescribed amount of recombinant Taq polymerase, and the PCR reaction profile detailed below. For each lot, see provided worksheet for the specific HLA alleles that can be identified by each probe using the procedures described below. For lot specific probe sites, refer to the ***Bead Probe Information*** document.


### B. Warning or Caution

1. FDA Designation: IVD
2. **Warning**: Ethidium bromide, which is used for gel staining and which is not included with this product, is a known carcinogen. Handle with appropriate caution. Can be harmful if absorbed through skin. Avoid splashing in eyes or on skin or clothing. Keep tightly sealed. Wash thoroughly after handling. Flush spill area with water spray.
3. **Warning**: Denaturation Buffer and Neutralization Buffer are corrosive and may cause burns. In case of contact, immediately flush eyes or skin with a copious amount of water for at least 15 minutes while also removing contaminated clothing and shoes (see MSDS).
4. **Caution**: LABType™ SSO Bead Mixture is light sensitive and must be protected from light.
5. **Caution**: Use LABType™ SSO Bead Mixture within three months after it is thawed.
6. Refer to the Material Safety Data Sheet for detailed information.

### C. Preparing Reagents for Use

See *Directions for Use* in this document.

### D. Storage Instructions

All of the LABType™ SSO Typing Tests can be safely stored frozen at -80° to -20°C in the product box. Avoid unnecessary handling. It is recommended to keep the entire package intact and frozen upon receipt until ready to use. See Table below for individual component storage conditions.

| Component | Storage Conditions |
|---|---|
| LABType SSO Bead Mixture | -80˚C to -20˚C<br>*Protect from light*<br>*After thawing store at 2˚C to 8˚C for 3 months*<br>*Do not refreeze and thaw* |
| Locus-Specific Primer Set | -80˚C to -20˚C<br>*May repeat freeze-thaw; store frozen* |
| Denaturation Buffer | -80˚C to 25˚C |
| Neutralization Buffer | -80˚C to 25˚C |
| Hybridization Buffer | -80˚C to 25˚C |
| Wash Buffer | -80˚C to 25˚C |
| SAPE Buffer | -80˚C to -20˚C<br>*After thawing store at 2˚C to 8˚C for 3 months* |
| Primer Set D-mix | -80˚C to -20˚C<br>*May repeat freeze-thaw; store frozen* |

### E. Instability Indications

1. Beads that exhibit discoloration, or aggregation that cannot be removed by vortexing, should be considered unusable.
2. If salts have precipitated out of any of the product reagents during shipping or storage, re-dissolve by extended vortexing at room temperature (20° to 25°C).
3. D-mix aliquots, upon thawing at room temperature (20° to 25°C), should be pink to light purple in color. Any D-mix aliquot without the specified coloration should be considered unusable.

## INSTRUMENT REQUIREMENTS

- LABScan™ 100 (Luminex 100/200) or LABScan3D™ (Luminex® FLEXMAP 3D®) flow analyzer
- Luminex® XY Platform (optional accessory for automated 96-sample reading on the LABScan™ 100 flow analyzer from Luminex Corporation)
- Centrifuge
- Rotor for 1.5 ml microfuge tube (14,000 to 18,000 g)
- Swing bucket rotor for 96-well microplate (1000 - 1300 g)
- Vortex mixer with adjustable speed
- Thermocycler - Veriti™ 96-Well Thermal Cycler or Thermocycler
  - Block format 0.2 mL alloy
  - Features Standard 0.2 mL 96-well format
  - Heated lid capable of maintaining 103°C
  - Max block ramp rate 3.90°C/sec
  - Max sample ramp rate 3.35°C/sec
  - Enabled to run 9600 emulation mode at sample ramp rate of +0.8°C/sec and -1.6°C/sec
  - Maximum temperature differential 25°C across whole block, 5°C zone-to-zone
  - Temperature accuracy ±0.25°C (35–99.9°C) zone
  - Temperature range 4.0°C to 99.9°C zone
  - Temperature uniformity <0.5°C (20 sec after reaching 95°C) zone
  - PCR volume range 10–80 µL zone

## SPECIMEN COLLECTION AND PREPARATION

**A.** DNA can be purified from sample sources including whole human blood, isolated lymphocyte cells (buffy coat) from blood, blood on filter paper, lymph nodes, buccal swabs, and bone marrow with validated method that meets the criteria below. The DNA sample to be used for PCR should be re-suspended in sterile water or in 10 mM Tris-HCl, pH 8.0 – 9.0 at an optimal concentration of 20 ng/µl with the A260/A280 ratio of 1.65 - 1.80. Other specifications used should be validated by the laboratory.

**B.** Samples should be free from any inhibitors of DNA polymerase, and should not be re-suspended in solutions containing chelating agents, such as EDTA, above 0.5 mM in concentration.

**C.** DNA samples may be used immediately after isolation or stored at -20°C or below for extended periods of time with no adverse effects on results.

**D.** DNA samples should be shipped at 4°C or below to preserve their integrity during transport.

## PROCEDURE

### A. Materials Provided

***NOTE*:** *The volumes provided are slightly more than the amount required for testing. This is to account for inadvertent losses that may result from pipetting. Do not mix components from different lots of products.*

| **100 Tests per Package** | | **20 Tests per Package** | |
|---|---|---|---|
| 2.25 ml Denaturation Buffer - 1 vial | 4.95 ml SAPE Buffer - 1 vial | 2.25ml Denaturation Buffer - 1 vial | 990 µl SAPE Buffer - 1 vial |
| 2.5 ml Neutralization Buffer - 1 vial | 1.38 ml Primer Set D-Mix - 2 vials of 690 µl each | 100 µl Neutralization Buffer - 1 vial | 276 µl Primer Set D-Mix - 1 vial |
| 3.4 ml Hybridization Buffer - 1 vial | 400 µl Locus-Specific Primer Set - 1 vial | 680µl Hybridization Buffer - 1 vial | 80 µl Locus-Specific Primer Set - 1 vial |
| 55 ml Wash Buffer - 1 bottle | Bead Mixture – 400 µl LABType™ SSO primary -1 vial * 20 µl Supplement – 1 vial* | 10 ml Wash Buffer - 1 vial | 80 µl LABType™ SSO or HD Bead Mixture - 1 vial |

**** NOTE:** LABType™ (100 test) kits may contain two bead vials as needed for continued optimum resolutions: primary bead mix and a supplement bead mix.*

### B. Materials Required, But Not Provided

1. Deionized water
2. 70% ethanol
3. 20% chlorine bleach
4. R-Phycoerythrin-Conjugated Streptavidin--SAPE
5. Sheath fluid (OLI Cat.#LXSF20 or LSXF20X5)
6. Recombinant Taq polymerase (OLI catalog IDs TAQ30, TAQ50 and TAQ75)
7. 15 - 50 ml disposable tubes
8. 96-well, thin-walled PCR tray, or tubes, and holder that can withstand 1000 – 1300 g in a centrifuge

   ***Caution:** PCR plate must have tight contact with heating block.*
9. Tray seal

   ***NOTE:** PCR trays (25) and tray seals (180) sufficient for 2400 samples can be ordered from One Lambda (OLI Cat. #PCRTRAC)*
10. Electrophoresis apparatus/power supply—150V minimum capacity UV transilluminator (Fotodyne FOTO/UV®21 or equivalent)
11. Photographic or image documentation system
12. Electrophoresis running buffer – example: 1x TBE buffer (89mM Tris-borate; 2 mM disodium EDTA, pH 8.0) with 0.5 µg/ml ethidium bromide or 5XTBE Buffer with ethidium bromide
13. Electrophoresis grade agarose (e.g., FMC Seakem® LE or equivalent)
14. PCR Pad
15. Crushed ice bath or equivalent.

### C. Directions for Use

***Caution:*** *Special care must be taken in the aliquoting process. Failure to follow the steps described below may result in reagent loss.*

1. **Bead Handling and Storage**
   a. Use of the recommended disposables (tubes, trays, and tips) can minimize loss of beads due to non-specific adhesion. (See "Material Required, but Not Provided.")
   b. LABType™ SSO beads can settle and aggregate if left in a tube. Beads must be evenly distributed before dispensing. Always mix beads vigorously by pipetting several times or by vortexing in horizontal position for 10 to 30 seconds, or as much as necessary, to obtain fully homogeneous mixture.
   c. For LABType™ SSO HD products, we recommend the following procedures to help prevent bead aggregation. Immediately after removal of supernatant in step 2f, 2g, and 3c in Test Procedure below, remove as much liquid as possible by inverting and very gently tapping tray on dry paper towel. Place a seal on tray and vortex thoroughly at low speed to loosen the pellets. Proceed to next step as described.
   d. LABType™ SSO beads are packaged in an aluminum foil bag. Do not remove beads from foil bag until ready to use.
   e. LABType™ SSO beads contain internal fluorescent dye, as well as HLA allele-specific probes, attached to their surfaces. To avoid photo bleaching of the beads, protect beads from light during usage and storage. Store beads at -20ºC in the tightly capped tube provided until ready to use. Cover beads with aluminum foil or equivalent during assay.

      ***Caution:***

      - *Once beads are thawed, store beads at 2° to 8ºC and use within 3 months. Do not refreeze beads.*
      - *Open bags containing Amplification Primer Mixture and D-Mix only in pre-amplication area. Store these items at* -80° to -20° C *in the pre-amplification area.*

2. **Amplification (Set up in pre-amplification area.)**
   a. Enter the "LABType™ PCR Program," into your thermal cycler as shown in **Table 2**. Confirm all parameters.
   b. Turn on the thermal cycler to warm up heated lid.
   c. Thaw DNA, Amplification Primers, and D-Mix. Keep on ice until use.
   d. Adjust the concentration of genomic DNA to 20 ng/μl using sterile water.
   e. Vortex D-mix and Amplification Primer for 15 seconds; centrifuge for 3-5 seconds.
   f. Using Table 1 below, mix indicated volume of D-mix and Primers. Vortex for 15 seconds, and place on ice. For accurate pipetting of Taq polymerase, it is recommended that you prepare master mix for at least 10 reactions.
   g. Add Taq polymerase immediately before use.

**Table 1: Amplification Mixture**

| # of Reactions | D-mix (μl) | Amplification Primer (μl) | Taq Polymerase (μl) |
|---|---|---|---|
| 1 | 13.8 | 4 | 0.2 |
| 10 | 138.0 | 40 | 2.0 |
| 50 | 690.0 | 200 | 10.0 |
| 96 | 1491.0 | 432 | 21.6 (22) |

   h. Pipette 2 μl of DNA (at 20 ng/μl) into the bottom of a tube (for final volume of 20 μl per PCR reaction). Store the tubes or tray partially covered to prevent evaporation and contamination.
   i. Add an appropriate amount of Taq polymerase (e.g., 0.2μl (typically at 5 U/ul) per 20 μl reaction) to the Amplification Mixture prepared in Step 2.f.
   j. Vortex for a few seconds, and centrifuge for 3-5 seconds.
   k. Aliquot 18 μl of Amplification Mixture into each well containing DNA.

      ***Caution:*** *To prevent cross-contamination, be sure not to touch the pre-aliquoted DNA at the bottom.*

   l. Cap or seal. If you are using a tray seal, make sure it is pressed tightly against the rim of each well. Place a PCR Pad appropriate for the thermal cycler on the tray before closing the lid. Close and tighten the lid of the thermal cycler.
   m. Run "LABType™ SSO PCR Program," shown in Table 2.

n. For Verti™ 96-Well Thermal Cycler, set “ramp speed” to the 9600 program. For other systems, consult the manufacturer’s documentation to adjust ramp speed to the specifications outlined in Instrument Requirements Use of a significantly different ramp speed will affect amplification efficiency and final results.

**Table 2: LABType™ SSO PCR Program**

| Step | Temperature and Incubation Time | # of Cycles |
|---|---|---|
| Step1: | 96ºC 03:00 | 1 |
| Step 2: | 96ºC 00:20 | 5 |
| | 60ºC 00:20 | |
| | 72ºC 00:20 | |
| Step 3: | 96ºC 00:10 | 30 |
| | 60ºC 00:15 | |
| | 72ºC 00:20 | |
| Step 4: | 72ºC 10:00 | 1 |
| Step 5: | 4ºC forever | 1 |

o. Amplified DNA is now ready to be tested using the Test Procedure in section D.

***NOTE:*** *It is recommended to first use 2 - 5 µl of amplified DNA for analysis by gel electrophoresis. Confirmation of an amplification product (band) prior to hybridization assay ensures generation of optimal signals.*

p. If the amplified product is not used immediately, store covered DNA tray at -80° to -20° C for up to one month.

**3. Test Set-Up**

a. Turn on the LABScan™ 100 and XY Platform or LABScan3D™ and follow the start-up procedure described in Section D of the Directions for Use. The LABScan™ 100 or LABScan3D™ requires at least 30 minutes to warm up.
b. Turn on thermal cycler and run program to 60°C HOLD, or equivalent, for at least 1.5 hours (or hold forever). Have a PCR Pad appropriate for your thermal cycler ready for use. Be sure to wait until the heated lid of the thermal cycler reaches the appropriate temperature before use. Use the appropriate 96-well PCR tray holder to ensure the proper incubation temperature.
c. Remove all reagents (except brown 100X SAPE bottle) from storage to room temperature. Aliquot necessary volumes of reagents into clean containers. (Use the tables below for reference). Be sure to prepare 1X SAPE during the third wash step. Remove the 100X SAPE bottle from storage only when needed, and return immediately to 2° to 8° C. Return any unused portions of the Bead Mixture to 2° to 8° C.
**Caution:** *Do not refreeze Bead Mixture after thawing.*

**4. LABType™ Bead Preparation (for 100-Test Kit containing two bead vials):**

a. For LABType™ kits containing 2 bead vials, give tubes a quick spin (10-15 seconds at 100 RCF (relative centrifugal force) in most small centrifuges) immediately after thawing.
b. Vortex vials at medium strength for 20 seconds, then give a quick spin again as described above.
c. Take the primary bead vial and slowly, but thoroughly, pipette up and down several times using P1000 or equivalent to mix bead solution and to prime the pipette tip.
d. Using the same pipette tip used in Step (c), carefully transfer the entire volume of the primary beads into the supplemental bead vial.
e. Discard the empty primary bead vial. The supplemental tube is labeled with the new lot/batch identifier for the combined beads. This lot number is associated with the correct analysis cat files and data sheets.
f. Mix the combined beads vigorously by vortexing the capped tube 3 times for 10 seconds each to obtain a homogenous bead mixture. Use immediately or store at condition described in page 2,

Storage Instructions. Be sure to vortex the bead vial at medium speed for at least 20 seconds immediately before use.

**Table 3: Reagent Preparation**

| Reagent | Amount per Test | Preparation Method and Suggestions |
|---|---|---|
| Bead Mixture | 4 µl | • Aliquot appropriate volume, plus extra volume*, for the required number of tests into a clean tube at room temperature.<br>• Protect from light. Use the entire contents of the Bead Mixture tube for 96 samples.<br>• Vortex immediately before use. |
| Hybridization Buffer | 34 µl | • Aliquot for exactly the same number of tests as used for the Bead Mixture.<br>• Add to pre-aliquoted Bead Mixture to prepare Hybridization Mixture.<br>• Keep at room temperature (20° to 25° C) until use. |
| Wash Buffer | 480 µl | • Aliquot appropriate volume, plus extra volume*, for the required number of tests, and keep at room temperature (20° to 25° C).<br>• Use the entire contents in a trough for 96 samples. |
| Denaturation Buffer | 2.5 µl | • Aliquot appropriate volume, plus extra volume*, for the number of tests.<br>• Use the entire contents in a trough for 96 samples. Keep at room temperature (20° to 25° C). |
| Neutralization Buffer | 5 µl | • Aliquot appropriate volume, plus extra volume*, for the number of tests.<br>• Use all 2.5 ml for 96 samples. Keep at room temperature (20° to 25° C). |
| SAPE Stock (100X) | 0.5 µl | • During the last centrifugation step, prepare 1X SAPE solution by making 1:100 dilution of SAPE Stock with SAPE Buffer for the appropriate number of tests, plus extra volume.* |
| SAPE Buffer | 49.5 µl | • Protect from light.<br>• Prepare enough 1XSAPE solution for 96 samples (around 110 sample worth depending on observed pipetting error).<br>• Keep SAPE Stock bottle at 2° to 8° C. |

***NOTE**: *The extra volume required depends on pipetting technique and calibration status of equipment. Use a full volume of Bead Mixture in the tube provided (enough for approximately 110 tests) for 96 tests. Prepare 1X SAPE for 115 tests, and use entire volume of other reagents to prevent a shortage. We recommend calibration of all pipetting devices and testing of these devices by aliquoting water. For reagents provided in excess volume, such as Denaturation and Neutralization Buffer, you may use a trough for multichannel pipetting.*

**Table 4: Reagent Volumes**

| Number of Tests | Denaturation Buffer (µl) | Neutralization Buffer (µl) | Hybridization Buffer (µl) | Wash Buffer (µl) Tray Method | Bead Mixture (µl) |
|---|---|---|---|---|---|
| 1 | 2.5 | 5 | 34 | 480 | 4 |
| 10 | 25 | 50 | 340 | 4800 | 40 |
| 20 | 50 | 100 | 680 | 9600 | 80 |
| 50 | 125 | 250 | 1700 | 24000 | 200 |
| 96 | 240 | 480 | 3264 | 46080 | 384 |

**Table 5: SAPE and SAPE Buffer Volumes**

| Number of Tests | SAPE Stock Volume (µl) | SAPE Buffer Volume (µl) |
|---|---|---|
| 1 | 0.5 | 49.5 |
| 10 | 5.0 | 495.0 |
| 20 | 10.0 | 990.0 |
| 50 | 25.0 | 2475.0 |
| 96 | 48.0 | 4752.0 |

***NOTE:*** *Volume of reagents in Tables 4 and 5 are for the exact number tests. The actual number of aliquots differs depending on pipetting accuracy. For a full 96-sample assay, we recommend using the entire bead mixture, the entire volume of hybridization buffer, 57.5 µl stock SAPE, and 5693 µl of SAPE buffer, which is slightly more than the exact amount required for the test.*

## D. Test Procedure

### TECHNICAL PRECAUTIONS

1. To assay a small number of samples (48 or fewer) you may use a 96-well tray, a tray that has been cut to the appropriate number of wells, or a 0.2 ml thin-wall PCR strip tube. Be sure to use a tube rack when using a cut-off tray or strip tube.
2. Mixing of samples in a 96-well tray involves sealing of the tray and low speed vortexing for a few seconds. Adjust the speed of the vortex mixer so that liquid inside the 96-well PCR tray is sufficiently agitated without excessive splashing. Note the speed setting, and use it for the 96-well tray method.
3. Sealing of the 96-well PCR tray should be done carefully and completely to prevent well-to-well sample contamination. Seal the tray by pressing the seal against each rim of the 96 wells. Do not re-use tray seals. Use a fresh seal for each step that requires application of a tray seal. A repeater pipette may be used where applicable; however, a repeater pipette is usually less accurate in volume delivery.
4. We recommend regular calibration and a manual volume check for each volume to be delivered. Do not use a repeater pipette for dispensing the Hybridization Mixture.

### 1. Denaturation/Neutralization

a. Prepare a crushed ice bath.
b. Place a clean 96-well plate in a tray holder.
c. Transfer 2.5 µl Denaturation Buffer into a well of a clean 96-well plate.
d. Add 5 µl of each amplified DNA. Make sure sample location and ID are noted. Mix thoroughly (preferably by pipetting up and down), and incubate at room temperature (20 - 25° C) for 10 minutes.

***NOTE:*** *Amplified DNA can be aliquoted first and subsequent addition of Denaturation Buffer.*

e. Add 5 µl Neutralization Buffer with pipette, and mix thoroughly (preferably by pipetting up and down). Note the color change from bright pink to pale yellow or clear.
f. Place PCR plate with neutralized PCR product on the ice bath.

***Caution:*** *Avoid contamination of PCR product with water.*

### 2. Hybridization

***NOTE:*** *Make sure that the thermal cycler has been turned on and the 60°C program has been started to warm the thermal block.*

a. Combine appropriate volumes of Bead Mixture and Hybridization Buffer to prepare Hybridization Mixture.
b. Add 38 µl Hybridization Mixture to each well.
c. Cover tray with tray seal and vortex thoroughly at low speed.
d. Remove from tray holder and place PCR tray into the pre-warmed thermal cycler (60° C).
e. Place PCR Pad on top of tray or caps on PCR tubes. Close and tighten lid. Incubate for 15 minutes.
f. Place tray in tray holder and remove tray seal. Quickly add 100 µl Wash Buffer to each well. Cover tray with tray seal. Centrifuge tray for 5 minutes at 1000 -1300 g. Place tray in tray holder and remove wash buffer.
g. Repeat step 2.f above two more times for a total of three wash steps. Remember to prepare 1X SAPE solution during third centrifugation.

### 3. Labeling

a. Place tray in tray holder. Add 50 µl of 1X SAPE solution to each well. Place tray seal on tray and vortex thoroughly at low speed. Place tray in the pre-heated thermal cycler (60° C). Place PCR Pad on top of tray or caps on PCR tubes. Close and tighten lid. Incubate for 5 minutes.
b. Remove tray. Place tray in tray holder. Remove seal and quickly add 100 µl Wash Buffer to each well.
c. Cover tray with tray seal. Centrifuge tray for 5 minutes at 1000 – 1300 g. Place tray in tray holder and remove supernatant.
d. Add 70 µl Wash Buffer to each well. Gently mix by pipetting. Transfer to reading plate using an 8- or 12-channel pipet. Avoid sample- to-sample contamination by using fresh pipette tips.
e. NOTE: Final volume should be at least 80 µl.
f. Cover tray with tray seal and aluminum foil. Keep tray in the dark and at 4°C until placed in the LABScan™ 100 or LABScan3D™ for reading.

g. For the best results, read samples as soon as possible. Prolonged storage of samples (more than 4 hours) may result in loss of signal. Store samples overnight at 4°C in the dark with a tray seal, if they cannot be read immediately. Be sure to thoroughly mix the samples immediately before reading.

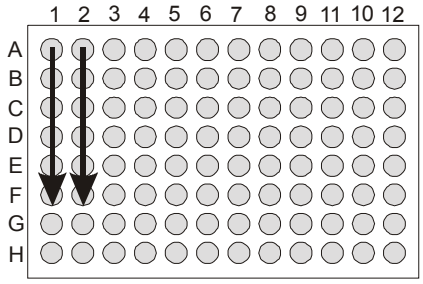


**Figure 1 The Luminex® XY Platform reads the sample in the following pattern: A1 to H1, A2 to H2, A12 to H12.**

### E. Data Acquisition

***NOTE:*** *Described below is a general guide to data acquisition. Details on the use of the LABScan™ 100 or LABScan3D™, may be found in the Luminex® 100/200[3] or Luminex® FLEXMAP 3D® User's Manual[4].*

1. Turn on the system and set up the LABScan™ 100 and/or LABScan3D™ for sample acquisition and calibration according to the Luminex User's Manual3.4 for the software version currently being used.
2. Choose a template/protocol according to the product catalog ID and lot number.
   a. Acquisition templates/protocols are available from One Lambda on a CD or are downloadable via the One Lambda website.
   b. To create your own acquisition template, follow the instructions in the Acquisition chapter of the" Luminex User's Manual." Start Up
3. Create a file name for the samples to be run.
4. Make sure all the template/protocol settings are correct.
5. Enter the sample IDs.

   ***Caution:*** *If the same sample is tested more than once, a different ID should be assigned.*
6. The plate is now ready to run.
7. Load the plate onto the XY platform and fill the reservoir with sheath fluid.
8. Click on the START button to initiate the session. After the samples have been run, the data output should be saved in a .csv file.
9. Wash the machine 2 times with sheath fluid at the end of the session.

   ***NOTE:*** *Luminex® software versions - LABScan 100 (IS 2.2/2.3, xPONENT 3.1, or xPONENT 4.2); LABScan 3D (xPONENT 4.0 or xPONENT 4.2) must be used. For LABType HD Analysis - Be sure to designate the new (supplemental) lot/ batch when reading and analyzing data. Capture and save the entire run file from the Luminex® flow analyzer for data analysis.*

## RESULTS

### A. Data Calculation

1. The mean fluorescence intensity (MFI) generated by the Luminex® Data Collector software, or equivalent, contains the FI for each bead (or probe bound to the bead) per sample. The percent positive value is calculated as:

   **Percent Positive Value = 100 x [MFI (Probe n) - MFI (Probe Negative Control)/ MFI (Probe Positive Control) - MFI (Probe Negative Control)]**

   The positive reaction is defined by the percent of positive values for the probe higher than the pre-set cut-off value for the probe. The negative reaction is defined as the percent of positive values lower than the cut-off value. Under the controlled product QC environment, the MFI for negative control is typically 0-100 and can vary between lots and locus-specific products. Signals outside of the range may represent inefficient controls of the assay parameters such as sample quantity and/or quality of sample, technique,

instrument calibration, and state of all reagents including amplified DNA, buffers, SAPE and the bead mixture.

2. Compare calculated percent positive values to the pre-determined cut-off values for each test probe. Assign a positive attribute to probes that have a percent positive above the cut-off and a negative attribute to those below the cut-off. The MFI of the positive control should be within 1200 - 7000 MFI. (The MFI value may fall outside of this range [see *Expected Values*, Section C] and varies for each positive control probe and lot.) The MFI of each probe is normalized against the positive control MFI and is expressed as a percentage of the positive control MFI. The pre-set cut-off value for each probe was established using a 100- to 200-sample DNA panel.

### B. Data Analysis

1. Determine HLA allele (or allele groups) of the sample by matching the pattern of positive and negative bead IDs with the information in the LABType™ SSO worksheet or using HLA Fusion™ Software.

   ***Note:*** *For LABType™ High Definition assays and LABType™ assays containing a supplemental bead vials it is necessary to use HLA Fusion™ software version 2.0 or higher for data analysis.*

## LIMITATIONS OF THE PROCEDURE

The LABType™ SSO system combines an HLA locus-specific DNA amplification process and DNA-DNA hybridization process. The procedure, as well as the equipment calibration described in this product, must be strictly followed.

DNA amplification is a dynamic process that requires highly controlled conditions to obtain PCR products that are specific to a target segment of HLA gene(s). The procedure provided for the DNA amplification process must be strictly followed. In particular, since sample DNA quantity and quality can significantly affect the amplification reaction, a standardized DNA extraction procedure and spectrophotometric measurement of DNA quantity and quality, followed by gel electrophoretic analysis, are strongly recommended.

In addition, to avoid contamination of initial materials with PCR products, all materials generated after DNA amplification (post-PCR materials, including reaction mixes; all disposable plastics; and equipment, such as pipetting devices and gel electrophoresis devices) must be physically separated from materials used before DNA amplification (pre-PCR materials including all disposable plastics, pipetting devices, sample DNAs, all other reagents used to set up amplification reactions).

Routine wipe testing of pre-amplification work area with validated detection method that is compliant with guidelines provided by concerning regulatory body is recommended.

The DNA-DNA hybridization-based assay using LABType™ SSO is a very temperature-sensitive process. The temperature used for the assay must be checked frequently (calibrated). Strict adherence to the temperatures and incubation times described in this procedure is critical for obtaining optimal results.

LABType™ SSO microspheres are light sensitive and must be protected from light as much as possible. Avoid freezing and thawing to ensure maximum shelf life.

The microsphere mixture provided contains a carefully optimized quantity of microspheres sets bearing HLA allele specific probes. Any alteration of the mixture would significantly affect the accuracy of the assay and would void the results. To minimize a loss of microspheres during the assay, follow the protocol described here and use only recommended pipette tips and tubes. The microsphere mixture provided contains a carefully optimized quantity of microspheres sets bearing HLA allele specific probes. Any alteration of the mixture would significantly affect the accuracy of the assay and would void the results.

When compared to SSP, SSO has more ambiguities because the probes used in SSO can interrogate sample DNA at only one region per test, and SSP can interrogate sample DNA at two regions per test. This is a basic limitation of the SSO method, which is well understood by the HLA professional. As mentioned previously, a list of Resolution Limitations is proved for each lot of the LABType™ SSO Typing Tests to aid in interpretation of the reaction pattern and assignment of HLA typing.

All instruments (e.g., thermal cycler, pipetting devices, LABScan™ 100 or LABScan3D™ and heat block) must be calibrated and/or verified according to the manufacturers' recommendations.

For lot-specific information, refer to the *Bead Probe Information* document.

Because of the complexity of the HLA allelic definitions, a certified HLA technician or specialist should review and interpret the data, and assign the HLA typing.

This test must not be used as the sole basis for making a clinical decision.

## EXPECTED VALUES

### A. Sample Amplification

1. The HLA locus-specific primer mix provided is expected to yield adequate quantity of amplified DNA. Failure to detect an amplification product by ethidium bromide stained agarose gel electrophoresis voids test results.
2. DNA amplification is subject to contamination by previously amplified DNA. Detection of contamination (by performing a control amplification using water or pre-established DNA wipe test for detection of contaminating amplification products) can void test results.

### B. LABScan™ 100 and LABScan3D™ Analyzer

1. The LABScan™ 100 or LABScan3D™ is advanced flow analyzers that requires daily maintenance and calibration and/or verification. Refer to the Luminex® 100/200 or Luminex® FLEXMAP 3D® User Manual for all necessary maintenance operation. Daily maintenance includes routine start-up and shut-down procedures. For best performance, calibrate the instrument as part of the start-up routine. Calibrate the instrument whenever the **Δ Cal Temp** temperature shown on the system monitor panel is more than ± 3ºC for the LABScan 100 or more than ± 5ºC for the LABScan 3D.
2. The instrument must pass a calibration test before LABType™ SSO samples are analyzed.

### C. Data Acquisition and Analysis

In order to obtain valid data, two parameters, count and Mean Fluorescence Intensity (MFI), must be monitored for each data acquisition. Count represents the total number of beads that has been analyzed, and the count should be above 100 ±25%. A significant reduction in the count suggests bead loss during sample acquisition or assay and can void test results.

MFI represents a PE signal detected within the counted beads. MFI varies based on reaction outcome. The MFI for the positive control probe could vary from lot to lot, and also due to sample quantity and/or quality, technique, instrument calibration, and state of all reagents including amplified DNA, buffers, SAPE and the bead mixture.

Product QC data information in analysis software presents lot-specific values obtained using DNAs that meet sample requirement (see *Specimen Collection and Preparation*).

***Users are strongly advised to determine their own range of the control value using reference sample validation tests for every lot. Significant reduction or elevation in MFI for the positive control probe, accompanied by non-assignable reaction patterns, may suggest inadequate sample quantity and/or quality, poor assay efficiency, or instrument failure, and can void test results.***

## SPECIFIC PERFORMANCE CHARACTERISTICS

In normal samples and using assay and data acquisition conditions that are within the specifications described in this product insert (e.g., starting genomic DNA concentration of 20 ng/µl and purity, OD260/280 of 1.65 to 1.80, hybridization incubation temperature and washing conditions, and the LABScan™ 100 or LABScan3D™ analyzer performance status), positive and negative reactions are determined by comparing the relative Mean Fluorescence Intensity (MFI) of a sample to its corresponding cut-off value. The cut-off value has been experimentally determined for a given lot of LABType™ SSO product, and the cut-off is used to distinguish between positive and negative signals, based on the HLA genotype of a sample. The results are expected to reflect the presence or absence of certain HLA allele(s), providing a clean-cut typing assignment.

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## TRADEMARKS AND DISCLAIMERS

The LABType™ typing reagents are manufactured and distributed by One Lambda, Inc., 21001 Kittridge Street, Canoga Park, CA 91303, U.S.A. Recombinant Taq polymerase is manufactured by F. Hoffmann-LaRoche.



## EUROPEAN AUTHORIZED REPRESENTATIVE

EC REP MDSS GmbH, Schiffgraben 41, 30175, Hannover, Germany

# Summary of Protocol for 96-Sample Assay

**A. Pre Set-Up**

1. Turn on LABScan™ 100 or LABScan™ 3D analyzer, and begin the start-up procedure. Turn on the thermal cycler, and start 60°C incubation program.
2. Prepare crushed ice bath (add small amount of water to allow PCR tray to stand straight on ice)
3. Thaw and vortex D-Mix and DNA.
4. Remove all reagents (except 100x SAPE bottle) from storage temperature and use at room temperature.
5. Thoroughly mix entire volume of Hybridization buffer and entire Bead Mixture in a clean tube; protect from light.

**B. Amplification**

1. Thaw all amplification reagents, and place on ice.
2. Aliquot 2 μl genomic DNA to each of 96 wells in a PCR tray.
3. Mix 432 μl of Primer Mix, 1491 μl of D-Mix, and 22 μl of Taq polymerase. Vortex well and give a quick spin.
4. Aliquot 18 μl of Amplification Mix from Step 3 into all 96 wells containing DNA.
5. Cap or seal the PCR tray.
6. Run the tray in a PCR oven using the LABType™ SSO PCR program.
6. Remove the PCR tray from the PCR oven, and check the amplified DNA on a 2.5% agarose gel (use 5 μl per well).

**C. Denaturation/Neutralization**

1. In a clean, thin-walled 96-well PCR tray, aliquot 2.5 μl of Denaturation Buffer per well.
2. Add 5 μl per well of amplified DNA. Note the sample locations in the 96 wells.

***NOTE:*** *Amplified DNA can be aliquoted first and subsequent addition of Denaturation Buffer.*

3. Mix thoroughly until the mixture changes to a bright pink color.
4. Incubate at room temperature (20° to 25° C) for 10 minutes.
5. Add 5 μl per well of Neutralization Buffer.
6. Mix thoroughly until the mixture turns clear or pale yellow.
7. Place tray carefully on the ice bath.

**D. Hybridization/Washing**

1. Aliquot 38 μl Hybridization Mixture (from A.5.above) per well into all neutralized DNA.
2. Place a seal on the tray and vortex thoroughly at low speed.
3. Incubate the tray in a 96-well block in a 60ºC thermal cycler (use PCR Pad) for 15 minutes.
4. Take out the tray. Add 100 μl of Wash Buffer to each well. Place a new seal on the tray, and spin at 1000 g for 5 minutes.
5. Remove supernatant, leaving approximately 10 μl or less.
6. Repeat Steps D.4 and D.5 two more times for total of 3 washes.
7. During the last centrifugation step, prepare 1X SAPE (57.5 µl Stock and 5693 µl SAPE Buffer) and leave covered at room temperature.

**E. Labeling**

1. After removal of supernatant from the third wash (D.6 above), add 50 μl 1X SAPE per well.
2. Place a seal carefully on the tray and vortex thoroughly at low speed.
3. Incubate at 60ºC in thermal cycler as above for 5 minutes.
4. Take out the tray, and add 100 μl Wash Buffer to each well. Place a new seal on the tray and spin at 1000 g for
5. 5 minutes.
6. Remove supernatant. Add Wash Buffer to make the final volume 80 μl.
7. Mix by pipetting and transfer all samples to a 96-well microplate for data acquisition.

## EXPLANATION OF SYMBOLS

| Symbol | Description |
|---|---|
| REF | Catalog number |
| IVD | In vitro diagnostic medical device |
|  | Consult instructions for use |
|  | Caution, consult accompanying documents |
|  | Biological risks |
|  | Temperature limitation |
| CE | CE mark |
| CE 0197 | CE mark of medical quality |
|  | Manufacturer |
| EC REP | Authorized representative in the European Community |

## REVISION HISTORY

| Revision | Date | Revision Description |
|---|---|---|
| 27 | 2016/11 | SAPE buffer storage temp change from -80°C to -20°C. Added product catalog IDs and their individual product descriptions. Updated onto current product insert template. |
| 28 | 2017/01 | Change storage of SAPE Buffer to store at 2-8°C upon thaw. Corrected typo in Materials Provided table. |

CE 0197

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*0197 Applies to Annex II List B products only