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PRODUCT INSERT

LIFECODES LSA™ Class I

LIFECODES LSA™ Class II

For Research Use only. Not for use in diagnostic procedures.

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DEFINITION OF SYMBOLS

(Product Labels and Supplemental Documents)

Batch Code	LOT	Catalog Number	REF	Use By Date		Temperature range (storage)	2°C 8°C
SAMPLE	SAMPLE	Manufacturer		MFI Threshold	MFI TH	Temperature (storage)	≤-65°C
Dilute Before Use	DIL	Light Sensitive (Keep away from light)		Sufficient for N tests	Σ	Consult Instructions for Use	
Name	NAME	Identification Number	ID#	Date	DATE	Technician	TECH
Bead	BEAD	Class I	CLI	Class II	CLII	Cut-off	CUT-OFF
Background	BKG	Antigen	AG	Median Fluorescence Intensity	MFI	Interpretation	INTRP
Negative Control Bead	NC	Positive Control Bead (Immunoglobulin G)	PC	Bleed date	BDT	Antigen ID	ANTIGEN ID
Lowest Ranked Antigen	LRA	MFI / Lowest Ranked Antigen	MFI/LRA	Warning	!	Observed Limits	OBSERVED LIMITS
Relative Antigen Density	RAD	Serological Equivalent	SERO				

SUMMARY AND EXPLANATION

Human leukocyte antigens (HLA) are a system of glycoproteins that have a functional role in the presentation of peptides to the immune system.[1,2] However, as a highly polymorphic system, HLA molecules can become the targets of antibody responses in people during pregnancy, transfusion of blood products, or organ transplant rejection. Generally, alloimmunization leads to the production of HLA antibodies in approximately 33% of exposed individuals.[3] The presence or absence of these HLA-specific antibodies has a role in determining the survival of transplant allografts.[4]

LIFECODES LSA™ Class I Beads are designed to detect IgG antibodies to HLA Class I glycoproteins. LSA Class I is composed of different Luminex Beads to which purified recombinant Class I HLA glycoproteins are conjugated.

LIFECODES LSA™ Class II Beads are designed to detect IgG antibodies to HLA Class II glycoproteins. LSA Class II is composed of different Luminex Beads to which purified recombinant Class II HLA glycoproteins are conjugated.

PRINCIPLES OF THE PROCEDURE

An aliquot of the Beads is allowed to incubate with a small volume of test serum sample. The sensitized beads are then washed to remove unbound antibody. An anti-Human IgG antibody conjugated to phycoerythrin is then added. After another incubation, the test sample is diluted and analyzed on the Luminex instrument. The signal intensity from each bead is compared to the signal intensity of the lowest ranked locus-specific bead included in the bead preparation to determine if the bead is positive or negative for bound alloantibody.

REAGENTS

A. Identification

265100R: LSA1 LIFECODES LSA™ Class I consists of five (5) components in sufficient quantities for 24 tests.

1. **265103 LSA1B LSA Class I Bead Mix** (960 µL): A blend of beads each conjugated with a different single Class I HLA glycoprotein plus control beads. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide and bovine proteins. LIGHT SENSITIVE. Keep routine exposure to light to three hours or less. **Store at ≤ –65ºC in the dark.**
2. **265002 LSACJ LSA Conjugate Concentrate** (120µL): Goat anti-Human IgG conjugated to phycoerythrin in a phosphate-based storage buffer containing NaCl, Tween-20 and sodium azide. **DIL** MUST BE DILUTED 1:10 in Wash Buffer prior to use. LIGHT SENSITIVE. Keep out of direct light for extended periods of time. **Store at 2 to 8°C in the dark.**
3. **628221 LMWB LIFECODES Wash Buffer** (30 mL): A phosphate-based buffer containing NaCl, Tween-20, sodium azide and bovine serum albumin. **Store at 2 to 8°C** and equilibrate to room temperature (20-24°C) prior to use.
4. **265101 LSAPC1 LSA Class I Positive Control** (100 µL): This serum or sera blend is obtained from individual(s) shown to be alloimmunized to HLA antigens and will react with most of the LSA Class I Beads. Contains 0.1% sodium azide as a preservative. **Store at 2 to 8°C.**
5. **265102 LSANC1 LSA Class I Negative Control** (100 µL): This serum or sera blend is obtained from individual(s) known to have no antibodies to HLA antigens and will react with few if any of the LSA Class I Beads. Contains 0.1% sodium azide as a preservative. **Store at 2 to 8°C.**

265200R: LSA2 LIFECODES LSA™ Class II consists of five (5) components in sufficient quantities for 24 tests.

1. **265203 LSA2B LSA Class II Bead Mix** (960 µL): A blend of beads each conjugated with a different single Class II HLA glycoprotein plus control beads. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide, and bovine proteins. LIGHT SENSITIVE. Keep routine exposure to light to three hours or less. **Store at ≤ –65ºC in the dark.**
2. **265010 LSACJ LSA Conjugate Concentrate** (120µL): Goat anti-Human IgG conjugated to phycoerythrin in a phosphate-based storage buffer containing NaCl, Tween-20 and sodium azide. **DIL** MUST BE DILUTED 1:10 in Wash Buffer prior to use. LIGHT SENSITIVE. Keep out of direct light for extended periods of time. **Store at 2 to 8°C in the dark.**
3. **628221 LMWB LIFECODES Wash Buffer** (30 mL): A phosphate-based buffer containing NaCl, Tween-20,sodium azide and bovine serum albumin. **Store at 2 to 8°C** and equilibrate to room temperature (20-24°C) prior to use.
4. **265201 LSAPC2 LSA Class II Positive Control** (100 µL): This serum or sera blend is obtained from individual(s) shown to be alloimmunized to HLA antigens and will react with most of the LSA Class II Beads. Contains 0.1% sodium azide as a preservative. **Store at 2 to 8°C.**
5. **265202 LSANC2 LSA Class II Negative Control** (100 µL): This serum or sera blend is obtained from individual(s) known to have no antibodies to HLA antigens and will react with few if any of the LSA Class II Beads. Contains 0.1% sodium azide as a preservative. **Store at 2 to 8°C.**

B. Warnings or Cautions

1. For Research Use only. Not for use in diagnostic procedures.
2. Human source material used in the production of this kit has been tested and found to be negative for antibody to HIV, HCV, and HBsAg by FDA-approved methods. However, no test method can offer complete assurance that infectious agents are absent. Therefore, **use Universal Precautions** when working with these materials.
3. Substitution of components other than those provided in this system may lead to erroneous results.
4. Reagents contain 0.1% sodium azide as a preservative, which may react with lead and copper plumbing to form explosive metal azides. Use large amounts of water when discarding materials down a sink.
5. Bacterial contamination of samples or the presence of immune complexes or other immunoglobulin aggregates can cause increased non-specific binding and erroneous results.
6. This product detects IgG antibodies that may or may not be lymphocytotoxic.
7. This product is not expected to detect antibodies of the IgA or IgM class of immunoglobulin.
8. These products are designed for use with the Luminex instrument according to the manufacturer's recommendations.
9. Dispose of all materials after use according to local regulations.
10. See Safety Data Sheets for additional information.

C. Storage Instructions

1. Refer to product labels for storage indications.
2. Beads and conjugate are LIGHT SENSITIVE. Keep routine exposure to light to three hours or less.

D. Purification or Treatment Required for Use

1. See "Specimen Collection and Preparation."
2. Conjugate Concentrate must be diluted 1:10 in Wash Buffer before use.

E. Instability Indications

1. Do not use components or controls that are turbid or beyond their expiration date.
2. Discard all unused diluted positive and negative controls and conjugate after use.

INSTRUMENT REQUIREMENTS

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

SPECIMEN COLLECTION AND PREPARATION

Blood should be collected without anticoagulant using aseptic technique and should be tested while still fresh to minimize the chance of obtaining false-positive or false-negative reactions due to improper storage or contamination of the specimen. Serum should be stored at 2 to 8°C for no longer than 48 hours. If serum is to be stored beyond 48 hours, it should be frozen at or below –20°C for up to 2 years. Individual laboratories should establish and validate methods for storing sera for more than 2 years. Serum should be separated from red cells when stored or shipped. Avoid repeated freezing and thawing of serum samples.

Do not use microbiologically contaminated, hemolyzed or lipemic, sera as these samples may give inconsistent results.

Prior to assaying, all samples should be vortexed and centrifuged briefly (30 seconds at 10,000xg) to pellet any particulate matter that may be present.

PROCEDURE

A. Materials Provided (See REAGENTS on page 2 for more specific information)

- LSA Bead Mix
- Conjugate Concentrate
- Wash Buffer
- Positive Control Serum
- Negative Control Serum
- Recording Sheet
- Plate Format Sheet

B. Materials, Reagents and Equipment *Required,* but Not Provided (as listed or equivalent)

- 5 µL – 50 µL adjustable pipets with appropriate pipet tips
- 250 µL multichannel pipet with matching tips and buffer trough
- 1.5 mL microcentrifuge tubes for conjugate dilutions
- Test tubes for patient and control samples
- Timer
- Marking pen
- Millipore multiscreen filter plates (Milllipore Cat# MSBVN1210, MSBVN1250, Lifecodes Cat # 888633 or 888633-50)
- Multiscreen vacuum manifold (Millipore Cat # MAVM 0960R, Qiagen Cat # 19504, Lifecodes Cat#888315)
- Luminex Sheath Fluid (Lifecodes Cat # 628005)
- Luminex Calibration Kits (Luminex 100/200 Calibration Kit, Luminex 100/200 Performance Verification Kit, Lifecodes Cat # 628018 and 628019 respectively)
- Distilled water
- Rotary Platform
- Adhesive plastic covers (Corning Cat # 6524 or 6570)

DIRECTIONS FOR USE

PRECAUTIONS:

- Care MUST be taken to avoid contamination of Wash Buffer and the anti-Human IgG reagent. Inadvertent contamination of these reagents with human serum will result in the neutralization of anti-Human IgG and subsequently result in test failure.
- Care must be taken to control vacuum strength. Strong vacuum pressure can cause beads to stick to the membrane causing bead count failure.
- Care must be taken during pipetting into the filter plate so that beads do not stick to the side of the microplate wells. Beads should be pipetted into the well being careful not to touch the membrane with the tip. Contacting the membrane with the pipet tip can lead to puncture of the membrane and subsequent failure of the assay.
- Care must be taken to ensure, during incubation steps, that the beads are not splashing and sticking to the sides of the wells. When running the assay for the first time, run a few positive and/or negative controls to determine the optimal speed for the rotary platform. A speed of approximately 200 rotations per minute with an orbit size of 19mm has been shown to be effective.
- The presence of significant levels of unbound antibody at the completion of the wash step, due to either excess serum or poor washing, may reduce the ability of the assay to detect IgG bound to sensitized beads and cause erroneous results.
- A sample of positive and negative control sera should be included with each test to help determine if technical error or reagent failures have occurred.
- The assay is validated with 10µL serum (Protocol 1) and 20µL serum (Protocol 2).

1. Take out the LSA Bead Mix from the freezer and store it in the dark at room temperature until thawed. Then place on ice and protect from light. **NOTE: The bead mix can be frozen and thawed a maximum of 6 times.**
2. Leaving other components at 2 to 8°C in the dark until required, bring the Wash Buffer to room temperature (20 to 24°C) prior to use. During this time, use the Plate Format Sheet to assign a position on the plate for each of the sera and controls to be analyzed. The control sera supplied in the kit are used to illustrate a broadly reactive positive alloserum and a negative serum.
3. Cover the unassigned wells of the Filter Plate with adhesive plastic cover. Pre-wet wells to be used with 100-300 µL of distilled water. After 2-5 minutes, remove water by gentle aspiration using the vacuum manifold. (See manufacturer's recommendations for proper use.)
4. Prepare the LSA Beads by briefly (30 seconds) centrifuging the vial at 600 – 800 xg to remove any beads or liquid from the cap or walls of the vial. Thoroughly vortex (~1 minute) to evenly resuspend the beads.
5. Add 40 µL of LSA Beads to each of the assigned wells. Re-vortex the LSA Bead vial every 2 minutes to keep the beads in suspension while distributing the beads, then add 10 µL of patient serum and control sera (Protocol 1) or 20 µL of patient serum and control sera (Protocol 2) and mix.

CAUTION: It is important to keep the beads resuspended to ensure sufficient beads are aliquoted into wells and to ensure low count times. Failure to vortex beads intermittently will cause beads to settle towards the bottom of the tube. This will result in differential amount of beads being dispensed into wells which may adversely affect run-times and analysis of results.

6. Cover the plate with adhesive plastic cover then foil or box to protect from light. Incubate for 30 minutes at room temperature (20-24°C) in the dark on a rotating platform. Return unused portions of control sera to storage at 2 to 8°C for future use. Return unused portions of LSA Bead Mix to storage at ≤ –65ºC in the dark for future use.
7. Dilute conjugate with Wash Buffer (5 µL conjugate to 45 µL Wash Buffer per sample). To accommodate pipetting losses, it is desirable to make up one (1) extra volume of diluted conjugate. Cover with foil and/or store in the dark at room temperature until used. Return the unused portion of Conjugate Concentrate to storage at 2 to 8°C in the dark for future use.
8. After the 30 minute incubation remove the adhesive plastic cover and add 100 µL of Wash Buffer to each well. Mix to resuspend the beads and gently aspirate the plate.

CAUTION: Use of excessive vacuum strength will cause beads to stick to the membrane and can result in sample failure. Apply the minimum vacuum pressure required to aspirate samples.

9. Add 250 µL of Wash Buffer to each well, mix to resuspend the beads, aspirate, and repeat two more times for a total of three washes.

CAUTION: Failure to wash completely may reduce the ability of the conjugate to detect IgG bound to sensitized beads and cause false negative results.

10. Add 50 µL of diluted conjugate to each well. Cover plate with foil or box to protect from light. Place on a rotating platform or gently vortex every 5-10 minutes. Incubate for 30 minutes at room temperature (20 to 24°C).
11. Using a clean pipette tip, add 130 - 150 µL of Wash Buffer to each well and mix to resuspend beads.
12. Collect data with Luminex instrument using the manufacturer's recommendations. Delays of greater than 3 hours may increase the chance of obtaining false-positive or false-negative reactions. Return the unused portion of Wash Buffer to storage at 2 to 8°C for future use.

RESULTS

Enter the Raw Median Fluorescence Intensity (MFI) values for each bead into the lot-specific Recording Sheet. To determine if a bead is positive, first establish if the MFI for each antigen bound bead is above the MFI Threshold found on the lot-specific Recording Sheet provided with the kit. If an antigen bound bead is above the MFI Threshold, divide the MFI by the MFI of the Lowest Ranked Antigen (LRA) of its respective locus to generate the MFI/Lowest Ranked Antigen (MFI/LRA) ratio. The LRA for each locus is the MFI value of the lowest ranked antigen bead for that locus.

Example: $\frac{\text{Individual Bead MFI}}{\text{LRA MFI for locus "1"}}$ = MFI/LRA for antigen "x" from locus "1"

$\frac{\text{Individual Bead MFI}}{\text{LRA MFI for locus "2"}}$ = MFI/LRA for antigen "y" from locus "2"

$\frac{\text{Individual Bead MFI}}{\text{LRA MFI for locus "3"}}$ = MFI/LRA for antigen "z" from locus "3"

Refer to the lot-specific Recording Sheet provided with the kit for the list of the antigens present on each bead and the cut-off for determining the positive/negative result with each antigen bound bead. An antigen bound bead is considered positive if the MFI value is above the MFI Threshold and the MFI/LRA ratio is above the cut-off value.

QUALITY CONTROL

Quality control of LSA Class I and Class II is built into the test system by the inclusion of Positive and Negative Control Sera. These controls should be included with each test run to help determine if technical errors or reagent failures have occurred. The Positive Control Sera will react with a large number HLA conjugated beads generating a pattern similar to that found in the lot-specific Recording Sheet. The Negative Control Sera will be negative and react with few if any of the HLA conjugated beads generating values ≤1000 MFI.

The bead sets include two control beads to monitor each sample's performance. The Positive Control Bead is coated with human IgG and should yield MFI values ≥10,000 with the control sera. If you obtain values less than 10,000 MFI with the control sera, your assay may be insufficiently washed or your conjugate may be compromised. ▲The Negative Control Bead should show low MFI values with the control sera. Refer to the lot specific recording sheet for observed limits for the control beads with control sera.

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

LIMITATIONS OF THE PROCEDURE

Erroneous results can occur from bacterial contamination of test materials, inadequate incubation periods, inadequate washing or decanting of beads, exposure of conjugate to stray light, or omission of test reagents or steps.

The presence of immune complexes or other immunoglobulin aggregates in the serum sample may cause an increased non-specific binding and produce erroneous results in this assay.

The antibodies detected by LSA Kits are those reactive within the population of available antigens listed on the Recording Sheet.

LIFECODES Single Antigen HLA Class I and II glycoproteins were obtained from cell lines expressing single HLA antigens.

Some IgG with low avidity or low titer, IgA, IgM and monospecific antibodies to antigens not included in the panel will not be detected with the LIFECODES Single Antigen assays.

Serum antibody titers are sample and time point specific. If many beads are producing MFI values above 15,000, it may be necessary to dilute the sera for better detection of IgG antibodies.

Due to the complex nature of HLA testing, qualified personnel should review data interpretation. The detection of antibody using LSA kits must take into consideration the results of all beads, including those that may be at or near the cut-off value. Knowledge of the sample history as well as an understanding of the cross-reactive groups can be useful in the evaluation of a specific serum.

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
Low Bead Count	Bead Mix not well suspended	Pulse vortex to completely resuspend
	Instrument failures - out of calibration	See Luminex Instrument Manual
	Instrument failures - sample flow blocked	See Luminex Instrument Manual
	Photobleached beads	Use new kit
	Vacuum pressure too strong/beads stuck to membrane	Reduce vacuum strength; Millipore Multiscreen Filter Plates recommend a vacuum of 271-406 millibar (8-12 in. Hg)
Negative Control Bead (NC) Threshold Surpassed with Control Sera	Poor washing	Repeat and monitor washes
	Incorrect sample added	Repeat with correct control sample
Positive Control Bead (PC) Threshold Failure with Control Sera	Compromised conjugate e.g. photobleaching	Use new kit
	Poor washing	Repeat and monitor washes
	Incorrect sample added	Repeat with correct control sample
Anomalous pattern for Positive Control Sera	Incorrect sample added	Repeat with correct control sample
	Poor washing	Repeat and monitor washes
Positive assignment for Negative Control Sera (>2 HLA conjugated beads) or >1000 MFI.	Incorrect sample added	Repeat with correct control sample
	Poor washing	Repeat and monitor washes to insure beads are re-suspended during washing
		Reduce vacuum strength
	Contamination of Bead Mix, Wash Buffer, Negative Control Sera or Conjugate Concentrate with positive sample	Use new kit
Clogged filter plate	Particulate matter in sample	Centrifuge sample approximately 5 minutes at 8,000 – 12,000xg

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