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## PRODUCT INSERT

**LIFECODES LifeScreen Deluxe (LMX)** is a Luminex® Screening Assay for the Detection of IgG Antibodies to HLA Class I and HLA Class II Molecules of Human Origin.



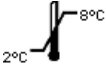

























### For In Vitro Diagnostic Use.

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### DEFINITION OF SYMBOLS

(Product Labels and Supplemental Documents)

Lot		Catalog Number	<b>REF</b>	Expiration Date		Temperature range (storage)	
Dilute Before Use		Light Sensitive (Keep away from light)		Sufficient for N tests		See instructions for use	
Patient Name		Identification Number	<b>ID#</b>	Date		Technician	
Bead		Ethnicity		Donor		Background Adjustment Factor	
Median Fluorescence Intensity		Score (see Results Section)		Interpretation		Negative Control Bead	
Positive Control Bead (Immunoglobulin G)		Class I		Class II		Sample	
Bleed Date		Number of Beads Possessing Antigen		Manufacturer		European Representative	
Expected Ranges		Controls					

## INTENDED USE

LIFECODES LifeScreen Deluxe is a qualitative bead-based immunoassay used to detect IgG antibodies to HLA Class I and Class II molecules.

## 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.<sup>1,2</sup> 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.<sup>3</sup> The presence or absence of these HLA-specific antibodies has a role in determining the survival of transplant allografts.<sup>4</sup>

LIFECODES LifeScreen Deluxe Beads are designed to detect IgG antibodies to HLA Class I and Class II antigens. LifeScreen Deluxe is composed of unique Luminex Beads to which affinity purified Class I HLA and 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 a negative control bead included in the bead preparation to determine if the bead is positive or negative for bound alloantibody.

## REAGENTS

### A. Identification

**628215:** **LMXB** LIFECODES LifeScreen Deluxe consists of five (5) components in sufficient quantities for 96 tests.

1. **LMXB** **LifeScreen Deluxe Beads** (480  $\mu$ L): A blend of beads conjugated with Class I HLA glycoproteins or Class II HLA glycoproteins plus four (4) control beads. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, ProClin300, and bovine serum albumin. LIGHT SENSITIVE. Keep routine exposure to light to 3 hours or less. Ready for use. Store at 2 to 8°C in the dark.
2. **LMCJS** **Conjugate Concentrate** (550  $\mu$ L): 10x stock of goat anti-Human IgG conjugated to phycoerythrin in a phosphate-based storage buffer containing NaCl, Tween-20, sodium azide, and bovine serum albumin. **DIL** MUST BE DILUTED 1 in 10 in Wash Buffer prior to use. LIGHT SENSITIVE. Keep routine exposure to light to 3 hours or less. Store at 2 to 8°C in the dark.
3. **LMWB** **Wash Buffer** (150 mL): A phosphate-based buffer containing NaCl, Tween-20, sodium azide, and bovine serum albumin. Ready for use. Store at 2 to 8°C and equilibrate to room temperature (20 to 24°C) prior to use.
4. **LMPC** **Positive Control Serum** (80  $\mu$ L): This serum or sera blend is obtained from individual(s) shown to be alloimmunized to HLA antigens. Contains sodium azide. Ready for use. Store at 2 to 8°C.
5. **LMNC** **Negative Control Serum** (80  $\mu$ L): This serum or sera blend is obtained from individual(s) known to have no antibodies to HLA antigens. Contains sodium azide. Ready for use. Store at 2 to 8°C.

### B. Warnings or Cautions

1. For In Vitro Diagnostic Use
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. Some 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 to be used with the Luminex instrument according to the manufacturer's recommendations.
9. Dispose of all materials after use according to local regulations.
10. See Material Safety Data Sheets for additional information.

### C. Storage Instructions

1. Refer to product labels for storage indications.
2. LifeScreen Deluxe Beads and Conjugate Concentrate are LIGHT SENSITIVE. Keep routine exposure to light to 3 hours or less.

### D. Treatment Required for Use

1. **LMCJS** **Conjugate Concentrate:** **DIL** MUST BE DILUTED 1 part added to 9 parts Wash Buffer prior to use. Prepare diluted Conjugate by adding 5  $\mu$ L of LMCJS to 45  $\mu$ L of Wash Buffer, for each well of testing. Cover with foil and/or store in the dark at room temperature (20 to 24°C) until used. Return the unused portion of LMCJS to storage at 2 to 8°C in the dark for future use.
2. **LMWB** **Wash Buffer:** Equilibrate to room temperature (20 to 24°C) prior to 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 conjugates after use.

## INSTRUMENT REQUIREMENTS

Luminex Instrument (Lifecodes Product Number 888300)

## 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 or -80°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, lipemic, or heat-inactivated sera as these samples may give inconsistent results.

In laboratory testing, Thymoglobulin (Genzyme) was shown to cause false positive results. Samples containing Thymoglobulin may give incorrect results.

Prior to assaying, all samples should be briefly vortexed (30 seconds) and centrifuged (4-5 minutes at 8,000 – 12,000 rcf) to pellet any particulate matter that may be present.

## PROCEDURE

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

- HLA Beads
- 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 - 2.0 mL microcentrifuge tubes
- Test tubes for patient and control samples
- Timer
- Marking pen
- Distilled water
- Rotator or vortex with plate adapter
- Millipore multiscreen filter plates (Cat # MSBVN 1210, Lifecodes #888633)
- Adhesive plate sealers (Lifecodes #888631)
- Multiscreen vacuum manifold (Millipore Cat #MAVM 0960R or Qiagen Cat#19504, Lifecodes Cat# 888315)
- Luminex Sheath Fluid (1x or 20x Lifecodes 628005, 628025)
- Luminex Calibration beads (CAL 1, CAL 2, CON 1, CON 2; Lifecodes 628006, 628007, 628008, 628009)
- Micro Centrifuge

## DIRECTIONS FOR USE

### PRECAUTIONS:

- Care **MUST** be taken to avoid contamination of Wash Buffer and the Conjugate Concentrate (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. Be careful not to touch the membrane with the pipet 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 or vortex mixer. A speed of approximately 200 rotations per minute has been shown to be effective with some instruments.
- The presence of significant levels of unbound antibody, 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.
- Do not mix components from different lots.

### Test Procedure:

1. 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.

- Cover the unassigned wells of the Filter Plate with adhesive plastic cover. Then pre-wet wells to be used with 100-300  $\mu\text{L}$  of distilled water. After 2-5 minutes, remove water by gentle aspiration using the vacuum manifold. (See manufacturer's recommendations for proper use.)
- Prepare the HLA Beads briefly (30 seconds) centrifuging the vial at 600 – 800 x g to remove any beads or liquid from the cap or walls of the vial. Thoroughly vortex (~1 minute) to evenly resuspend the beads
- Add 40  $\mu\text{L}$  of Wash Buffer to each test well of the Filter Plate, then 12.5  $\mu\text{L}$  of patient serum or control serum and mix.
- Add 5  $\mu\text{L}$  of HLA Beads to each of the assigned wells. Re-vortex the HLA Bead vial every 2 minutes to keep the beads in suspension while distributing the beads.

**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.

- 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 (200 rotations per minute). Return unused portions of control sera and beads to storage at 2 to 8°C in the dark for future use.
- Dilute conjugate with wash buffer and store in the dark (5  $\mu\text{L}$  conjugate to 45  $\mu\text{L}$  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.
- After the 30 minute incubation remove the adhesive plastic cover and add 100  $\mu\text{L}$  of Wash Buffer to each well. Mix by tapping the side of the plate 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.

- Add 250  $\mu\text{L}$  of Wash Buffer to each well, 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.

- Add 50  $\mu\text{L}$  of diluted conjugate to each well. Cover plate with foil or box to protect from light. Place on a rotating platform (set at 200 rotations per minute) or gently vortex every 5-10 minutes. Incubate for 30 minutes at room temperature (20 to 24°C).
- Using a clean pipette tip, add 150  $\mu\text{L}$  of Wash Buffer to each well and mix to resuspend beads.
- 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.

## QUALITY CONTROL

Quality control of product performance is included in LIFECODES LifeScreen Deluxe. The product includes a Positive and Negative Control Serum. These control sera should be included with each test run to help determine if technical errors or reagent failures have occurred. The Positive Control Serum will react with both the HLA Class I (PROBE I-01) and Class II (PROBE II-01) conjugated beads giving a minimum median fluorescence intensity of 10,000. The Negative Control Sera will be negative for both HLA Class I and HLA Class II antibodies. If these controls fail to meet these criteria then your assay or conjugate may be compromised.

In addition, the bead blend includes three negative control beads [CON] and one positive control bead [Probe 77]. The CON beads measure the background in the assay and are used to normalize the signal of the HLA beads. These beads should show low MFI values. Refer to the lot specific recording sheet for expected ranges for the negative Control Beads. Data outside of the range should be examined carefully.

The Positive Control Bead [Probe 77] is conjugated with human IgG and will indicate that the Conjugate (LMCJS) has been added to a test well. When testing with either of the control sera, Probe 77 should show values  $\geq 10,000$  MFI. If you obtain values less than 10,000 MFI, your assay may be insufficiently washed or your conjugate may be compromised. Patient samples show a wide range of reactivity with Probe 77, but should produce a signal of  $>3500$  MFI. In the absence of sera, Probe 77 will give strong signal while the rest of the beads will average approximately 50 MFI or less. If a test sample produces a similar pattern of values, then the sample may have been inadvertently omitted. These samples should be repeated.

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.

## RESULTS

To determine if a test sample is positive for class I or class II HLA-specific antibodies, each individual HLA bead is first evaluated to determine if it is positive or negative for IgG antibodies.

To determine if an individual HLA bead is positive, first divide the individual bead MFI by the MFI for each Negative Control Bead (CON1, CON2, CON3). From these quotients, subtract the Background Adjustment Factor (BAF) for the appropriate bead/CON combination. The BAF is a pre-determined MFI ratio for each bead/CON combination to compensate for background noise due to bead variation. See the lot-specific Recording Sheet provided with the kit for BAF values.

Example:	$\frac{\text{Individual Bead MFI}}{\text{Negative Control Bead 1 (CON1) MFI}}$	- (BAF) = Adjusted ratio 1
	$\frac{\text{Individual Bead MFI}}{\text{Negative Control Bead 2 (CON2) MFI}}$	- (BAF) = Adjusted ratio 2
	$\frac{\text{Individual Bead MFI}}{\text{Negative Control Bead 3 (CON3) MFI}}$	- (BAF) = Adjusted ratio 3

- For PROBE I-01 and PROBE II-01, a positive value for any one of the calculations indicates a positive bead reaction.
- For all remaining beads, a positive value for any two of the calculations indicates a positive bead reaction.
- A negative value for all three calculations indicates a negative bead reaction.

**Evaluating Test Sample-**

- A sample is considered to be positive for class I HLA-specific antibodies if at least one of the seven (7) class I HLA beads is positive.
- A sample is considered to be positive for class II HLA-specific antibodies if at least one of the five (5) class II HLA beads is positive.
- A sample is considered to be negative for HLA-specific IgG antibodies if all the HLA beads are found to be negative.

**LIMITATIONS OF THE PROCEDURE**

The results of this test must not be used as the sole basis for making a clinical decision.

Erroneous results can occur from bacterial contamination of test materials, inadequate incubation periods, inadequate washing 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 patient sample may cause an increased non-specific binding and produce erroneous results in this assay.

This product detects IgG antibodies that may or may not be lymphocytotoxic. The antibodies detected are those reactive within the population of available antigens listed on the Recording Sheet.

LifeScreen Deluxe is composed of unique Luminex beads to which affinity purified Class I HLA and Class II HLA glycoproteins are conjugated. For PROBE I-01, affinity purified HLA Class I glycoproteins are obtained from platelets of 100 Caucasians, 100 African Americans, and 100 Hispanic blood donors. The HLA Class I glycoproteins affinity-purified from EBV-transformed B lymphocytes and attached to PROBE I-02 through I-07 are listed on the lot-specific recording sheet. For PROBE II-01, affinity purified HLA Class II glycoproteins used in the manufacture of this product are derived from EBV transformed B lymphocytes representing the following HLA class II antigens: DR1, DR4, DR7, DR8, DR9, DR10, DR11, DR12, DR13, DR14, DR15, DR16, DR17, DR18, DR103, DR51, DR52, and DR53. The HLA Class II glycoproteins affinity-purified from EBV-transformed B lymphocytes and attached to PROBE II-01 through II-05 are listed on the lot-specific recording sheet.

Some low avidity, some low titer, IgA, IgM and antibodies to rare alleles will not be detected with the LIFECODES LifeScreen Deluxe assays.

Due to the complex nature of HLA testing, qualified personnel should review data interpretation. The determination of antibody reactivity using LIFECODES LifeScreen Deluxe kits must take into consideration the results of all beads, including those that may be at or near the cutoff value. Knowledge of the patient history as well as an understanding of the cross-reactive groups can be useful in the assignment of antibody reactivity to 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 Instrument Manual
	Instrument failures – sample flow blocked	See Instrument Manual
	Photobleached beads	Use new kit
	Vacuum pressure too strong/beads stuck to membrane	Reduce vacuum strength
Control Threshold Surpassed	Poor washing	Repeat and monitor washes
	Poor sample quality	Redraw
	Photobleached conjugate	Use new kit
Positive Control Threshold Failure	Poor washing	Repeat and monitor washes

**SPECIFIC PERFORMANCE CHARACTERISTICS**

When LIFECODES LifeScreen Deluxe kits are used according to the procedure described, the results reveal the presence or absence of HLA IgG antibodies. In the detection of Class I HLA-specific antibodies, the LifeScreen Deluxe kits showed 100% co-positivity (97.6-100%), 91% co-negativity (87.3-94.1%), and 95% agreement for 417 sera samples evaluated when compared to results obtained with LifeScreen method (95% confidence limits). In the detection of Class II HLA-specific antibodies, the LifeScreen Deluxe kits showed 100% co-positivity (97.8-100%), 93% co-negativity (89.5-95.8%), and 96% agreement for 424 sera samples evaluated when compared to results obtained with LifeScreen method (95% confidence limits).

## REFERENCES

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3. Rodey, GE. HLA Beyond Tears (2<sup>nd</sup> Edition). DeNovo, Inc. Durango, CO. 2000; 163.
4. McKenna, RM; Takemoto, SK; Terasaki, PI. Anti-HLA Antibodies after Solid Organ Transplantation. Transplantation 2000; 69:319.

## MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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