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PRODUCT INSERT

LIFECODES Donor Specific Antibody (DSA) is a bead-based immunoassay using the Luminex® for the qualitative detection of IgG antibodies to donor-specific Class I and Class II Human Leukocyte Antigens.


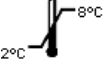





For Research Use Only. Not for use in Diagnostic Procedures

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DEFINITION OF SYMBOLS

(Product Labels and Supplemental Documents)

Lot	LOT	Catalog Number	REF	Expiration Date		Temperature range (storage)	
Dilute Before Use	DIL	Light Sensitive (Keep away from light)		Sufficient for N tests		See instructions for use	
Name	NAME	Identification Number	ID#	Date	DATE	Technician	TECH
Bead	BEAD	Ethnicity	ETHN	Donor	DONOR	Rehydrate before Use	REH
Background Adjustment Factor	BAF	Median Fluorescence Intensity	MFI	Score (see Results Section)	SCORE	Interpretation	INTRP
Negative Control Bead	CON	Positive Control Bead (Immunoglobulin G)	IgG	Antigen	AG	Class I	CLI
Class II	CLII	Sample	SAMPLE	Row	ROW	Column	COL
Bleed Date	BDI	Number of Beads Possessing Antigen		Manufacturer			
Expected Values	Expected Values						

SUMMARY AND EXPLANATION

Donor Specific Antibody (DSA) is a bead-based immunoassay used for the qualitative detection of IgG antibodies to donor-specific Class I and Class II Human Leukocyte Antigens.

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

Donor Specific Antibody (DSA) provides the materials and methods required to test a serum for IgG antibody against the Class I or Class II HLA of a specific donor.

PRINCIPLES OF THE PROCEDURE

Donor lymphocytes isolated from peripheral blood or spleen are used as the source material for HLA. The isolated cells are solubilized with a non-ionic detergent. Following a centrifugation step to remove cell debris and fragments, the lysate can be used immediately or stored frozen for future use.

DSA includes a single blend of Luminex beads. Two of the beads in the blend are conjugated with monoclonal antibodies specific for Class I HLA or Class II HLA. When mixed with a lysate, these two beads will capture the solubilized HLA, making a donor-specific HLA target for antibodies in a serum sample. The bead blend also includes control beads to monitor the amount of background in the assay and to assure that the appropriate conjugate has been used in the assay.

After capture of the donor HLA, the beads are transferred to a filter plate and washed. Serum, diluted in the Specimen Diluent, is then added and incubated with the beads for 30 minutes. Following another wash, the diluted anti-human IgG phycoerythrin (PE) conjugate is added to the beads. After a final 30 minute incubation, wash buffer is added to the wells, the plate is placed in the Luminex instrument, and data is collected for analysis.

DSA includes a control reagent to be run with each lysate. To assure that HLA has been captured, the Lysate Control Reagent is tested in parallel with the sera samples. The Lysate Control Reagent is a mixture of biotinylated monoclonal antibodies that are specific for and bind to Class I HLA and Class II HLA. The biotinylated monoclonal antibodies are detected by the diluted Streptavidin-PE conjugate.

DSA also includes a control system to verify that the entire assay is working properly: a Dried Lymphocyte Control, a Negative Control Serum, and a Positive Control Serum, matched to the Dried Lymphocyte Control containing antibodies to both class I HLA and class II HLA. The control system is run with every assay.

REAGENTS

A. Identification

628230: **DSA** Donor Specific Antibody consists of ten (10) components in sufficient quantities for 96 tests.

1. **LMDB** **Capture Beads** (665 µL): A blend of beads conjugated with monoclonals specific for Class I HLA glycoproteins or Class II HLA glycoproteins plus five (5) 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 3 hours or less. Ready for use. Store at 2 to 8°C in the dark.
2. **LMCJS** **Conjugate Concentrate** (550 µL): 10x stock of goat anti-Human IgG conjugated to phycoerythrin in a phosphate-based storage buffer containing NaCl, Tween-20, ProClin 300, and bovine serum albumin. Store at 2 to 8°C in the dark.
3. **LMSA** **SA-PE Concentrate** (285 µL): 10x stock of streptavidin conjugated to phycoerythrin in a phosphate-based storage buffer containing NaCl, Tween-20, ProClin 300, and bovine serum albumin. LIGHT SENSITIVE. Keep routine exposure to light to 3 hours or less. Store at 2 to 8°C in the dark.
4. **LMLLB** **Lymphocyte Lysis Buffer** (2.5 mL): 10x stock of tris-buffered solution containing a non-ionic detergent and sodium azide. Store at 2 to 8°C.
5. **LMSD** **Specimen Diluent** (5 mL): Phosphate-buffered solution containing bovine serum albumin and mouse serum and sodium azide. Ready for use. Store at 2 to 8°C.
6. **LMLCR** **Lysate Control Reagent** (285 µL): 10x stock of biotinylated monoclonal antibodies specific for Class I and Class II HLA. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide, and bovine serum albumin. Store at 2 to 8°C.
7. **LMWB** **Wash Buffer** (250 mL): A phosphate-based buffer containing NaCl, Tween-20, sodium azide, and bovine serum albumin. Ready for use. Store at 2 to 8°C.
8. **LMPC** **Positive Control Serum** (228 µL): This serum or sera blend containing sodium azide is selected to give an example of a test sample that will react with both the Class I and Class II HLA captured from the Dried Lymphocyte Control Pellet (LMDLC). Ready for use. Store at 2 to 8°C.
9. **LMNC** **Negative Control Serum** (228 µL): This serum or sera blend containing sodium azide is selected to give an example of a test sample that will not react with the Class I or Class II HLA captured from the LMDLC. Ready for use. Store at 2 to 8°C.
10. **LMDLC** **Dried Lymphocyte Control Pellet** (3 vials; 1 pellet each): Dried Lymphocytes to be made into a lymphocyte lysate. Store at 2 to 8°C.

B. Warnings or Cautions

1. For Research Use Only (RUO). 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. Some reagents contain 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. Dispose of all materials after use according to local regulations.
6. See Material Safety Data Sheets for additional information.

C. Storage Instructions

1. Refer to product labels for storage indications.
2. Capture Beads, Conjugate Concentrate, and SA-PE 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 µL of LMCJS to 45 µL 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. **LMSA SA-PE Concentrate:** **DIL** MUST BE DILUTED 1 part added to 9 parts Wash Buffer prior to use. Prepare diluted SA-PE by adding 5 µL of LMSA to 45 µL of Wash Buffer, for each well of testing. Cover with foil and/or store in the dark at room temperature until used. Return the unused portion of LMSA to storage at 2 to 8°C in the dark for future use.
3. **LMLLB Lymphocyte Lysis Buffer:** **DIL** MUST BE DILUTED 1 part added to 9 parts deionized or distilled water before use. Add 10 µL of stock to 90 µL of deionized or distilled water. Store diluted lysis buffer on ice for up to 4 hours. Return the unused portion of LMLLB to storage at 2 to 8°C for future use.
4. **LMSD Specimen Diluent:** Equilibrate to room temperature (20 to 24°C) prior to use.
5. **LMLCR Lysate Control Reagent:** **DIL** MUST BE DILUTED 1 part added to 9 parts Specimen Diluent prior to use. Prepare diluted LMLCR by adding 5 µL of the LMLCR to 45 µL of Specimen Diluent, for each well of testing. Store at room temperature until used. Return the unused portion of LMLCR to storage at 2 to 8°C for future use.
6. **LMWB Wash Buffer:** Equilibrate to room temperature (20 to 24°C) prior to use.
7. **LMDLC Dried Lymphocyte Control Pellet :** **REH** MUST BE REHYDRATED and made into a lysate prior to use. See "Lysate Preparation"

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, lymphocyte lysis buffer, and conjugates after use.

INSTRUMENT REQUIREMENTS

Luminex Instrument (Lifecodes Product Number 888300)

SPECIMEN COLLECTION AND PREPARATION

Cells: Spleen should be harvested according to local procurement procedures. Spleen should be processed within 72 hours of harvesting. Peripheral blood lymphocytes should be collected in sodium heparin or ACD tubes using aseptic technique, stored at room temperature, and should be processed into a lysate within 72 hours of collection.

Sera: 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. Prior to use, vortex serum samples to thoroughly mix then centrifuge at 8,000 to 12,000 rcf for 4 to 5 minutes. Avoid any pelleted material when withdrawing an aliquot for testing.

Do not use microbiologically contaminated, hemolyzed, lipemic, or heat-inactivated sera.

PROCEDURE

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

- Capture Beads
- Conjugate Concentrate
- SA-PE Concentrate
- Lymphocyte Lysis Buffer
- Specimen Diluent
- Lysate Control Reagent
- Wash Buffer
- Positive Control Serum
- Negative Control Serum
- Dried Lymphocyte Control Pellet
- 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 sample 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 Omega Bio-Tek Cat # Vac 01)
- Luminex Sheath Fluid (1x or 20x Lifecodes 628005, 6280025)
- Luminex Calibration beads (CAL 1, CAL 2, CON 1, CON 2; Lifecodes 628006, 628007, 628008, 628009)
- Complete cell culture media
- Lymphocyte density separation media (1.077 g/mL)
- 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 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.
- Do not mix components from different lots.
- The lysate prepared from the Dried Lymphocyte Control should be tested with the Positive Serum Control and Negative Serum Control for each assay to detect technical errors or reagent failures.
- Include a Lysate Control Reagent well for each donor lysate to confirm that lysate has been added and HLA has been captured.
- Care must be taken to minimize the number of red blood cells and platelets when isolating lymphocytes to prepare a lysate.

Lysate Preparation (Donor and Dried Lymphocyte Control):

Donor and control lysate may be prepared ahead of time and aliquots may be frozen at -70 to -80°C. If donor and control lysate have been previously prepared, go to "Test Procedure".

Rehydrate Dried Lymphocyte Control

1. Add 500 µL of cell culture media to the dried pellet of cells.
2. Allow to stand at room temperature (20 to 24°C) for at least 1 hour.
3. Dislodge the button of cells with the aid of a pipet tip and vortex to obtain a uniform suspension of cells.
4. Centrifuge the mixture at 1,000 to 1,500 rcf for 5 minutes to pellet the cells.
5. Discard the Supernatant.

Prepare Dried Lymphocyte Control Lysate

1. Prepare 500 µL of *diluted* lysis buffer by adding 50 µL of lysis buffer (LMLLB) to 450 µL reagent grade water.
2. Add 500 µL of *diluted* lysis buffer to the Dried Lymphocyte Control pellet that was previously rehydrated. The rehydrated lymphocyte control provided contains approximately a 50 µL pellet.
3. Mix and vortex the cell suspension to completely lyse the cells.
4. Centrifuge the mixture at 1,000 to 1,500 rcf for 3-5 minutes to sediment the cell membranes.
5. Transfer the supernatant (lymphocyte lysate) into a clean labeled tube.

Note: The lymphocyte lysates should be held either at 2 to 8°C or preferably on ice for a short period of time not exceeding 4 hours. Alternatively, promptly freeze any remaining undiluted control lymphocyte lysate in small aliquots (single use) in sealed and labeled tubes at -70 to -80°C for up to two years. If frozen lysate is being used, thaw an aliquot just prior to use. Vortex the thawed lysate to thoroughly mix, then centrifuge at 8,000-12,000 rcf for 4-5 minutes. Avoid any pelleted material when withdrawing an aliquot for testing.

Isolate Donor Lymphocytes

1. Layer whole blood or spleen cell suspension on a lymphocyte density separation media (1.077 g/mL)
 - a. To create a cell suspension from a spleen, macerate the tissue in complete cell culture media.
 - b. When isolating lymphocytes from whole blood, add an equal volume of complete culture media. Layer the whole blood/complete cell culture media suspension over the density separation media.
2. Centrifuge at 1,000 to 1,500 rcf with the break off for 15 to 20 minutes.
3. Collect the layer of cells at the density separation media interface and transfer to a large tube. Care should be taken to minimize the number of red cells and platelets in the preparation.
4. Centrifuge at 1,000 to 1,500 rcf for 5 to 10 minutes to pellet the cells. Discard the supernatant.
5. Wash the cells three times
 - a. Add a volume of complete cell culture media equal to at least 5 times the volume of the pellet. Gently resuspend the cells.
 - b. Centrifuge at 1,000 to 1,500 rcf for 5 to 10 minutes to pellet the cells. Discard the supernatant.

- c. Repeat two more times for a total of three washes
6. Estimate the total packed cell pellet volume available.
7. If the cells are to be used in testing other than DSA, add an equal volume of media to the packed cell pellet and resuspend the cells. A portion of the cell suspension will be moved to a new tube to make the lysate.

Determine the Volume of Lymphocytes Required for Testing

1. Referring to the test procedure, determine the number of wells that will be required for each donor or control lysate. Include 1 extra well for every 8 wells required.
2. Each well of testing requires 8 μL of lysate which is generated from $\sim 0.7 \mu\text{L}$ of packed lymphocyte pellet, 1.4 μL of a 50% cell suspension, or $\sim 2.2 \times 10^6$ cells. Refer to the following table for additional information.

Volume of Packed Lymphocytes	Estimated # Lymphocytes Obtained from Peripheral blood	Estimated # Lymphocytes Obtained from Spleen cells
10 μL	30×10^6 cells	30×10^6 cells
20 μL	50×10^6 cells	60×10^6 cells

Prepare Donor Lymphocyte Lysate

1. Determine the volume of lysate to be used in the DSA assay.
2. Determine the volume of packed cells that is required to make the lysate. For every 10 μL of packed cells, 100 μL of *diluted* lysis buffer is required.
3. Transfer the volume of 50% cell suspension (from the Isolating Donor Lymphocyte section) that will deliver the required packed cell volume to a new microfuge tube.
4. Centrifuge the transferred volume cell suspension at 1000-1500 rcf for 5-10 minutes to pellet the cells. Discard the supernatant.
5. Add the required volume of *diluted* lysis buffer to the cell pellet. For every 10 μL of packed cells, 100 μL of *diluted* lysis buffer is required. To make 100 μL of *diluted* lysis buffer, add 10 μL of lysis buffer (LMLLB) to 90 μL of reagent grade water.
6. Mix and vortex the cell suspension to completely lyse the cells.
7. Centrifuge the mixture at 1,000 to 1,500 rcf for 3-5 minutes to sediment the cell membranes.
8. Transfer the supernatant (lymphocyte lysate) into a clean labeled tube.

Note: The lymphocyte lysates should be held either at 2 to 8°C or preferably on ice for a short period of time not exceeding 4 hours. Alternatively, promptly freeze any remaining undiluted donor lymphocyte lysate in small aliquots (single use) in sealed and labeled tubes at -70 to -80°C for up to two years. If frozen lysate is being used, thaw an aliquot just prior to use. Vortex the thawed lysate to thoroughly mix, then centrifuge at 8,000-12,000 rcf for 4-5 minutes. Avoid any pelleted material when withdrawing an aliquot for testing.

Test Procedure:

1. Bring Wash Buffer and Specimen Diluent to room temperature (20 to 24°C). Leave other components at 2 to 8°C in the dark until required.
2. Using the Plate Format Sheet, assign a position on the filter plate for each of the following.
 - a) For the Dried Lymphocyte Control (LMDLC) lysate, assign one well for the Lysate Control Reagent (LMLCR), one well for the supplied Positive Control Serum (LMPC) and one well for the supplied Negative Control Serum (LMNC).
 - b) For each test lysate, assign one well for the Lysate Control Reagent (LMLCR) and one well for each serum being tested on the lysate.
3. Prepare the lysates by centrifuging the vials at 8,000-12,000 rcf for 4-5 minutes.
4. Prepare the test sera by centrifuging the vials at 8,000-12,000 rcf for 4-5 minutes.
5. Prepare the beads by centrifuging (30 seconds) the vial at 600 – 800 rcf to remove any beads or liquid from the cap or walls of the vial. Thoroughly vortex (~ 1 minute) to evenly resuspend the beads.
6. Add the required volume of each lysate and required volume of beads to a labeled microfuge tube. 8 μL of lysate and 5 μL of beads are required per well of testing. To allow for losses due to pipetting, add an extra well for every 8 wells. Repeat for each donor and control lysate. Vortex each tube briefly to mix the beads and lysate together.
7. Incubate the lysates and bead mixtures for 30 minutes in the dark at room temperature (20 to 24°C). Vortex the bead/lysate blends every 5-10 minutes.
8. Prepare the required total volume of *diluted* LMLCR. 50 μL of diluted LMLCR is required per well of testing; one for each donor and control lysate. To accommodate pipetting losses, add one (1) extra volume of diluted LMLCR for every 8 wells required. Prepare diluted LMLCR by adding 5 μL of the LMLCR to 45 μL of Specimen Diluent, for each well of testing. Store in the dark at room temperature until used. Return the unused portion of LMLCR to storage at 2 to 8°C for future use.
9. Prepare the wells of the filter plate. Referring to the Plate Format Sheet, cover the unassigned wells of the Filter Plate with adhesive plastic sealer. Add 100-300 μL of distilled water to the assigned wells. After 2-5 minutes, aspirate the water from the plate by using the vacuum manifold. (See manufacturer's recommendations for proper use).
10. After the 30 minute incubation, add the correct volume of Wash Buffer to the appropriate bead/lysate mixture. 42 μL of Wash Buffer is required per well of testing. To allow for losses due to pipetting, add one (1) extra well for every 8 wells required for each lysate. Vortex 30 seconds to mix.
11. Transfer 55 μL of each diluted bead/lysate suspension to the wells of the filter plate assigned to that lysate. Refer to the Plate Format Sheet.
12. Add 100 μL of Wash Buffer to each well. Mix by tapping the side of the plate and aspirate the plate.
13. Add 250 μL of Wash Buffer to each well, aspirate, and repeat two more times for a total of three 250 μL washes.
14. Add 50 μL of *diluted* LMLCR (step 8) to the designated wells. Refer to the plate format sheet.
15. Add 38 μL of Specimen Diluent to each test well of the Filter Plate, then 12 μL of serum or control serum.

16. Cover the plate with adhesive plastic sealer then foil or box to protect from light.
17. Incubate for 30 minutes at room temperature (20 to 24°C) in the dark on a rotating platform (200 rotations per minute).
18. Prepare the required total volume of *diluted* SA-PE. Prepare diluted SA-PE by adding 5 µL of LMSA to 45 µL of Wash Buffer, for each well of testing (see step 23). To accommodate pipetting losses, add one (1) extra volume of diluted LMSA for every 8 wells required. Cover with foil and/or store in the dark at room temperature until used. Return the unused portion of LMSA to storage at 2 to 8°C in the dark for future use.
19. Prepare the required total volume of *diluted* Conjugate. Prepare diluted Conjugate by adding 5 µL of LMCJS to 45 µL of Wash Buffer, for each well of testing (see step 24). To accommodate pipetting losses, add one (1) extra volume of diluted LMCJS for every 8 wells required. Cover with foil and/or store in the dark at room temperature until used. Return the unused portion of LMCJS to storage at 2 to 8°C in the dark for future use.
20. After the 30 minute incubation, carefully remove the adhesive plate sealer from the filter plate.
21. Add 100 µL of Wash Buffer to each well. Mix by tapping the side of the plate and then aspirate the plate.
22. Add 250 µL of Wash Buffer to each well, mix and aspirate. Repeat two more times for a total of three washes.
23. Add 50 µL of diluted SAPE to the wells that previously contained diluted LMLCR. Refer to the plate format sheet.
24. Add 50 µL of diluted Conjugate to the wells that previously contained diluted serum samples and serum controls. Refer to the Plate Format Sheet.
25. Cover the plate with an adhesive plate sealer then foil or box to protect from light.
26. Incubate for 30 minutes at room temperature (20-24°C) in the dark on a rotating platform (200 rotations per minute).
27. After the 30 minute incubation, carefully remove the adhesive plastic sealer from the filter plate.
28. Add 150 µL of Wash Buffer to each well. Mix by tapping the side of the plate.
29. Collect data with Luminex instrument using the manufacturer's recommendations.
30. Return the unused portion of reagents to storage at 2 to 8°C for future use.

QUALITY CONTROL

The bead set includes control beads to monitor assay performance. The CON beads measure the background in the assay and are used to normalize the signal of the capture beads. Probe 77 is conjugated with human IgG and will indicate that the Conjugate (LMCJS) has been added to the well. Probe 78 is conjugated with a biotinylated protein and will indicate that the SA-PE (LMSA) has been added to the well.

Quality control of Donor Specific Antibody is built into the test system by the inclusion of Positive and Negative Control Sera matched to the provided Dried Lymphocyte Control. These controls should be included with each test run to help determine if technical errors or reagent failures have occurred. With both of these control sera samples, Probe 77 should show values $\geq 10,000$ MFI. When the dried lymphocyte control is tested with the lysate control reagent, the LMLCR will react with both the Class I and Class II HLA capture beads giving a median fluorescence intensity of $\geq 10,000$. Probe 78 should show values $\geq 8,000$ MFI. If these controls fail to meet these criteria then your assay, conjugate, or SA-PE may be compromised.

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 capture bead is positive or negative for donor specific antibodies, the MFI value of each capture bead is compared to three cutoff values (background adjustment factors; BAFs). The three cutoff values are calculated from the background measured on the three CON beads in each test well. Each CON bead has an equation for calculating the cutoff value for the Class I HLA capture bead and a separate equation for calculating the cutoff value for the Class II HLA capture bead. The two equations for each CON bead are lot-specific and can be found on the Recording Sheet. The cutoff value calculated for a CON bead is subtracted from the MFI value of the capture bead. The process is repeated for each of the remaining two CON beads to obtain three results (Adjusted MFI Values). A sample is considered to be positive for donor specific antibodies if two or more Adjusted MFI Values are positive. A sample is considered to be negative for donor specific antibodies if two or more Adjusted MFI Values are negative.

Example: Class I HLA BAF for CON1 = $49.465(\text{CON1})^{0.5312}$

Class I HLA BAF for CON2 = $34.622(\text{CON2})^{0.5673}$

Class I HLA BAF for CON3 = $29.615(\text{CON3})^{0.6532}$

Example: Class I HLA Capture Bead MFI - (BAF for CON1) = Adjusted MFI 1

Class I HLA Capture Bead MFI - (BAF for CON2) = Adjusted MFI 2

Class I HLA Capture Bead MFI - (BAF for CON3) = Adjusted MFI 3

- A sample is considered positive for donor specific antibodies if two or more adjusted MFI values are positive.
- A sample is considered negative for donor specific antibodies if two or more adjusted MFI values are negative.

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, or SA-PE to stray light, or omission of test reagents or steps.

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

The quality of this product is evaluated using lysates matched to sera with antibodies reactive against HLA from the A, B, and DR loci. Bacterial contamination of samples can cause increased non-specific binding and erroneous results.

This product detects IgG antibodies that may or may not be lymphocytotoxic.

This product is not designed to detect IgA or IgM antibodies.

These products are designed to be used with the Luminex instrument according to the manufacturer's recommendations.

TROUBLESHOOTING

To be determined

EXPECTED VALUES

To be determined

SPECIFIC PERFORMANCE CHARACTERISTICS

The specific performance characteristics for this product have not yet been determined.

REFERENCES

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MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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