ONE LAMBDA
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APPLICATION NOTE

LABType® XR and CWD

REF **Catalog #'s:** RSSOW1AR, RSOW1ATR, RSSOW1BR, RSOWIBTR, RSSOW1CR, RSOW1CTR, RSSOW2B1R, RSOW2B1TR, RSSOX1AR, RSOX1ATR, RSSOX1BR, RSOX1BTR, RSSOX1CR, RSOX1CTR, RSSOX2B1R, RSOX2B1TR
For Research Use Only. Not for use in diagnostic procedures.

REAGENTS

A. Storage Instructions

1. All of the LABType® XR and CWD reagents and buffers can be safely stored frozen at -80° to -20°C in the product box. Avoid unnecessary handling. It is recommended that you keep the entire package intact and frozen upon receipt until ready to use. However, once the bead mixtures are thawed for use, they should be stored at 2° to 8°C and should never be refrozen.
2. A brief summary of the required storage and handling conditions necessary to ensure optimal stability for the LABType® XR and CWD reagents follow below.

LABType® XR and CWD Buffers:
All of the LABType® XR and CWD buffers, with the exception of the SAPE buffer, have an allowable temperature range of -80° to 25° C and can be refrozen. The SAPE Buffer may not be refrozen. The SAPE buffer must be stored at -80° to 8° C. Once thawed, the SAPE Buffer must be stored refrigerated at 2° to 8° C.

LABType® XR and CWD SSO Bead Mixtures:
The LABType XR and CWD Bead Mixtures are most stable frozen. We recommend initial storage of the beads at -80° to -20° C until ready to use. Once the beads have been thawed for use, they should be kept at 2° to 8° C for up to 3 months.
Important: To prolong the shelf life of the beads, do not refreeze and thaw the beads again.

LABType® XR and CWD Primer Sets and D-mix:
LABType® XR and CWD Primer Sets and D-mix are most stable frozen at -80° to -20° C. Both reagents may undergo repeated freeze-thaw cycles. Thus, we recommend storage at -80° to -20°C at all times.

INSTRUMENT REQUIREMENTS

A. LABScan 3D™ analyzer

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.

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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

1. Pre-optimized and tested mixture of microspheres with probes covalently attached
2. Denaturation Buffer to denature double-stranded DNA
3. Neutralization Buffer neutralize the pH
4. Hybridization reaction buffers to facilitate the binding of target DNA to the probe
5. Wash Buffer to wash off unbound DNA
6. SAPE buffer for diluting Stock SAPE solution
7. DNA amplification reagents (pre-optimized HLA loci-specific primer mix): These reagents are lot specific and are not interchangeable between lots.
8. D-mix (specially formulated amplification buffer mix).

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
9. Caution: PCR plate must have tight contact with heating block.
10. Tray seal (OLI Cat. #SSPSEA300 or equivalent)
11. Note: PCR trays (25) and tray seals (180) sufficient for 2400 samples can be ordered from One Lambda (OLI Cat. #PCRTRAC)
12. Electrophoresis apparatus/power supply—150V minimum capacity (Micro SSP™ Gel System, OLI Cat. #MGS108 or equivalent)
13. UV transilluminator (Fotodyne FOTO/UV®21 or equivalent)
14. Photographic or image documentation system
15. 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)
16. Electrophoresis grade agarose (e.g., FMC Seakem® LE or equivalent)
17. PCR Pad
18. Crushed ice

C. Materials Recommended, but not provided:

1. 1.5 ml microfuge tube
2. Pipette tips (Rainin -GPS 10G, 250, 1000)

3. 96-well, 250 µl V-bottom, white polystyrene microplate with a non-treated surface

D. Directions for Use

1. Pre Set-Up
   a. Turn on LABScan3D™, FlexMap 3D analyzer, and begin the start-up procedure. Turn on the thermal cycler, and start 60°C incubation program.
   b. Prepare crushed ice bath (add small amount of water to allow PCR tray to stand straight on ice)
   c. Thaw and vortex D-Mix and DNA.
   d. Remove all reagents (except 100x SAPE bottle) from storage temperature and use at room temperature.
   e. Thoroughly mix entire volume of Hybridization buffer and entire Bead Mixture in a clean tube; protect from light.
2. **Amplification** *(for 96-Sample Assay)*
   a. Thaw all amplification reagents, and place on ice.
   b. Aliquot 2 µl genomic DNA to each of 96 wells in a PCR tray.
   c. Mix 432 µl of Primer Mix, 1491 µl of D-Mix, and 22 µl of Taq polymerase. Vortex well and give a quick spin.
   d. Aliquot 18 µl of Amplification Mix from Step 3 into all 96 wells containing DNA.
   e. Cap or seal the PCR tray.
   f. Run the tray in a PCR oven using the LABType® XR and CWD PCR program.

Table 1: LABType® XR CWD 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

   g. Remove the PCR tray from the PCR oven, and check the amplified DNA on a 2.5% agarose gel (use 5 µl per well).

3. Denaturization/Neutralization

   a. In a clean, thin-walled 96-well PCR tray, aliquot 2.5 µl of Denaturation Buffer per well.
   b. 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.
   c. Mix thoroughly until the mixture changes to a bright pink color.
   d. Incubate at room temperature (20° to 25° C) for 10 minutes.
   e. Add 5 µl per well of Neutralization Buffer.
   f. Mix thoroughly until the mixture turns clear or pale yellow.
   g. Place tray carefully on the ice bath.

4. Hybridization/Washing
   a. Aliquot 38 µl Hybridization Mixture (from A.5.above) per well into all neutralized DNA.
   b. Place a seal on the tray and vortex thoroughly at low speed.
   c. Incubate the tray in a 96-well block in a 60°C thermal cycler (use PCR Pad) for 15 minutes.
   d. 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.
   e. Remove supernatant, leaving approximately 10 µl or less.

f. Repeat Steps D.4 and D.5 two more times for total of 3 washes.
g. During the last centrifugation step, prepare 1X SAPE (57.5 µl Stock and 5693 µl SAPE Buffer) and leave covered at room temperature.

5. Labeling
   a. After removal of supernatant from the third wash (D.6 above), add 50 µl 1X SAPE per well.
   b. Place a seal carefully on the tray and vortex thoroughly at low speed.
   c. Incubate at 60°C in thermal cycler as above for 5 minutes.
   d. 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.
   e. Remove supernatant. Add Wash Buffer to make the final volume 80 µl.
   f. Mix by pipetting and transfer all samples to a 96-well microplate for data acquisition.

RESULTS

A. Data Acquisition

1. Read plate on LABScan 3D™.

B. Data Analysis

1. The LABType XR and CWD products require the use of HLA Fusion Beta version for RUO.

LIMITATIONS OF THE PROCEDURE

- **For Research Use Only.** This product is not intended to provide information for the diagnosis, prevention or treatment of disease or to aid in the clinical decision making process. This product is not cleared or approved for clinical use by the FDA or approved in the EU as an in vitro diagnostic assay, nor is it CE marked.

TRADEMARKS AND DISCLAIMERS

The LABType® typing reagents are manufactured and distributed by One Lambda, Inc., now part of Thermo Fisher Scientific., at 21001 Kittridge Street, Canoga Park, CA 91303, U.S.A.
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EXPLANATION OF SYMBOLS

Symbol	Description
REF	Catalogue number
	Temperature limitation
	Manufacturer

REVISION HISTORY

Revision	Date	Revision Description
0	2013/12	Initial Release
1	2015/03	Transferred to new template; added Denaturation Buffer to Materials Provided.