LABType™ XR and CWD DNA Typing Test

REF

Catalog ID	Product Name
RSSOW1A	LABType™ CWD Class I A Locus Typing Test
RSSOW1B	LABType™ CWD Class I B Locus Typing Test
RSSOW2B1	LABType™ CWD Class II DRB1 Typing Test
RSSOX1A	LABType™ XR Class I A Locus Typing Test
RSSOX1B	LABType™ XR Class I B Locus Typing Test
RSSOX2B1	LABType™ XR Class II DRB1 Typing Test

Catalog ID	Product Name
RSOW1AT	LABType™ CWD Class I A Locus Typing Test – 20 tests
RSOW1BT	LABType™ CWD Class I B Locus Typing Test – 20 tests
RSOW2B1T	LABType™ CWD Class II DRB1 Typing Test – 20 tests
RSOX1AT	LABType™ XR Class I A Locus Typing Test – 20 tests
RSOX1BT	LABType™ XR Class I B Locus Typing Test – 20 tests
RSOX2B1T	LABType™ XR Class II DRB1 Typing Test – 20 tests

IVD For In Vitro Diagnostic Use in EU only. Not for distribution in the USA or Canada.

INTENDED USE

DNA typing of HLA Class I or Class II alleles

SUMMARY AND EXPLANATION

LABType XR and CWD DNA Typing Test products encompass exons 2-5 for A and B loci and exon 2 for DRB1 locus and also contains probes that will type for Common and Well-Documented HLA alleles based on the current CWD catalog available on the IMGT/HLA database. The typing test products are used in conjunction with the LABScan 3D instrument (Luminex® FLEXMAP 3D® instrument). The typing tests apply Luminex technology to the reverse SSO DNA typing method. The LABScan 3D instrument combines dyed fluorescent microsphere sets to allow multiplexing of up to 500 unique assays within a single sample.

In this assay, 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 strepavidin (SAPE). The PCR product is denatured and allowed to rehybridize to complementary DNA probes conjugated to fluorescently coded microspheres. A bench-top analyzer (LABScan 3D) identifies the fluorescent intensity of PE on each microsphere. Positive reactions are identified by comparing the fluorescent signal for each test probe as a percent of positive internal control probe signal to a given cut-off value.

Separately available analysis software (HLA Fusion) can be used to assist in determining HLA typing.

PRINCIPLE(S)

HLA antigens are polymorphic heterodimers encoded by genes located on the short arm of chromosome 6 and regulate the immune response to pathogens and distinguish "self" from "non-self" in transplantation immunology. Histocompatibility testing allows the matching of organ and bone marrow recipients and donors with a degree of matching accuracy capable of impacting the clinical outcome of organ and bone marrow transplantation. Molecular-based typing methods have been refined for practical testing in the clinical lab setting. LABType XR and CWD DNA Typing Test 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 tube, makes this method suitable for large-scale testing.

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REAGENTS

A. Identification

The LABType XR and CWD DNA Typing Tests provide 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 the 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): 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. **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.
2. **Warning**: Refer to the Safety Data Sheet for detailed information.
3. **Caution**: LABType Bead Mixture is light sensitive and must be protected from light.
4. **Caution**: Use LABType Bead Mixture within three months after it is thawed.

C. Preparing Reagents for Use

See *Directions for Use* below.

D. Storage Instructions

All of the LABType XR and CWD DNA 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 XR and CWD DNA Typing Test Bead Mixture	-80˚C to -20˚C *Protect from light* *After thawing store at 2˚C to 8˚C for 3 months*
Locus-Specific Primer Set	-80˚C to -20˚C *After thawing store at 2˚C to 8˚C for 3 months*
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 *After thawing store at 2˚C to 8˚C for 3 months*
Primer Set D-mix	-80˚C to -20˚C *After thawing store at 2˚C to 8˚C for 3 months*

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

A. LABScan 3D™ (Luminex FLEXMAP 3D) flow analyzer

B. Centrifuge
- Rotor for 1.5 ml microfuge tube (14,000 to 18,000 g)
- Swing bucket rotor for 96-well microplate (1000 - 1300 g)

C. Vortex mixer with adjustable speed

D. Thermocycler - Veriti™ 96-Well Thermal Cycler or Thermocycler capable of the following parameters:
- Block format 0.2 mL alloy
- Features Standard 0.2 mL 96-well format
- Heated lid capable of maintaining 103°C
- 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 any human cells 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 loss 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	2.25 ml 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	680 µl Hybridization Buffer – 1 vial	80 µl Locus-Specific Primer Set – 1 vial
55 ml Wash Buffer – 1 bottle	400 µl LABType XR and CWD DNA Typing Test Bead Mixture – 1 vial	10 ml Wash Buffer – 1 vial	80 µl LABType XR or CWD DNA Typing Test Bead Mixture – 1 vial

B. Materials Required, But Not Provided

1. Deionized water
2. 70% ethanol
3. 20% chlorine bleach (or equivalent)
4. R-Phycoerythrin-Conjugated Streptavidin--SAPE) (OLI Cat. #LT-SAPE)
5. Sheath fluid (OLI Cat.#LXSF20 or LSXF20X5)
6. Recombinant Taq polymerase (AmpliTaq Polymerase, OLI catalog IDs TAQ30, TAQ50 and TAQ75 or equivalent)
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 seals (OLI Cat. #SSPSEA300 or equivalent)

   Note: PCR trays (25) and tray seals (180) sufficient for 2400 samples can be ordered from One Lambda (OLI Cat. #PCRTRAC)
10. PCR Pad (OLI Cat. # SSPPADTN, SSPPADTN5, or equivalent)
11. Crushed ice
12. 1.5 ml microfuge tube
13. P10, P200, and P1000 pipettes
14. Pipette tips for P10, P200, and P1000 pipettes
15. 96-well, 250 µl V-bottom, white polystyrene microplate with a non-treated surface (or equivalent)

C. Optional Materials, Not Provided

1. Electrophoresis apparatus/power supply—150V minimum capacity
2. Micro SSP™ Gel System (OLI Cat. #MGS108 or equivalent)
3. UV transilluminator (Fotodyne FOTO/UV®21 or equivalent)
4. Photographic or image documentation system
5. 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 (OLI Cat. # 5XTBE100 or equivalent)
6. Electrophoresis grade agarose (e.g., FMC Seakem® LE or equivalent)

D. 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 plastic ware (tubes, trays, and tips) can minimize loss of beads due to nonspecific adhesion. (See "Material Required, but Not Provided.")
b. LABType XR and CWD DNA Typing Test bead mix 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 XR and CWD DNA Typing Test products, we recommend the following procedures to help prevent bead aggregation. Immediately after removal of supernatant in step 2f, 2g, and 3c of Test Procedure > Hybridization and Labeling 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 XR and CWD DNA Typing Test bead mix are packaged in an aluminum foil bag. Do not remove beads from foil bag until ready to use.
e. LABType XR and CWD DNA Typing Test bead mix 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 -80 to -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 2º to 8º C and use within 3 months. Do not refreeze beads.*
- *Open bags containing Primer Mix and D-Mix only in pre-amplification area. Store these items at* -80 *º* to -20 *º* 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	40	2
50	690	200	10
96	1491	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 your thermal cycler on the tray before closing the lid. Close and tighten the lid of the thermal cycler.
m. Run "LABType 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 PCR Program

Step	Temperature and Incubation Time	# of Cycles
Step 1:	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 E.

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 LABScan3D™ and follow the start-up procedure. The 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 and the SAPE Buffer to 2° to 8° C.
Caution: *Do not refreeze Bead Mixture after thawing.*

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. • Protect from light. Use the entire contents of the Bead Mixture tube for 96 samples. • Vortex immediately before use.
Hybridization Buffer	34 µL	• Aliquot for exactly the same number of tests as used for the Bead Mixture. • Add to pre-aliquoted Bead Mixture to prepare Hybridization Mixture. • 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). • 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. • 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. • 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.* • Protect from light. • Prepare enough 1XSAPE solution for 96 samples (around 110 sample worth depending on observed pipetting error). • Keep SAPE Stock bottle at 2° to 8° C.
SAPE Buffer	49.5 µL	

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	380	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.*

E. 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.
NOTE: *Final volume should be at least 80 µl.*

e. Cover tray with tray seal and aluminum foil. Keep tray in the dark and at 4°C until placed in the LABScan3D™ for reading.
f. 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.

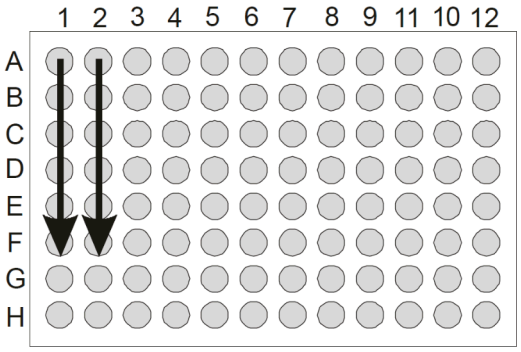

Figure 1: The Luminex XY Platform reads the sameple in the following Pattern: A1 to H1, A2 to H2, A12 to H12 etc.

RESULTS

A. Data Acquisition

NOTE: *Described below is a general guide to data acquisition. Details on the use of the LABScan3D™ and xPONENT 4.2 software, may be found in the Luminex® FLEXMAP 3D® Hardware User Manual and Luminex® xPONENT® Software User Manual.*

1. Turn on the system and set up the LABScan3D™ for sample acquisition and calibration according to the Luminex User's Manual for the software version currently being used.
2. Choose a Protocol according to the product catalog ID and lot number.
   a. Acquisition Protocols are available from One Lambda via the One Lambda website.
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 DI water.
8. Click on the RUN button to initiate the session. After the samples have been run, the data output will be saved in a .csv Output file as well as a Run CSV (RCSV) file folder.
   NOTE: *Run CSV (RCSV) files are required for import into HLA Fusion (refer to the HLA Fusion User Manual for instructions on configuration and import)*
   NOTE: *Luminex software versions - LABScan 3D (xPONENT 4.2 or higher) must be used.*

B. Data Calculation

1. The mean fluorescence intensity (MFI) generated by the Luminex xPONENT software, or equivalent, contains the MFI 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 p1ercent of positive values for the probe higher than the pre-set cutoff 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 greater than 1000 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.

C. 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 XR and CWD DNA Typing Test worksheet or by using HLA Fusion™ Software version 4.0 or higher, reference the HLA Fusion User Manual.

LIMITATIONS OF THE PROCEDURE

The LABType XR and CWD DNA Typing Test combine 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 XR and CWD DNA Typing Test 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 XR and CWD DNA Typing Test 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.

All instruments (e.g., thermal cycler, pipetting devices, and LABScan3D™) 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. LABScan3D™ Analyzer

1. The LABScan3D™ is an advanced flow analyzers that requires daily maintenance and calibration and/or verification. Refer to the 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 ± 5ºC for the LABScan 3D.
2. The instrument must pass a calibration test before LABType XR and CWD DNA Typing Test 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 greater than or equal to 75 beads. 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 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 XR and CWD DNA Typing Test 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.

Performance evaluation of the LABType XR and CWD DNA Typing Test products were tested for their ability to obtain correct typing results using 90-96 approved reference DNA samples. The results demonstrated 100% concordance to the reference typing. The reproducibility of the LABType XR and CWD DNA Typing Test was tested. Based on the data generated from 90 different sessions, the LABType XR and CWD DNA Typing Tests are highly reproducible as the lower bound of the one-sided 95% confidence interval for total reaction pattern all exceeded 0.95 between technicians, days, and runs.

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TRADEMARKS AND DISCLAIMERS

The LABType™ typing reagents are manufactured and distributed by One Lambda, Inc., a Thermo Fisher Scientific Brand., at 21001 Kittridge Street, Canoga Park, CA 91303, U.S.A.

EUROPEAN AUTHORIZED REPRESENTATIVE

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Summary of Protocol for 96-Sample Assay

A. Pre Set-Up

1. Turn on 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 pulse 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 PCR program.
7. 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 º to 25º 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 1000-1300 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 minutes.
5. Remove supernatant. Add Wash Buffer to make the final volume 80 µL.
6. 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
0	2015/08	New Product Insert
1	2016/12	Update IFU for SAPE Buffer to add to Store at 2-8c for 3 months upon thaw. Added Catalog IDs and descriptions to pg 1. Corrected typos in Materials Provided table.
2	Current	Correct Expected Values, Section C, Data Acquisition and Analysis from "greater than 75 beads" to greater or equal to".

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