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PRODUCT INSERT

LIFECODES Class I ID: A Luminex[®] Screening Assay for the Qualitative Detection of IgG Panel Reactive Antibodies to HLA Class I Molecules.

LIFECODES Class II ID: A Luminex[®] Screening Assay for the Qualitative Detection of IgG Panel Reactive Antibodies to HLA Class II Molecules.



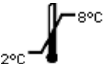




























For In Vitro Diagnostic Use.

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DEFINITION OF SYMBOLS

(Product Labels and Supplemental Documents)

Lot		Catalog Number	REF	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		Date		Technician	
Bead		Ethnicity		Donor		Bleed Date	
Background Adjustment Factor		Median Fluorescence Intensity		Panel Reactive Antibodies		Interpretation	
Negative Control Bead		Positive Control Bead (Immunoglobulin G)		Antigen		Sample	
Class I		Class II		Row		Column	
Number of Beads Possessing Antigen		Expected Ranges		Manufacturer		European Representative	

INTENDED USE

LIFECODES Class I and Class II ID are bead-based immunoassays used to qualitatively detect Panel Reactive HLA IgG antibodies (PRA).

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 Class I ID Beads are designed to detect IgG antibodies to HLA Class I glycoproteins. LIFECODES Class I ID is composed of different Luminex Beads to which affinity purified Class I HLA glycoproteins from different individuals are conjugated.

LIFECODES Class II ID Beads are designed to detect IgG antibodies to HLA Class II glycoproteins. LIFECODES Class II ID is composed of different Luminex Beads to which affinity purified Class II HLA glycoproteins from different individuals 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

628200: LM1 LIFECODES Class I ID consists of five (5) components in sufficient quantities for 24 tests.

1. **LM1B HLA I Beads** (120 µL): A blend of beads each conjugated with Class I HLA glycoproteins from a different individual plus four (4) control beads. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide, bovine serum albumin, and glycerol. LIGHT SENSITIVE. Keep routine exposure to light to three hours or less. Store at 2 to 8°C in the dark.
2. **LMCJ Conjugate Concentrate** (170 µL): 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 1:10 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. **LMWB 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. **LMPC Positive Control Serum** (80 µL): This serum or sera blend is obtained from individual(s) shown to be alloimmunized to HLA antigens (>85% PRA). Store at 2 to 8°C.
5. **LMNC Negative Control Serum** (80 µL): This serum or sera blend is obtained from individual(s) known to have no antibodies to HLA antigens (<10% PRA). Store at 2 to 8°C.

628220: LM2 LIFECODES Class II ID consists of five (5) components in sufficient quantities for 24 tests.

1. **LM2B HLA II Beads** (120 µL): A blend of beads each conjugated with Class II HLA glycoproteins from a different individual plus four (4) control beads. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide, and bovine serum albumin. LIGHT SENSITIVE. Keep routine exposure to light to three hours or less. Store at 2 to 8°C in the dark.
2. **LMCJ Conjugate Concentrate** (170 µL): 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 out of direct light for extended periods of time. Store at 2 to 8°C in the dark.
3. **LMWB 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. **LMPC Positive Control Serum** (80 µL): This serum or sera blend is obtained from individual(s) shown to be alloimmunized to HLA antigens (>85% PRA). Store at 2 to 8°C.
5. **LMNC Negative Control Serum** (80 µL): This serum or sera blend is obtained from individual(s) known to have no antibodies to HLA antigens (<10% PRA). 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. 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. Determination of % PRA is not the sole basis for a clinical decision affecting a patient's treatment. A final crossmatch is routinely performed prior to transplant.
9. These products are designed to be used with the Luminex instrument according to the manufacturer's recommendations.
10. Dispose of all materials after use according to local regulations.
11. See Material 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)

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.

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)

- 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 mL microcentrifuge tubes for conjugate dilutions
- Test tubes for patient and control samples
- Timer
- Marking Pen
- Adhesive Plastic Covers
- Luminex Sheath Fluid (1x or 20x, Lifecodes Cat. No. 628005 or 628025)
- Luminex Calibration beads (CAL 1, CAL 2, CON 1, CON 2; Lifecodes Cat #'s 628006, 628007, 628008, 628009)
- Distilled water
- Rotator or vortex with plate adapter
- Millipore Multiscreen filter plates (Millipore Cat #MSBVN1210, Lifecodes Cat# 888633)
- Multiscreen vacuum manifold (Millipore Cat# MAVM 0960R, Qiagen Cat#19504, Lifecodes Cat# 888315)

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, that beads do not stick to the side of the microplate wells. Beads should be pipetted into the solution already present in the well, while 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 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 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.

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.
2. Cover the unassigned wells of the Filter Plate with adhesive plastic cover. Then 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.)
3. 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.
4. Add 40 µL of Wash Buffer to each test well of the Filter Plate, then 12.5 µL of patient serum or control serum and mix.
5. Add 5 µL 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.

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 (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.
7. Dilute conjugate with wash buffer and store in the dark (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 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.

9. Add 250 µL 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.

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 (set at 200 rotations per minute) 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

To determine if a bead is positive, first divide the individual bead median 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 and can be found on the lot-specific Recording Sheet provided with the kit.

Example:

$$\frac{\text{Individual Bead MFI}}{\text{Negative Control Bead 1 (CON1) MFI}} - (\text{BAF}) = \text{Adjusted ratio 1}$$

$$\frac{\text{Individual Bead MFI}}{\text{Negative Control Bead 2 (CON2) MFI}} - (\text{BAF}) = \text{Adjusted ratio 2}$$

$$\frac{\text{Individual Bead MFI}}{\text{Negative Control Bead 3 (CON3) MFI}} - (\text{BAF}) = \text{Adjusted ratio 3}$$

The Class I & II ID kits also employ a fourth negative control calculation, "CalcCON", based on performance of the non-reacting antigen-containing beads. CalcCON is determined as follows: CalcCON = MFI lowest ranked antigen bead + 20. Thus, the fourth calculation is:

$$\frac{\text{Individual Bead MFI}}{\text{CalcCON}} - (\text{BAF}) = \text{Adjusted ratio 4}$$

- A positive value for two or more of the four calculations indicates a positive bead reaction.
- A negative value for three or more of the four calculations indicates a negative bead reaction.

The percent PRA (Panel Reactive Antibodies) for the ID kits is calculated by:

$$\% \text{ PRA} = \frac{\text{Number of positive bead reactions}}{\text{Number of beads in the assay (not including the control beads)}} \times 100$$

Refer to the lot-specific Recording Sheet provided with the kit for the list of the antigens present on each bead to determine specificity.

QUALITY CONTROL

Quality control of Class I ID and Class II ID 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. For the ID kits, the Positive Control Sera will react with most or all of the HLA conjugated beads (>85%) with the peak HLA conjugated bead producing a minimum median fluorescence intensity of $\geq 10,000$. The Negative Control Sera will be negative (<10%).

The bead sets include four 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. Patient samples show a wide range of reactivity with the positive Control Bead, but should produce a signal of > 3500 MFI. Similarly, the Negative Control 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. In the absence of sera, the positive control will give strong signal while the rest of the beads will average approximately 50 MFI or less. If a sample produces these very low 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.

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 patient sample may cause an increased non-specific binding and produce erroneous results in this assay.

For ID kits, the antibodies detected are those reactive within the population of available antigens listed on the Recording Sheet.

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

Due to the complex nature of HLA testing, qualified personnel should review data interpretation. The determination of antibody specificity using LIFECODES ID 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 specificity 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
Control Threshold Surpassed	Vacuum pressure too strong/beads stuck to membrane	Reduce vacuum strength
	Poor washing	Repeat and monitor washes
	Poor sample quality	Redraw
Positive Control Threshold Failure	Photobleached conjugate	Use new kit
	Poor washing	Repeat and monitor washes

SPECIFIC PERFORMANCE CHARACTERISTICS

When LIFECODES ID kits are used according to the procedure described, the results reveal the presence or absence of HLA IgG antibodies. Using a 10% PRA cutoff, the Class I ID kit showed 97.1% co-positivity (90.2-99.2%), 86.4% co-negativity (75.5-93.0%), and 92.2% agreement (86.3-95.7%) for 129 samples evaluated when compared to results obtained using flow cytometric methods (95% confidence limits). Using a 12% PRA cutoff, the Class II ID kit showed 90.9% co-positivity (82.4-95.5%), 82.4% co-negativity (69.7-90.4%), and 87.5% agreement (80.7-92.2%) for 128 samples evaluated when compared to results obtained using flow cytometric methods (95% confidence limits).

REFERENCES

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3. Rodey, GE. HLA Beyond Tears (2nd 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.

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