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PRODUCT INSERT

LIFECODES LSA™-MIC: A Luminex® screening assay for the qualitative detection of anti-MICA IgG antibodies.

For Research Use only.

TABLE OF CONTENTS

Definition of Symbols............................ **1**
Intended Use... **2**
Summary and Explanation..................... **2**
Principles of the Procedure.................... **2**
Reagents... **2**
A. Identification................................... 2
B. Warnings and Cautions..................... 2
C. Storage Instructions......................... 2
D. Purification or Treatment for Use........ 2
E. Instability Indications........................ 3
Instrument Requirements...................... **3**
Specimen Collection and Preparation...... **3**
Procedure.. **3**
A. Materials Provided.......................... 3
B. Materials Required, but not Provided.. 3
Directions for Use.................................. **3**
Results.. **4**
Quality Control..................................... **4**
Limitation of the Procedure................... **4**
Troubleshooting.................................... **5**
Expected Values................................... **5**
Specific Performance Characteristics..... **5**
References... **5**

DEFINITION OF SYMBOLS

(Product Labels and Supplemental Documents)

Meaning	Symbol	Meaning	Symbol	Meaning	Symbol	Meaning	Symbol
Lot	LOT	Catalog Number	REF	Expiration Date	[hourglass]	Temperature range (storage)	2°C – 8°C
Relative amount of bead bound Antigen	AG-O	Manufacturer	[factory]	European Representative	EC REP	Temperature (storage)	-80C
Dilute Before Use	DIL	Light Sensitive (Keep away from light)	[no light]	Sufficient for N tests	Σ	See instructions for use	[book i]
Name	NAME	Identification Number	ID#	Date	DATE	Technician	TECH
Bead	BEAD	Lifecodes Single Antigen	LSA	MHC class I chain-related antigen A	MICA	Bleed Date	BDT
Background Adjusted Value	BAV	Adjusted Value	AV	Median Fluorescence Intensity	MFI	Interpretation	INTRP
Negative Control Bead	CON	Positive Control Bead (Immunoglobulin G)	IgG	Antigen	AG	Sample	SAMPLE
Calculated Control	CalcCON	Suggested Cutoff	SCO	Probe	PROBE	Expected Result	EXPECTED RESULT

LC1017RUO.4 (12/09)

INTENDED USE

LIFECODES LSA™-MIC is a bead-based immunoassay used to qualitatively detect anti-MICA IgG antibodies.

SUMMARY AND EXPLANATION

Major histocompatibility complex class I chain-related antigen A (MICA) proteins are similar to the HLA class I gene products. However, MICA proteins do not associate with beta$_2$-microglobulin and have a groove too narrow to accommodate peptides for the presentation to T cells. MICA has a functional role as a ligand for the activating receptor NKG2D. Similar to the highly polymorphic HLA system, MICA molecules can become the targets of antibody responses in people during pregnancy, transfusion of blood products, or organ transplant rejection. Generally, immunization leads to the production of anti-MICA antibodies in a significant fraction of exposed individuals. The presence or absence of these MICA-specific antibodies may play a role in determining the survival of organ transplants.

LIFECODES LSA-MIC™ Beads are designed to detect IgG antibodies to MICA proteins. LSA-MIC is composed of different Luminex Beads to which purified recombinant MICA proteins 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 a second 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 negative control sera and the negative control bead included in the bead preparation to determine if the bead is positive or negative for bound antibody.

REAGENTS

A. Identification

265300: LSAMIC LIFECODES LSA™-MIC consists of five (5) components in sufficient quantities for 24 tests.

1. **LSAMICB** **LSA-MIC Bead Mix** (960 µL): A blend of beads each conjugated with a different single MICA glycoprotein plus four (4) control beads. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide and bovine serum. LIGHT SENSITIVE. Keep routine exposure to light to three hours or less. **Store at -80℃ in the dark.**
2. **LSACJM** **LSA-MIC Conjugate Concentrate** (120µL): Donkey F(ab')$_2$ 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℃ in the dark.
3. **LSAWB** **LSA Wash Buffer** (25 mL): A phosphate-based buffer containing NaCl, Tween-20 and sodium azide. Store at 2 to 8℃ and equilibrate to room temperature (20-24°C) prior to use.
4. **LSAPCM** **LSA-MIC Positive Control** (50 µL): This serum or sera blend is obtained from individual(s) shown to be immunized to MICA antigens and will react with most of the LSA-MIC Beads. Contains 0.1% sodium azide as a preservative. Store at 2 to 8℃.
5. **LSANCM** **LSA-MIC Negative Control** (50 µL): This serum or sera blend is obtained from individual(s) known to have no antibodies to MICA antigens and will react with few if any of the LSA-MIC Beads. Contains 0.1% sodium azide as a preservative. Store at 2 to 8℃.

B. Warnings or Cautions

1. For Research Use only.
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 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 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-MIC 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 samples and control
- Timer
- Marking pen
- Millipore multiscreen filter plates (Milllipore Cat# MSBVN1210, Lifecodes Cat # 888633)
- Multiscreen vacuum manifold (Millipore Cat # MAVM 0960R, Qiagen Cat # 19504, Lifecodes Cat#888315)
- Luminex Sheath Fluid (1x or 20x, Lifecodes Cat # 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
- Adhesive plastic covers

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 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. Take out the LSA-MIC 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 at least 6 times without affecting performance.**
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 serum 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-MIC 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-MIC Beads to each of the assigned wells. Re-vortex the LSA-MIC Bead Mix vial every 2 minutes to keep the beads in suspension while distributing the beads, then add 10 µL of serum or control serum 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 (200 rotations per minute). Return unused portions of control sera to storage at 2 to 8°C for future use. Return unused portions of LSA-MIC Bead Mix to storage at -80°C 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 by tapping the side of the plate or pipetting 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 by tapping the side of the plate or pipetting, 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

Enter the Raw Median Fluorescence Intensity (MFI) values for each bead into the worksheet. To determine if a bead is positive, first subtract the Background MFI from the RAW MFI to generate the Adjusted Value 1 for each individual bead. The Background MFI is the background noise due to bead variation and can be found on the lot-specific Recording Sheet provided with the kit. Divide the Adjusted Value 1 by the MFI of the Calculated Control (CalcCON) to generate the Adjusted Value 2. The CalcCON is the Raw MFI value of the lowest ranked antigen bead.

Example:

$$\frac{\text{Individual Bead Raw MFI} - \text{Background MFI}}{\text{CalcCON MFI}} = \text{Adjusted Value 2 for antigen "x"}$$

To generate Adjusted Value 3, normalize Adjusted Value 2 by dividing this number by the relative amount of antigen on each bead as found in the lot-specific Recording Sheet.

Refer to the lot-specific Recording Sheet provided with the kit for the list of the antigens present on each bead and the suggested cutoff for estimating the positive/negative result. The bead is considered positive if two or more of the adjusted values are above the cutoff values. Higher or lower sensitivities can be obtained by adjusting the cutoff.

QUALITY CONTROL

Quality control of LSA-MIC 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 of the LSA-MIC conjugated beads, generating a pattern similar to that found in the lot specific worksheet. The Negative Control Sera will react with few if any of the LSA-MIC conjugated beads typically generating raw MFI values under 1000 MFI.

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

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

LIFECODES Single Antigen MICA proteins were obtained from cell lines expressing single MICA 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 MICA testing, qualified personnel should review data interpretation.

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 (CON) Threshold Surpassed	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
Anomalous pattern for Positive Control Sera	Incorrect sample added	Repeat with correct control sample
	Poor washing	Repeat and monitor washes
High MFI for Negative Control Sera (adj. val. 1 >300MFI)	Poor washing	Repeat and monitor washes to insure beads are re-suspended during washing
		Reduce vacuum strength

EXPECTED VALUES

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 > 2,000 with the control sera. If you obtain values less than 2,000 MFI with the control sera, your assay may be insufficiently washed or your conjugate may be compromised. Similarly, the Negative Control Bead typically shows low MFI values with the Positive Control Sera (see lot specific worksheet).

SPECIFIC PERFORMANCE CHARACTERISTICS

When LSA-MIC kits are used according to the procedure described, the results reveal the presence or absence of anti-MICA IgG antibodies. Specific performance characteristics are to be determined.

REFERENCES

1. Gao, X. *et al.* (2006). Diversity of MICA and Linkage Disequilibrium with HLA-B in Two North American Populations. *Human Immunology*, 67,152-158.
2. Mizutani, K. *et al.* (2006). Association of Kidney Transplant Failure and Antibodies Against MICA. *Human Immunology*, 67, 683-691.

MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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