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PRODUCT INSERT

LIFECODES® C3d Detection

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

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DEFINITION OF SYMBOLS

(Product Labels and Supplemental Documents)

Batch Code	LOT	Catalog Number	REF	Use By Date		Temperature range (storage)	8°C 2°C
Manufacturer		Light Sensitive (Keep away from light)		Temperature (storage)	≤ -65°C	Sufficient for N tests	Σ
Warning							

SUMMARY AND EXPLANATION

This product may be used to detect C3d complement bound to antibody/antigen complex.
This product contains PE-conjugated anti-human C3d antibody, positive control bead, complement containing human sera and wash buffer.

PRINCIPLES OF THE PROCEDURE

An aliquot of bead bound HLA antigens is allowed to incubate with a small volume of test serum sample. After this initial incubation, negative serum reagent is added as a source of complement for an additional incubation. The sensitized beads are then washed to remove unbound antibody. An anti-Human C3d antibody conjugated to phycoerythrin is then added. After another incubation, the test sample is washed, diluted and analyzed on the Luminex instrument. The signal intensity from each bead, for the test sample, is compared to the signal intensity of negative control sera to determine if the sample should be considered positive or negative for C3d bound to antibody/antigen complex.

LC1495RUO.2 (08/15)

REAGENTS PROVIDED

A. Identification and storage conditions
C3d Detection Product Number 265400R

1. **C3dCJ** C3d Conjugate (P/N 265410; 1200 µL). PE-conjugated Anti-human C3d antibody in a ready to use phosphate-based storage buffer containing NaCl, Tween-20 and sodium azide. LIGHT SENSITIVE. Keep out of direct light for extended periods of time. **Store at 2 to 8°C in the dark for up to 3 months or at ≤-65°C up to expiration date**. It can be re-frozen up to 6 times at ≤-65°C after initial thaw.
2. **C3dCS** Complement Serum (P/N 265415 ; 2 vials x 360 µL). Serum from non-transfused male donor. **Store at ≤-65°C**. It can be re-frozen up to 4 times after initial thaw.
3. **C3dPCB** C3d Positive Control Bead (P/N 265405; 24 µL). The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide and bovine proteins. LIGHT SENSITIVE. **Store at ≤-65°C**. It can be re-frozen up to 6 times after initial thaw.
4. **LSAWB** LSA Wash Buffer (P/N 265001; 25 mL). A phosphate-based buffer containing NaCl, Tween-20 and sodium azide. **Store at 2 to 8°C** and equilibrate to room temperature (20-24°C) prior to use.

B. Warnings or Cautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. Human source material used in the production of this kit has been tested and found to be negative for antibody to HIV, HCV, and HBsAg by FDA-approved methods. However, no test method can offer complete assurance that infectious agents are absent. Therefore, **use Universal Precautions** when working with these materials.
3. 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.
4. Bacterial contamination of samples or the presence of immune complexes or other immunoglobulin aggregates can cause increased non-specific binding and erroneous results.
5. Dispose of all materials after use according to local regulations.
6. See Safety Data Sheets for additional information.
7. Complement Sera left at 2 to 8°C for extended periods of time demonstrate reduced complement activity.
8. Beads and conjugate are LIGHT SENSITIVE. Keep routine exposure to light to three hours or less.

C. Purification or Treatment Required for Use

1. See “Specimen Collection and Preparation.”
2. All components are ready for use and no dilution is required.

D. Instability Indications

1. Do not use components or controls that are turbid or beyond their expiration date.

MATERIALS, REAGENTS AND EQUIPMENT Required but NOT PROVIDED

- LIFECODES LSA Class I (P/N 265100R, LSA1) or LIFECODES LSA Class II (P/N 265200R, LSA2) Kits.
- Equipment required to perform the LIFECODES LSA Class I or Class II assay (see corresponding Product Insert, LC976RUO)

SPECIMEN COLLECTION AND SERUM PREPARATION

Blood should be collected without anticoagulant using aseptic technique and should be tested while still fresh or properly stored to minimize the chance of 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 up to 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.

CAUTION: 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.

DIRECTIONS FOR USE

PRECAUTIONS:

- Care MUST be taken to avoid contamination of Wash Buffer and the anti-Human C3d reagent. Inadvertent contamination of these reagents with human serum may subsequently result in test failure.
- A sample of positive and negative control sera should be included with each test to help determine if technical error or reagent failures have occurred.
- The Complement Serum is a negative serum reagent required for the C3d Detection Assay as a standard source of complement.
- In addition, follow the general precautions described in the LIFECODES LSA Product Insert (LC976RUO).

1. Turn on the Luminex instrument to allow for 30 minute warm-up.
2. Remove LSA Bead Mix, C3d Positive Control Bead (C3dPCB), C3d Complement Serum (C3dCS) and C3d Conjugate (C3dCJ) from the -65ºC freezer and store in the dark at room temperature until thawed. Once thawed, place immediately on ice and protect from light.
3. Bring the Wash Buffer to room temperature (20 to 24°C) prior to use. During this time, use the Plate Format Sheet (LC979) to assign a position on the plate for each of the sera and controls to be analyzed. **The Negative Control Sera (provided with LSA1 and LSA2 kits) is used as the negative control**.
4. 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).
5. Prepare the LSA Beads by 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. In a separate vial combine 1 µL/sample of C3dPCB with the appropriate volume of LSA beads (40 µL/sample). Thoroughly vortex (~1 minute) to evenly resuspend the beads.
6. Add 40 µL of LSA Bead Mix with C3dPCB to each of the assigned wells. Re-vortex the LSA Bead vial every 2 minutes to keep the beads in suspension while distributing the beads. Then centrifuge serum (30 seconds at ~10,000 x g) and 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 bead 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.

7. 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 Bead Mix and C3dPCB to storage at ≤-65ºC in the dark for future use.
8. After the 30 minute incubation, remove the adhesive plastic cover and add 30 µL of the C3dCS to each well including the negative control well. Return **the C3dCS to storage at ≤-65°C immediately after use.** 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).
9. 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.

10. Add 250 µL of Wash Buffer to each well, aspirate, and repeat three more times.

CAUTION: Failure to wash completely may reduce the ability of the conjugate to detect C3d bound to antibody/antigen complex and cause false negative results.

11. Centrifuge C3dCJ for 30 seconds in a microcentrifuge (~600 – 800 x g). C3dCJ is ready to use and no dilution is required. Add 50 µL of C3dCJ to each well. Store remaining C3dCJ at 4°C up to three months or store it at ≤-65ºC up to expiration date.
12. 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).
13. 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.
14. Add 250 µL of Wash Buffer to each well and aspirate.
15. Using a clean pipette tip, add 200 µL of Wash Buffer to each well and mix to resuspend beads.
16. Collect data with the Luminex instrument using the manufacturer's recommendations and **use a Luminex C3d template (refer to LIFECODES LSA for the lot specific information)**. Delays of greater than 3 hours at room temperature 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

C3d Detection: To analyze the results for a batch of samples:

1. Create a worksheet in Excel by opening a copy of the output CSV file with the results from the Luminex batch and "Save as" an Excel file. This file will be used for the calculations used to analyze the results.
2. From the lot specific Recording Worksheet provided with the LSA Kit, copy the name of the antigen that corresponds to each bead.
3. Next, subtract the MFI values of the Negative Control serum (MFI of NC serum) from the RAW MFI for each individual bead to calculate the Background Adjusted MFI (BG Adjusted).

   (a) BG Adjusted MFI = MFI of a sample – MFI of NC serum

4. Then, divide the BG Adjusted MFI by the MFI of the Calculated Control (CalcCON) of its respective locus to generate the Background Corrected ratio (BCR-Neg). The CalcCON for each locus is the Raw MFI value of the lowest ranked antigen bead for that locus.

   (b) BCR-Neg = $\frac{\text{BG Adjusted MFI of antigen}}{\text{Lowest Raw Value MFI of locus}}$

5. Last, divide the BG Adjusted MFI of antigen by the corresponding MFI value of the antigen for the LSA Negative Control (NC) serum to generate the relative strength (R-Strength).

   (c) R-Strength = $\frac{\text{BG Adjusted MFI of antigen}}{\text{Raw Value MFI of antigen}}$

A 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. Due to the complex nature of HLA testing and the multiple factors that may affect the complement cascade leading to the formation of C3d, qualified personnel should review and interpret the results. Refer to the Certificate of Analysis of the C3d Detection product (265400R) for the positive and negative default cut-offs.

QUALITY CONTROL

Quality control of C3d Detection is built into the test system by the inclusion of the Positive Control Bead and Positive and Negative Control Serum (provided with LSA1 and LSA2 kits). These controls 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 a number of LSA conjugated beads, generating a pattern similar to that found in the C3d detection graph. The Negative Control Serum will react with few if any of the LSA conjugated beads.
The C3d Positive Control Bead should yield MFI values ≥10,000 with an assay using the negative control serum. Sample values lower than 10,000 MFI may indicate that insufficient C3dCJ has been added, the assay may be poorly washed or the C3dCJ may be compromised.

LIMITATIONS OF THE PROCEDURE

For Research use only. The results of tests performed with these reagents are not intended to be used for any type of diagnosis, patient evaluation, clinical decisions or prevention of disease.

Erroneous results can occur from bacterial contamination of test materials, inadequate incubation periods, inadequate washing or decanting of beads, exposure of C3dCJ 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.

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.

TROUBLESHOOTING

(Also refer to LIFECODES LSA Class I and Class II Product Insert LC976RUO).

PROBLEM	POSSIBLE CAUSE	SOLUTION
Low Bead Count only for C3dPCB	Insufficient beads added to LSA Bead Mix	Pulse vortex to completely resuspend, avoid pippeting <3 µL, use calibrated pipettes
	Instrument failures - out of calibration	See Luminex Manual
	Photobleached beads	Use new vial of C3dPCB
C3d Positive Control MFI values with Serum <10,000 MFI	Photobleached or insufficient C3dCJ added to reaction	Repeat assay. Use new vial of C3dCJ
	Poor washing	Repeat assay and monitor washes
Low MFI for Positive Control Serum	Incorrect sample added	Repeat assay with correct control sample
	Insufficient C3dCJ added to reaction	Repeat assay with correct amount of C3dCJ
	Insufficient C3dCS or it was not added to the reaction	Repeat assay adding C3dCS
	Low assay temperature	For higher MFI repeat assay at 22°C-24°C; for better temperature control it is suggested to use a thermomixer or equivalent
Anomalous pattern for Positive Control Sera	Incorrect sample added	Repeat assay with correct control sample
	Poor washing	Repeat assay and monitor washes
High MFI for Negative Control Sera (>1500 MFI)	Poor washing	Repeat assay and monitor washes to insure beads are re-suspended during washing
		Reduce vacuum strength

SPECIFIC PERFORMANCE CHARACTERISTICS

Specific performance characteristics have not been determined.

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