LABScreen®

Antibody Detection

Features & Benefits

Quality

Proven Accuracy

- Distinguishes HLA Class I and Class II antibodies
- Contains a defined pool of HLA antigens, including rare alleles
- Eliminates false positive reactions due to non-HLA antibodies or auto antibodies
- Single antigen assay identifies negative or safe antigens, even for high PRA patients
- Detects IgG antibodies

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Location: Moldova

Premier Automation

- Based on Luminex® xMAP® technology
- Provides software-driven data acquisition

Maximum Consistency

- High reproducibility
- Delivers reaction-to-reaction consistency

PRA Screening and Specificity Assignment Utilizing Advanced Flow Analysis Technology

ONE LAMBDA
A Thermo Fisher Scientific Brand

LABScreen®

Using Luminex® xMAP® technology, 100 or more analytes may be tested simultaneously. The LABScreen® product line consists of color-coded microbeads coated with purified HLA Class I and Class II antigens. The beads are analyzed using the LABScan™ 100 flow analyzer and interpretation software.

LABScreen® Single Antigen

Single antigen assays provide a unique solution to the dilemma presented by high PRA patients. In these patients, antibody reactive to one or more dominant epitopes can mask the presence of additional antibody specificities. These specificities can now be identified by single antigen technology.

LABScreen® Single Antigen Supplement

A single antigen panel designed to screen antibodies against HLA antigens found in higher frequencies among certain ethnic populations.

LABScreen® Single Antigen MICA

A single antigen panel designed to identify MICA antibodies.

LABScreen® PRA

Determines percent PRA and identifies antibody specificities using HLA antigens purified from different cells. HLA Class I and Class II PRA tests may be used separately or together.

LABScreen® Mixed

Tests for the presence of HLA Class I and Class II antibodies, as well as MICA antibodies, with a single tube protocol. Well suited for monthly patient antibody screens in both low and high throughput laboratories.

LABScreen® Multi

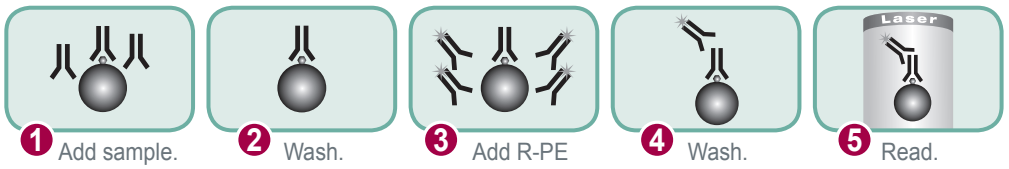
Tests for the presence of HLA Class I and Class II, and identifies HNA antigens.

LABScreen® Singles

HLA Class I and Class II antigens are also offered as Singles. Each LABScreen® Single Antigen is packaged separately, allowing you to order only the antigens needed.

Principle

Detection by R-Phycoerythrin Conjugated Antibody



LEGEND: – sample antibody – R-PE conjugated Anti-IgG – LABScreen® bead with HLA antigens

To learn more about LABScreen® and other quality products, contact your One Lambda representative today, or visit us at ***www.onelambda.com***

ONE LAMBDA
A Thermo Fisher Scientific Brand
21001 Kittridge St., Canoga Park, CA 91303-2801, USA
TEL: 818.702.0042 800.822.8824 (except greater L.A. area)
FAX: 818.702.6904 800.992.2111 (U.S. and Canada only)
INTERNATIONAL: Contact your local distributor

CE 0197 TÜV ISO 13485 Registered POSITIVE IVD

Order Information

For In Vitro Diagnostic Use.
(Unless otherwise stated)

LABScreen® Single Antigen

Class I

LS MICA SAg - Group 1 – 25 tests
(Catalog ID: LSMICA001)

LS SAg - Combi – (A Locus/B Locus/ C Locus) – 25 test
(Catalog ID: LS1A04)

LS SAg Class I Supplement – Group 1 – 25 tests
(Catalog ID: LS1ASP01)

Class II

LS SAg - Group 1 – (DRB1/DRB3,4,5/ DQB1) – 25 tests
(Catalog ID: LS2A01)

LS SAg Class II Supplement – Group 1 – 25 tests
(Catalog ID: LS2ASP01)

LABScreen® PRA

LS PRA HLA Class I – 25 tests
(Catalog ID: LS1PRA)

LS PRA HLA Class II – 25 tests
(Catalog ID: LS2PRA)

LS PRA HLA Class I & II – 25 tests
(Catalog ID: LS12PRA)

LABScreen® Mixed

LS Mixed HLA Class I & II – 100 tests
(Catalog ID: LSM12)

LABScreen® Multi

LABScreen Multi – 100 tests
(Catalog ID: LSMUTR)

This product is not associated with the diagnosis or treatment of TRALI.

LABScreen® Singles - 10 tests

(visit www.onelambda.com for details)

Ancillary Products

Adsorb Out – 25 tests*
(Catalog ID: ADSORB)

LS Negative Control Serum – 20 tests
(Catalog ID: LS-NC)

PE – Conjugated Goat Anti-Human, Lyophilized, 1ml – 1000 tests
(Catalog ID: LS-AB2)

Quantiplex Beads**
(Catalog ID: LXQNTPLX)

*For General Laboratory Use.
**For Investigational Use Only.

LABScreen™

REF

Catalog ID	Product Name
LS1PRA*	LABScreen™ PRA Class I
LS2PRA*	LABScreen™ PRA Class II
LS12PRA*	LABScreen™ PRA Class I & II
LSM12*	LABScreen™ Mixed Class I & II
LS1A04*	LABScreen™ Single Antigen HLA Class I - Combi
LS1ASP01*	LABScreen™ Single Antigen HLA Class I Supplement - Group 1
LS2A01*	LABScreen™ Single Antigen HLA Class II - Group 1
LS2ASP01*	LABScreen™ Single Antigen HLA Class II Supplement - Group 1
LSMICA001	LABScreen™ MICA Single Antigen - Group 1
LSPWABUF	LABScreen™ Wash Buffer

IVD For In Vitro Diagnostic Use.

INTENDED USE

LABScreen products are intended for use in detection of HLA antibody using flow cytometric technology

SUMMARY AND EXPLANATION

LABScreen products use microbeads coated with purified Class I or Class II HLA antigens and pre-optimized reagents for the detection of Class I or Class II HLA antibodies in human sera. LABScreen products utilize the LABScan™ 100 (Luminex® 100/200) or LABScan3D™ (Luminex® FLEXMAP 3D®) for analysis of up to 100 or 500 bead regions, respectively, in a single test.

The Mixed assay detects the presence of antibody to Class I and/or Class II HLA antigens. The PRA tests can detect antibodies and their specificities against the HLA antigens in each LABScreen panel. The Single Antigen assay allows confirmation of antibody specificity suggested by a previous PRA test, while individual Singles beads are used to focus on reactions against one or a few antigens, e.g. to compare reactivity of different serum samples from the same individual. A negative control serum is used to establish the background value for each bead in a test batch.

PRINCIPLE(S)

Test serum is incubated with LABScreen beads. Any HLA antibodies present in the test serum bind to the antigens on the beads and then are labeled with R-Phycoerythrin (PE)-conjugated goat anti-human IgG. The LABScan™ 100 or LABScan3D™ flow analyzer(s) simultaneously detects the fluorescent emission of PE and a dye signature from each bead, allowing almost real-time data acquisition. To assign PRA and HLA specificity, the reaction pattern of the test serum is compared to the lot-specific worksheet defining the antigen array.

REAGENTS

A. Identification

See LABScreen Reference Table for product description.

IVD B. Warning or Caution

One Lambda Inc., A Thermo Fisher Scientific Brand
21001 Kittridge Street, Canoga Park, CA 91303 U.S.A.
T: 818.702.0042 F:818.702.6904

1. **Warning**: LABScreen PRA test reagents contain 0.1% sodium azide (NaN_3) as a preservative. Under acidic conditions, sodium azide yields hydrazoic acid, an extremely toxic compound. Dilute reagents containing sodium azide in running water before discarding, to avoid deposits in plumbing where explosive conditions may develop. (Refer to Material Safety Data Sheet for detail.)
2. **Warning**: All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
3. **Caution**: For manual flicking of trays, use a quick downward arm motion without wrist movement to prevent repetitive motion effects.
4. Refer to the Material Safety Data Sheet for detailed information.

C. Preparing Reagents for Use
   1. See Directions for Use, below.
   2. If buffer salts have precipitated out of solution during shipment or storage, re-dissolve by gently warming before preparing working dilution.

D. Storage Instructions
   1. LABScreen products are shipped to the end user on dry ice. The entire package may be stored in a freezer at -65° C or below until first use, up to the labeled expiration date.
   2. Once beads are thawed, DO NOT REFREEZE. Store at 2 - 8° C for up to three months or until the expiration date (if earlier).
   3. After first use, store wash buffer at 2 - 8°C for up to three months or until the expiration date, if earlier.

E. Purification or Treatment Required for Use
   See Directions for Use, below.

F. Instability Indications
   None

INSTRUMENT REQUIREMENTS

A. Required Equipment

- LABScan™ 100 flow analyzer (Luminex® 100/200) with Luminex® XY platform (for automated 96-sample data acquisition) and sheath fluid delivery system (OLI Cat. # LABSCNXS3) OR LABScan3D™ flow analyzer (Luminex® FLEXMAP 3D®) with XY platform and sheath fluid delivery system (OLI Cat. # LABSCNXS4)
- Centrifuge
- Rotor for 1.5 ml microcentrifuge tube (9,300 g), or a swinging bucket rotor for 96-well microplate (1,300 g)
- Vortex mixer
- Plate shaker or rotating platform

For Filter Plate Option:

- Vacuum manifold, 96-well (Millipore Cat. # MAVM0960R or equivalent)
- Vacuum pump with a pressure less than 100 mm Hg
- Plate shaker or rotating platform

B. Equipment Calibration

Follow manufacturer's instructions for calibration of the LABScan™ 100 or LABScan3D™ flow analyzer.

C. Recommended Software

HLA Fusion™ (OLI Cat. # FUSPGR)

SPECIMEN COLLECTION AND PREPARATION

- Unopened blood specimens may be kept at room temperature up to 4 days. Separated serum (from clotted samples) or plasma (in ACD or K-EDTA) may be refrigerated up to 7 days, or aliquots may be frozen at -20°C or below and thawed just before the assay. Aggregates should be removed from the test serum/plasma by centrifugation (8,000 – 10,000 g for 10 minutes) or filtration (0.2 µm) prior to testing. Any aggregates or contamination of the sample may generate invalid results.
- Samples may be treated or diluted to reduce non-specific background or to remove inhibitory factor – see limitation section.

  Note:

  - Test serum or plasma should not be heat inactivated, because it may give a high background in the test.
  - Undiluted serum or plasma is generally used for the test. However, if a high background serum sample is diluted for this assay, the negative control serum should be tested at the same dilution.

PROCEDURE

A. Materials Provided

1. See the LABScreen Reference Table (LS-RFTB-PI-XX-00) in Product Documentation on the One Lambda, Inc. web site for a list of materials provided for each product.
2. The volumes provided exceed the amount required for testing by a small amount to allow for pipetting losses.

B. Materials Required, But Not Provided

1. PE–Conjugated Goat Anti-Human IgG (OLI Cat. # LS-AB2)
2. PBS, filtered [USA Scientific Cat. # 9242 (500 ml 10X) or equivalent]
3. 1.5 ml microcentrifuge tube (USA Scientific Cat. # 1415-2500 or equivalent)
4. Pipette tips (Rainin GPS)
5. Negative Control Serum, containing no HLA antibody when tested by LABScreen method (OLI Cat. # LS-NC or equivalent)

If the test is performed in a 96-well microplate:

1. 96-well microplate, 250 µl, non-treated surface (Whatman Cat. # 7701-3250 or equivalent)
2. Tray seal (OLI Cat. # SSPSEA300 or equivalent)

For the Filter Plate option:

1. Filter plate (Multiscreen-BV, Millipore Cat. No. MABVN1250 or equivalent)

C. Directions for Use

Notes:

- Take special care in the aliquoting process. Failure to follow the steps described below may result in reagent loss.
- Sections A through C indicate the volumes of reagents needed for testing a single bead group. If you are running a combined test, see Section D before proceeding.
- Turn on the LABScan 100™ or LABScan3D™ flow analyzer at least 30 minutes before starting the assay.
- Create a filename and sample code sheet for each test tray.

I. For each test batch, test a negative control serum (e.g. OLI Cat. # LS-NC or equivalent) to establish background values. To complete the test in a 1.5 ml microcentrifuge tube

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.
2. Incubate 5 µl of LABScreen beads with 20 µl of test serum in a 1.5 ml micro-centrifuge tube for 30 minutes, in the dark at 20 - 25º C with gentle shaking.
3. Dilute 10X wash buffer (OLI Cat. # LSPWABUF) in distilled water to make a 1X solution.
4. Add 1 ml of 1X wash buffer to each bead/serum solution tube and vortex. Centrifuge at 9,300 g for 2 minutes. Aspirate and discard the supernatant.
5. Repeat Step 4 twice.
6. Dilute 1 µl per test of 100X PE-conjugated anti-human IgG (OLI Cat. # LS-AB2) with 99 µl of 1X wash buffer to make a 1X solution.
7. Add 100 µl of 1X PE-conjugated anti-human IgG to each tube. Vortex and then incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
8. Repeat Step 4 twice.
9. Add 80 µl 1X PBS to each tube. Proceed to data acquisition and analysis, or store tray at 2 - 8º C in the dark for up to 24 hours before analysis.

II. To complete the test in a 96-well plate

Caution: Seal the 96-well tray carefully and completely to prevent well-to-well sample contamination 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.

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.
2. Incubate 5 µl of LABScreen beads with 20 µl of test serum in each well of a 96-well plate for 30 minutes, in the dark at 20 - 25º C with gentle shaking.
3. Dilute 10X wash buffer (Cat. # LSPWABUF) in distilled water to make a 1X wash solution.
4. After incubation, add 150 µl of 1X wash buffer to each well of the plate. Cover with tray seal (OLI Cat. # SSPSEA300 or equivalent) and vortex. Centrifuge at 1,300 g for 5 minutes.
5. Remove wash buffer from wells of plate by flicking or with vacuum aspiration.
6. Add 200 µl of 1X wash buffer to each well of the plate. Cover with a new tray seal and vortex. Centrifuge at 1,300 g for 5 minutes.
7. Remove supernatant from wells of plate by flicking or with vacuum aspiration.
8. Repeat Steps 6 and 7.
9. Dilute 1 µl per test of 100X PE-conjugated anti-human IgG (OLI Cat. # LS-AB2) with 99 µl of 1X wash buffer to make a 1X solution.
10. Add 100 µl of 1X PE-conjugated anti-human IgG to each well. Cover with tray seal and vortex. Incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
11. Centrifuge at 1,300 g for 5 minutes.
12. Remove supernatant from wells of plate by flicking or with vacuum aspiration.
13. Repeat Steps 6 and 7 twice.
14. Add 80 µl 1X PBS to each well. Cover with a new tray seal and vortex. Proceed to data acquisition and analysis, or store tray at 2 - 8º C in the dark for up to 24 hours before analysis.

III. To complete the test in a 96-well filter plate

1. Mix the LABScreen beads well by gently vortexing or pipetting up and down several times prior to use.
2. Dilute 10X wash buffer (OLI Cat. # LSPWABUF) in distilled water to make a 1X solution (approximately 3.2 ml/tray/wash).

3. Cover any wells of the plate that will remain unused during the test with a tray seal to assure that the unused wells remain dry. Pre-wet filters in the filter plate by dispensing 300 µl wash buffer into only those wells that will be used for the assay.
4. Incubate the plate for 10 minutes on a platform plate shaker at low speed.
5. Aspirate all wash buffers from the wells using a Millipore vacuum manifold. Do not exceed 100 mm Hg vacuum pressure!
6. Add 5 µl of LABScreen beads and 20 µl of test serum per test well.

   Note: During bead and sample dispensing steps, press pipette tip gently against filter plate well to avoid filter rupture.
7. Incubate the plate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
8. Add 175 µl wash buffer per well.
9. Turn on vacuum pump. Press the plate firmly on the vacuum manifold. Make sure liquid drains out slowly. Make sure all liquid has drained from the wells before proceeding.

Caution: Do not exceed 100 mm Hg vacuum pressure. A rapid vacuum will cause loss of beads due to be entrapment in the pores of the filter paper.

10. Repeat Steps 8 and 9, above, four times.
11. Add 100 µl of 1X PE-conjugated anti-human IgG to each well.
12. Incubate in the dark for 30 minutes at 20 - 25º C with gentle shaking.
13. Repeat Steps 8 and 9 five times.
14. Add 80 µl of 1X PBS to each well.
15. Read sample on the LABScan 100™ or LABScan3D™ flow analyzer, adjusting probe height if necessary.

IV. Combined tests

Any of the above protocols can be used for a combined test of certain LABScreen products.

- For acceptable lot combinations of LS12PRA see www.onelambda.com
  (Antibody Detection>LABScreen>LABScreen PRA/ Product Documentation: LABScreen Bead Combo – Multiple IDs DataSheet).
- Do not combine LABScreen Single Antigen Class I Combi and Class II panels (bead IDs would overlap).

1. Mix equal volumes of beads. Then dispense the appropriate aggregate amount (10 or 15 µl) of bead mixture per test.
2. Bead combinations and amounts to dispense are listed in the table below.

Catalog ID	Bead volume per Test	Control (NC/PC) Beads	Test Serum per Test
LS12PRA (CI and CII beads)	5 µl + 5 µl	Included	40 µl
LS1A04	5 µl	Included	20 µl

RESULTS

A. Data Acquisition

1. Set up the LABScan 100™ or LABScan3D™ flow analyzer for sample acquisition and calibration according to the Luminex User's Manual.[1]
2. Choose a template according to product kit catalog ID and lot number.
   a. Acquisition templates are available from OLI by CD or via our download website.
   b. To create your own acquisition template, follow instructions in the Acquisition chapter of the Luminex User's Manual.

c. Luminex software versions - LABScan 100 (xPONENT 3.1 or xPONENT 4.2); LABScan 3D (xPONENT 4.2) must be used.

NOTE: Luminex xPONENT 3.1 templates will not be supported after March 30, 2018.

3. Create a file name for the samples to be run.
4. Make sure all the template settings are correct. Template specifications are:
   a. Set sample volume to 50 µl.
   b. Set sample time-out to 80 seconds.
   c. Set doublet discriminator gate to 8,000 (low limit) and 16,000 (high limit).
   d. Set number and ID of beads selected according to the product-specific worksheet provided with the product.
   e. Set minimum events collected to 100 per bead.
5. Enter the sample IDs (if the same sample is tested more than once, assign a different ID).
6. Load the plate onto the XY platform and fill the reservoir with sheath fluid.
7. Click the START button to initiate the session. After the samples have finished running, save the data output in a .csv file.
8. Wash the machine twice with sheath fluid at the end of the session.

B. Data Analysis

1. The reactivity of a test sample is calculated from the "raw" fluorescence values recorded by the LABScan device (.csv file) for each HLA coated bead.
2. Calculate anti-HLA serum reactivity by correcting for non-specific binding to the negative control bead and background values (obtained by testing with a negative control serum (OLI Cat. # LS-NC) to determine the normalized background ratio (NBG ratio). See Calculations, below.
3. For LABScreen PRA or LABScreen Single Antigen, the normalized fluorescent value for each HLA coated bead equals the value of that bead divided by the value of the NC bead. (For LABScreen Mixed, the normalized fluorescent signal equals the value of the Class I or Class II coated bead minus the value of the NC bead.)

   Note: The fluorescent signal (value) can be either the trimmed mean or median value.

C. Calculations

1. The abbreviations used in this section are defined below:

NBG ratio	Normalized Background ratio used to assign strength of each anti-HLA reaction
S#N	Sample-specific fluorescent value for bead #N
SNC bead	Sample-specific fluorescent value for Negative Control bead
BG#N	Background NC Serum fluorescent value for bead #N
BGNC bead	Background NC Serum fluorescent value for Negative Control bead
NC Serum	Negative Control Serum (OLI Cat. # LS-NC) validated for a given lot of LABScreen beads

2. For LABScreen PRA or LABScreen Single Antigen:

$$\text{NBG ratio} = \frac{\text{S\#N / SNC bead}}{\text{BG\#N / BGNC bead}}$$

For LABScreen Mixed:

$$\text{NBG ratio} = \frac{\text{S\#N - SNC bead}}{\text{BG\#N - BGNC bead}}$$

Note: If (BG#N-BGNC bead) <50 then use 50 as a default threshold value.

D. Determination of Positive/Negative Cut-Off

1. For LABScreen PRA and LABScreen Mixed:
   a. Select the NBG ratio that gives a significant shift over background fluorescent value when the background value is obtained using the negative control serum in 3 - 5 replicate tests. If you prefer,

test 5 - 10 serum samples from non-transfused, non-transplanted male donors to obtain an average background value.

b. Validate the cut-off using 5 - 10 reference alloserum samples with defined HLA antibody specificity. The NGB ratio values for expected positive antigen reactions should be above the cut-off.

c. Additional positive/negative reactions may be noted. If necessary, adjust the LABScreen assay cut-off to match the sensitivity of a previously accepted antibody detection assay.

d. For high PRA serum, the patient's own antigen(s) may show weak positive reactions. For such cases, the fluorescence value for the patient's own antigen may be used as the cut-off.

2. For LABScreen Single Antigen:

a. Test negative control serum or several negative serum samples (see 1a, above).

b. Define working range:

Working Range = NBG ratio maximum - NBG ratio minimum

c. Define cut-off points within the working range:

Relative NBG ratio cut-off = X% (working range) + NBG ratio minimum, where X% = user-defined percent cut-off point within the working range for negative (1), gray area(2), weak positive (4) and strong positive (8).

d. Set criteria to define positive vs. negative reactions, for example:

(1) If [NBG ratio max/NBG ratio min] >8, apply the calculation in 2c.

(2) If [NBG ratio max/NBG ratio min] <8 AND

(a) NBG ratio max >5, then NBG ratio min should be adjusted to one half of the NBG ratio max and the relative NBG ratio cut-off should be re-computed (as in 2c) based on the adjusted NBG ratio min. The reaction is then scored as above.

(b) NBG ratio max <5, then the reaction of the test serum with that bead is negative. Assign a score of "1".

e. Test several reference allosera as in 1b above, using the relative NBG ratio to validate the cut-off.

(1) Establish a strong and weak reactivity cut-off based on the performance of the reference allosera, relative to an established assay.

(2) It may be helpful to plot the NBG ratio values in a histogram for visualization of the HLA reactivity pattern of each serum.

3. Higher or lower sensitivities can be obtained by adjusting the cut-off.

4. Optional analysis – HLA Fusion™ software.

LIMITATIONS OF THE PROCEDURE

- Sera or plasma samples that contain contaminants or aggregates may clog the LABScan flow analyzer and generate inaccurate data. Aggregates in the test specimen should be removed by centrifugation or filtering the serum prior to testing.
- The presence of IgG-IgM immune complex may cause inhibition in some patient samples. Samples should be treated to reduce this presence according to the protocols determined by the laboratory, however, samples should not be heat treated as they may cause non-specific background – please reference bibliography section for more information.[7,8,9,10, 11]
- Ambient temperature may affect LABScan 100™ and LABScan3D™ performance. If the ambient temperature changes, the machine may need to be re-calibrated. Consult the manufacturer's manual for more information.
- The LABScan 100™ and LABScan3D™ flow analyzer must be properly calibrated and maintained. If insufficiently flushed, aggregates of the sample may cause the machine to clog and generate invalid data.

- Assignment of antibody specificity is limited to the HLA antigens presented in each bead panel (see lot-specific worksheet).
- The bead region used for each antigen and the antigen composition of the panel may change from lot-to-lot of product (see the lot specific worksheet).
- 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. LABScreen PRA Class I or Class II

- The reactivity strength of a test serum to each bead can be compared to distinguish the strong positive, weak positive and negative reactions. Reactivity ratios can be ranked within different ranges, if a scoring system is desired.
- Our data show NBG ratios > 1.5 in the LABScreen PRA test (using the LABScan™ 100) correlate well with positive reactions in the FlowPRA test.
- For calculation of percent PRA (Panel Reactive Antibody), divide the number of positive reactions by the number of valid reactions for that test serum.
- To determine the specificity of HLA antibody, enter the reaction score into the lot specific Worksheet to analyze the reaction pattern.

B. LABScreen Mixed

- Score HLA Class I and Class II reactions separately, according to reactivity strength of the serum for each bead set.
- If anyone bead in the mixed assay is positive, then the result should be assigned as positive.
- Our data show that NBG ratios >2.2 in the LABScreen Mixed test (using the LABScan™ 100) correlate well with positive reactions in LAT™ Mixed.

C. LABScreen Single Antigen

- Allosera may produce signal/background ratios that are much higher than those obtained in the PRA assay. Establishing the assay cut-off(s) using the relative NBG ratio is one way of normalizing the data (see Results, Section D-2c).
- Our data show that a positive/negative cut-off or relative NBG ratio >15% of the working range NBG ratio calculated for each test serum (using the LABScan™ 100) will give results comparable to the LABScreen PRA assay.

D. General Guidelines

- Each bead count should be over 50. A lower bead count may be due to sample loss during the wash steps. It could also be due to improper calibration or clogging of the LABScan™ 100 or LABScan3D™ flow analyzer, or by photo-bleached beads that dropped out from the mapped region.
- Signal values are the fluorescence intensity of each bead set vs. the test serum. A negative control serum should be tested with the same batch of samples to establish the background value(s) for that test run.
- Negative Control Serum (OLI Cat. # LS-NC or equivalent) is recommended. Using any other negative control serum may require adjustment of cut-off values.
- Negative Control Beads (Ag ID = NC) are not coated with HLA antigen. The fluorescence value may vary among different sera due to non-specific binding of the sera or to insufficient washing. The NC value is usually less than 500 except for serum samples with a high background. It should always be lower than 1500 and less than or equal to half of the PC value.
- Positive Control Beads are coated with purified human IgG, which should bind to the secondary antibody to produce a positive signal. The PC value should be over 500 and at least twice the NC value.

E. Validation of the Assay

- The cut-off value of signal to background should be validated if a new negative control serum is used.
- For a given serum, the value for PC/NC should be greater than 2. A lower value may be due to an extremely high NC bead background value for the test serum, a high HLA bead signal for the NS control, or a low signal from the secondary antibody or the LABScan™ 100 and LABScan3D™ flow analyzer. In this case, the data may have to be confirmed.
- Each user should evaluate the performance of the assay in their laboratory to validate the cut-off value(s) selected.
- Plasma samples may give lower FI or higher background values than serum. The user may wish to normalize the data if comparing results between sera and plasma samples (see Reference 5) for the same or different test subjects.

SPECIFIC PERFORMANCE CHARACTERISTICS

A. Using the assay cut-offs referenced under Expected Values, above, LABScreen assays have given results comparable to the results of the One Lambda FlowPRA® and LAT™ assays. However, HLA antibody patterns may be quite complex. A given test sample may contain several HLA Class I and Class II antibody specificities, each with a different avidity; however, not all specificities will be recognized in assays with lesser sensitivity. Therefore, each laboratory should establish and validate the assay cut-offs for their own use based on their expertise in recognizing HLA CREG patterns and an evaluation of the assay performance using HLA allosera with defined specificities.

B. Comparison of serum vs. plasma for 1,000 blood donors in the NIHLBI REDS-II study (5) showed good correlation within the working range of the assay. For anti-HLA CI and CII antibodies the R2 values were 0.88 and 0.91, respectively. However, the NBG ratio was generally 1.3-fold higher for serum samples.

C. If high background is seen, this may indicate improper washing during the test protocol. High negative control background may cause inaccurate normalized MFI values.

D. Clinical performance testing was conducted for LABScreen products at three different clinical sites, using 240 random samples – See Table A. Clinical Performance.

E. Clinical reproducibility testing was conducted for LABScreen products at three different clinical sites using 16 (LS1PRA, LS2PRA, LS1A04) and 32 (LSM12) samples, consisting of 10 runs each, in triplicate – see Table B. Clinical Reproducibility.

F. Clinical testing used a default cut off value, with scores of >4 were considered positive.

Table A - Clinical Performance

LSM12

LABScan 100	LABScan 3D +	LABScan 3D -
+	573	11
-	4	119
Undefined		40
Total Defined		707

	Positive Agreement	Negative Agreement	Overall Agreement (excluding undefined)	Overall Agreement (including undefined)
Point estimate	98%	97%	98%	93%
One-sided 95% lower confidence limit	97%	93%	97%	91%

LS2PRA

LABScan 100	LABScan 3D +	LABScan 3D -
+	939	57
-	187	7781
Total		8964

	Positive Agreement	Negative Agreement	Overall Agreement
Point estimate	94%	98%	97%
One-sided 95% lower confidence limit	93%	97%	97%

LS1PRA

	LABScan 3D +	LABScan 3D -
+	2060	260
-	446	16905
Total		19671

	Positive Agreement	Negative Agreement	Overall Agreement
Point estimate	89%	97%	96%
One-sided 95% lower confidence limit	88%	96%	96%

LS1A04

	LABScan 3D +	LABScan 3D -
+	3245	214
-	682	12062
Total		16203

	Positive Agreement	Negative Agreement	Overall Agreement
Point estimate	94%	95%	94%
One-sided 95% lower confidence limit	93%	94%	94%

Table B - Clinical Reproducibility

LSM12	Overall Agreement (excluding undefined)	Overall Agreement (including undefined)
Point estimate	98%	93%
One-sided 95% lower confidence limit	97%	93%

LS1PRA	Overall Agreement	LS2PRA	Overall Agreement	LS1A04	Overall Agreement
Point estimate	99%	**Point estimate**	99%	**Point estimate**	98%
One-sided 95% lower confidence limit	99%	**One-sided 95% lower confidence limit**	99%	**One-sided 95% lower confidence limit**	98%

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2. Luminex® FLEXMAP 3D® Hardware User Manual, Luminex Corporation PN 89-00002-00-187 Rev. B
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TRADEMARKS AND DISCLAIMERS

All One Lambda products are designed to assist personnel experienced in HLA analysis by suggesting typing results or antibody assignments. All test results must be carefully reviewed by such qualified personnel to assure correctness.

EUROPEAN AUTHORIZED REPRESENTATIVE

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

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
	Manufacturer
EC REP	Authorized representative in the European Community

REVISION HISTORY

Revision	Date	Revision Description
25	2016/08	Addition of xPONENT version 4.2 for the LABScan 100
26	2016/10	Update to product limitation sections and bibliography section.
27	2017/05	Added catalog IDs and product descriptions. Added note to RESULTS section: Luminex xPONENT 3.1 templates will not be supported after March 30, 2018. Added catalog IDs and product descriptions.
28	Current	added details to the specimen and collection preparation section

CE 0197 CE

*0197 Applies to Annex II List B products only.

LABScreen™

PRA Screening and Specificity Assignments Utilizing Flow Analysis Technology

Key Benefits

Proven Accuracy

- Distinguishes HLA Class I and Class II antibodies
- Contains a purified single antigen or a defined pool of HLA antigens, including rare alleles
- Eliminates false positive reactions due to non-HLA antibodies or auto antibodies
- Single antigen assay identifies negative or safe antigens, even for high PRA patients
- Detects IgG antibodies

Premier Automation

- Based on Luminex® xMAP® technology
- Provides software-driven data acquisition

Maximum Consistency

- High reproducibility
- Delivers reaction-to-reaction consistency

PRA Screening and Specificity Assignment Utilizing Advanced Flow Analysis Technology

LABScreen reagents are powered by Luminex xMAP technology, a microbead platform used to deliver multiplex antibody assays. This antigen-bead based assay allows for a precise determination of antibody profiles against HLA and MICA. The proven reliability of LABScreen's consistency, high sensitivity and robustness for PRA screening has gained rapid momentum in the transplant community.

The LABScreen product line consists of color-coded microbeads coated with purified HLA Class I, Class II and MICA antigens. The beads are analyzed using Luminex xMAP multiplex technology.

LABScreen Single Antigen

Single antigen assays provide a unique solution to the dilemma presented by high PRA patients. In these patients, antibody reactive to one or more dominant epitopes can mask the presence of additional antibody specificities. These specificities can now be identified by single antigen technology.

LABScreen Single Antigen Supplement

A single antigen panel designed to screen antibodies against HLA antigens found in higher frequencies among certain ethnic populations.

LABScreen Single Antigen MICA

A single antigen panel designed to identify MICA antibodies.

LABScreen PRA

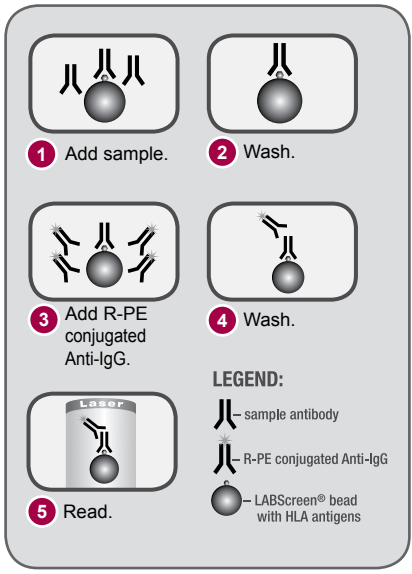
Determines percent PRA and identifies antibody specificities using HLA antigens purified from different cells. HLA Class I and Class II PRA tests may be used separately or together.

LABScreen Mixed

Tests for the presence of HLA Class I and Class II antibodies, as well as MICA antibodies, with a single tube protocol. Well suited for monthly patient antibody screens in both low and high throughput laboratories.

Principle

Detection by R-Phycoerythrin Conjugated Antibody



Ordering Information

Product	Tests	Cat. No.
For In Vitro Diagnostic Use. (Unless otherwise stated.) CE 0197		
LABScreen™ Single Antigen Class I		
LABScreen™ MICA Single Antigen - Group 1	25 tests	LSMICA001
LABScreen™ Single Antigen HLA Class I - Combi	25 tests	LS1A04
LABScreen™ Single Antigen HLA Class I Supplement - Group 1	25 tests	LS1ASP01
LABScreen™ Single Antigen Class II	25 tests	
LABScreen™ Single Antigen HLA Class II - Group 1		LS2A01
LABScreen™ Single Antigen HLA Class II Supplement - Group 1	25 tests	LS2ASP01
LABScreen™ PRA		
LABScreen™ PRA Class I	25 tests	LS1PRA
LABScreen™ PRA Class II	25 tests	LS2PRA
LABScreen™ PRA Class I & II	25 tests	LS12PRA
LABScreen™ Mixed		
LABScreen™ Mixed Class I & II	100 tests	LSM12
Ancillary Products		
LABScreen™ Negative Control	20 tests	LS-NC
PE Conjugated Goat Anti-Human IgG	1000 tests	LS-AB2

Find out more at **onelambda.com**

ONE LAMBDA
A Thermo Fisher Scientific Brand

21001 Kittridge Street
Canoga Park, CA 91303-2801, USA
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(except greater LA area)
FAX: 818.702.6904 | 800.992.2111
(U.S. and Canada Only)
INTERNATIONAL: Contact your local distributor

TÜV ISO 13485 Registered

LABType SSO tests

Class I and Class II typing

Key benefits

Proven accuracy

- Typing spans A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1/DQB1, DPA1/DPB1, and KIR loci

Maximum functionality

- Reduced testing time compared to a multi-well format assay
- Processing 96 samples at one time dramatically reduces labor and reaction-to-reaction inconsistencies
- Rapid data acquisition with either the One Lambda™ LABScan™ 100 or LABScan3D™ System and Luminex® xPONENT® Software
- Software-aided data analysis and HLA allele assignment using One Lambda™ HLA Fusion™ Software

Revolutionary rSSO DNA typing

Well suited for low- and high-throughput laboratories, One Lambda™ LABType™ sequence-specific oligonucleotide (SSO) tests are based on the reverse SSO (rSSO) method. A suspension array platform employs fluorescently coded microspheres as a solid support to immobilize oligonucleotide probes. Target DNA is amplified with biotinylated, locus specific primers. The amplified product is then denatured and hybridized with the oligonucleotide probes. Amplified product hybridized to microsphere-bound oligonucleotide probes are labeled with R-phycoerythrin–conjugated streptavidin (SAPE) and then processed by the LABScan system. The assay takes place in a single well of a 96-well PCR plate.

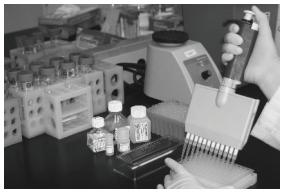
Multiplexing saves labor costs and processing time. Bead improvements and additions for every new lot are provided to address nomenclature updates. Tests are available for Class I and Class II alleles in 20 test or 100 test packages (40 test package for KIR).

LABType SSO tests for DNA typing

Workflow

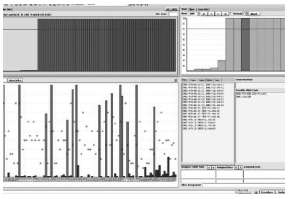
1. Perform PCR

2. Hybridization of PCR product to beads



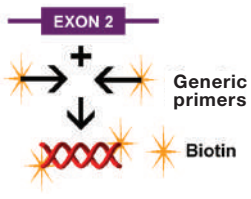
3. Detection using LABScan100 or LABScan3D System

4. Software analysis and HLA assignment with HLA Fusion Software

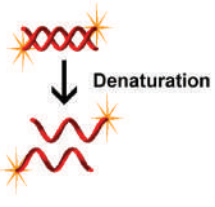


Procedure

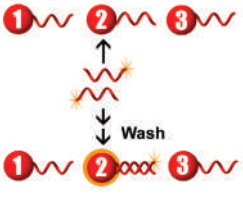
1. Amplification



2. Denaturation



3. Hybridization and wash



4. Labeling

ONE LAMBDA
A Thermo Fisher Scientific Brand

Ordering information

Product	Quantity	Cat. No.
LABType SSO HLA A Locus Typing Test	20 tests	RSO1AT
	100 tests	RSSO1A
LABType SSO HLA B Locus Typing Test	20 tests	RSO1BT
	100 tests	RSSO1B
LABType SSO HLA B7 Supplement Typing Test	20 tests	RSO1S1T
LABType SSO HLA Bw4 Supplement Typing Test	20 tests	RSO1S4T
LABType SSO HLA C Locus Typing Test	20 tests	RSO1CT
	100 tests	RSSO1C
LABType SSO HLA Exon 4 to 7 Supplement Typing Test	20 tests	RSO1E47T
	100 tests	RSSO1E47
LABType SSO HLA DRB1 Typing Test	20 tests	RSO2B1T
	100 tests	RSSO2B1
LABType SSO HLA DRB3, 4, 5 Typing Test	20 tests	RSO2345T
	100 tests	RSSO2345
LABType SSO HLA DQA1/DQB1 Typing Test	20 tests	RSO2QT
	100 tests	RSSO2Q
LABType SSO HLA DPA1/DPB1 Typing Test	20 tests	RSO2PT
	100 tests	RSSO2P
LT RSSO KIR (For Research Use Only)	40 tests	RSSOKIR
PE-Conjugated Streptavidin	20 tests	LT-SAPE

For *In Vitro* Diagnostic Use Only. Unless otherwise stated.

Product	Quantity	Cat. No.
Taq Polymerase, 30 µL	40 tests	TAQ30
Taq Polymerase, 50 µL	40 tests	TAQ50
Taq Polymerase, 75 µL	40 tests	TAQ75

For General Laboratory Use.

Find out more at **thermofisher.com/onelambda**

ThermoFisher SCIENTIFIC

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