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PRODUCT INSERT

REF

LAMBDA CELL TRAY™

Catalog #s LCT-30ABC, LCT60-ABC, LCT-30D, LCT-72ABC, LCT1W30, LCT1W60

IVD **For In Vitro Diagnostic Use.**

INTENDED USE

For the screening of sera for Class I and Class II antibodies with a complement-dependent microlymphocytotoxic technique.

The intended use of the Lambda Cell Tray™ 30T and 36T (OLI Cat. #s LCT30ABC, LCT1W30) is for the screening and assignment of broad HLA Class I specificities.

The intended use of the Lambda Cell Tray™ 60T and 72T (OLI Cat. #s LCT60ABC, LCT72ABC, and LCT1W60) is for the screening and assignment of HLA Class I specificities. The intended use of the Lambda Cell Tray 30B (OLI Cat. #LCT30D) is for the assignment of HLA Class II specificities.

SUMMARY AND EXPLANATION

The Lambda Cell Trays™ (LCT™) are used to detect the presence of HLA cytotoxic antibodies. Each well on the tray contains approximately 1 µl of human lymphocytes suspended in dimethyl sulfoxide (DMSO) in McCoy's media. LCT1W trays contain a proprietary freezing media to increase stability and to maintain lymphocyte viability. To prevent evaporation, 5 µl of heavy mineral oil is placed in each well.

The LCT™ ABC trays contain T cell lymphocytes representing the HLA Class I antigens from selected individuals in addition to a negative and positive control. The LCT30ABC, and LCT1W30 will process two samples with each sample assigned to either rows 1-5 and 1-6 or rows 6-10 and 7-12. The LCT60ABC, LCT72ABC and LCT1W60 will process one sample.

The LCT30D has B cell lymphocytes representing the HLA DR, DQ antigens from 28 different selected individuals, in addition to a positive and a negative control. These B cell lymphocytes are isolated from individuals who have Chronic Lymphocytic Leukemia. The LCT30D will process two samples and each sample is assigned to either row 1-5 or row 6-10 for testing.

Positive and negative control sera should be run with every batch of LCT™ trays tested. The positive control serum is used to determine cell reactivity. The positive control can be an antilymphocyte serum that is produced in rabbits or mice that is a monoclonal anti-lymphocyte antibody, or a high PRA human sera. The negative control serum is used to determine lymphocyte viability. This negative control antiserum must be from a healthy male blood type AB and must have no cytotoxic reactivity in tests with random lymphocyte donors.

PRINCIPLE(S)

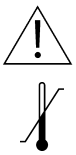
Lymphocytes with known antigens are incubated with an unknown serum and rabbit complement. If the serum contains antibodies that are able to bind specifically to those antigens present on the lymphocyte surface, complement mediated cytolysis will occur. If the unknown serum contains HLA antibodies, they can be characterized.

REAGENTS

A. Identification
Each well on the tray contains approximately 3,000 – 5,000 frozen human lymphocytes per well. To prevent evaporation, 5 µl of heavy mineral oil is placed in each well.

B. Warning or Caution
1. For In Vitro Diagnostic Use.
2. **Warning:** All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
3. Refer to Material Safety Data Sheet for detailed information.

C. Instructions for Use
See "Directions for Use."



D. Storage Instructions
1. Store reagents at temperature indicated on package. Use before printed expiration date.
2. **For LCT-30ABC, LCT-30D, LCT-60ABC and LCT-72ABC:** Trays must be stored in the bottom of the freezer at -65° C or below for no longer than two weeks or in the vapor phase (VP) of liquid nitrogen for no longer than three months.
3. **For LCT1W30 and LCT1W60:** Trays must be stored in the bottom of the freezer at -65° C or below for the entire product shelf life (approximately six months). Do not store in vapor phase of liquid nitrogen.
4. Do not refreeze. Do not store trays in dry ice.

E. Purification or Treatment Required for Use
LCT trays must be washed prior to use. Refer to "Directions For Use" below.

F. Instability Indications
Bacterial contamination and/or exposure to carbon dioxide will cause cell suspensions to change pH. This pH change is indicated by a yellow color cell suspension. If such a change occurs, the trays must be discarded.

SPECIMEN COLLECTION AND PREPARATION

Specimen required for testing is 1 ml of serum. This can be obtained by drawing a 5 ml sample of blood into a red (no anticoagulant), green (Sodium Heparin), purple (EDTA), or Yellow (ACD) Vacutainer® tube.

Preparation of Serum and Plasma Specimens

A. **Red Top Tubes:** Since there is no anticoagulant, a red cell clot should form. Remove the clot with a wooden applicator stick. Centrifuge the tube for 10 minutes at 1000 g. Transfer the serum to a clean tube.
B. **Green Top Tubes:** Centrifuge the tube for 10 minutes at 1000 g. Pipette plasma into an appropriate size tube. Add to plasma 1 drop of bovine thrombin (10,000 U.S. units per 6 ml of sterile water) per ml of plasma. Mix well and remove the fibrin clot. Centrifuge the tube for 10 minutes at 1000 g. Transfer the serum to a clean tube.
C. **Purple and Yellow Top Tubes:** Centrifuge sample for 10 minutes at 1000 g. Pipette plasma into an appropriate size tube. Add to plasma 1 drop of calcium chloride (1% solution by weight) per ml of plasma. Mix thoroughly. Add 1 drop of bovine thrombin (10,000 units per 6 ml of sterile water) per ml of plasma. Mix well and remove the fibrin clot. Centrifuge the tube for 10 minutes at 1000 g. Transfer the serum into a clean tube.

PROCEDURE

A. Materials Provided

1. Lambda Cell Tray(s)
2. Worksheets identifying antigen specificity
3. Instructions for Use.

B. Materials Required, But Not Provided

1. Phase Contrast inverted microscope with 40x objective
2. Microsyringes
3. Light Box/Fluorescent light table
4. Insta-Seal™ (OLI Cat. #TIS250U) cover slides or glass slides and petrolatum (Vaseline™)
5. 5% HIFCS McCoy's 5A
6. Stain and Fix Reagents
   a. **For Dye Exclusion Testing:** Eosin Y (sodium base) and formaldehyde, or Stain-Fix™ (OLI Cat. #SF-500).
   b. **For Fluorescence Testing:** FluoroQuench™ AO/EB (OLI Cat. #FQAE-500)

7. Positive and Negative Controls
8. Rabbit Complement
9. Calcium Chloride Solution ($CaC1_2$)
10. Bovine Thrombin
11. Beckman TJ6 Centrifuge or equivalent
12. 5 ml tubes
13. Pipettes
14. Bulbs
15. Beckman tubes
16. Wooden applicator sticks

C. Step-by-step procedure.
See "Directions For Use."

DIRECTIONS FOR USE

For optimal results, these procedures must be strictly followed. Use trays before the printed expiration date on the package.

A. Directions for HLA Class I Screening
1. Thaw the trays at 37 C for 2 - 5 minutes. ***For LCT1W30 and LCT1W60 only,*** thaw trays at 37° C for 5 minutes.
2. Add 20 µl of 20° - 25°C 5% HIFCS McCoy's 5A medium (RPMI can also be used) to each well and flick.
***Note for LCT1W30 and LCT1W60 only*:** Add 20 µl of 37° C 5% HIFCS McCoy's 5A medium (RPMI can also be used) to each well and incubate at 20 - 25° C for 5 - 10 minutes and flick.*

Note: *If total removal of DMSO and media cannot be accomplished without loss of lymphocytes by the flicking technique, centrifuge tray at 850 - 1000 g for 30 seconds. This will cause lymphocytes to adhere to the bottom of the well and flicking the tray will not cause any cell loss.*

3. Add approximately 5 µl of mineral oil to each well to prevent evaporation.
4. Add 1 µl of serum to each well**. Do not add serum to the control wells.** Add 1 µl 5% HIFCS McCoy's 5A medium to the control wells.
5. Mix the microdroplets together using an electrostatic mixer or a wire.
6. Incubate the tray at room temperature (20° - 25°C) for 30 minutes.
7. Add 5 µl of ABC rabbit complement to each well.
8. Incubate the trays at room temperature (20° - 25° C) for 1 hour.
9. After incubation, stain and fix the cells:
   a. For dye exclusion testing, add to each well:
      - 5 µl of eosin dye, followed 2 minutes later by 5 µl of formaldehyde, or
      - 10 µl Stain-Fix™ (OLI Cat. #SF-500)
   b. For fluorescence testing, add 5 µl FluoroQuench™ AO/EB (OLI Cat. #FQAE-500) to each well.
10. Cover the trays with Terasaki Insta-Seal™ (OLI Cat. #TIS250U). Let trays stand at room temperature for 15 minutes to allow lymphocytes to settle. Dye exclusion trays may be stored at 2 – 5° C for up to 2 weeks. Fluorescent trays may be stored at 2 - 5° C in the dark for up to 2 days. Trays covered with Terasaki Insta-Seal™ cover slides must be read the same day they are prepared for testing.

B. Directions for HLA Class II Screening (Using OLI Cat. #LCT-30D)
1. Follow procedure steps 1 - 5 in "Directions for HLA Class I Screening" above.
2. Incubate for 1 hour at 37° C.

**To flick, hold a tray in a level upright position and sharply snap the tray in a counter-clockwise motion (right-handed user). Failure to remove all the DMSO can cause some viability problems.*

3. Add 5 µl of DR rabbit complement to each well and incubate for 2 hours at room temperature (20 - 25° C).
4. Stain and fix the cells using Stain-Fix™, eosin/formalin or FluoroQuench™ AO/EB according to Step 9 above and read.

LIMITATIONS OF THE PROCEDURE

This test does not detect non-cytotoxic antibodies. However, goat anti-human globulin (AHG) may be used to augment cytotoxicity (OLI Cat. # AHG1).

RESULTS AND EXPECTED VALUES

Interpretation of Results (for each sample):

1. Using the appropriate worksheet, record baseline viability from normal serum control, and test serum reactions in the appropriate columns alongside the panel specification.

2. Count the number of recorded positive reactions. Using the formulas below, compute the percentage of positive reactions and the strength of the serum.

$$\text{PRA (\%Pos.)} = \frac{\text{No. of positive reactions}}{\text{No. of cells in panel}} \times 100$$

$$\text{Strength Index} = \frac{\text{No. of "8" reactions}}{\text{No. of positive reactions}} \times 100$$

3. The following is a simple method for determining the most probable HLA specificities present in the test serum:
   a. Mark all antigens present in the positive cells on the line opposite each cell in the matrix to produce a "serograph."
   b. For each antigen appearing in the matrix, count the number of "positive" reactions, and record the total number on the line labeled "positive rxns. (++)" below the matrix. If the number of positives is more than half the number of times an antigen appears in the panel, then the corresponding antibody is presumed to be present in the serum.
   c. Next, eliminate all specificities whose reaction pattern is wholly included within a longer pattern. Although an included specificity may be present, it would be necessary to test this serum against cells that do not bear antigens recognized by the longer reaction pattern.
   d. Specificities partially included should be checked with cells not bearing more than one antigen recognized by the serum, especially if the frequency of the antigen(s) in the panel is low.
   e. For each of the remaining probable specificities, count the number of false positives in the raw data (positive reactions that do not include the antigen in question) and record on the line below the antigen list labeled "false positives (-/+)." Then count the number of false negatives, and record on the line labeled "false negatives (+/-)."
   f. Calculate for each specificity, the r value or correlation coefficient.

$$r = \frac{(AD) - (BC)}{[(A+B)(C+D)(A+C)(B+D)]^{1/2}}.$$

A = Number of cells with the antigen and positive with the serum
B = Number of cells with the antigen but are negative (false negative)
C = Number of cells without the antigen but are positive (false positive)
D = Number of cells without the antigen and negative with the serum

Note: *A serum with an r value of 0.85 or more is a potentially useful typing reagent.*

   g. For sera containing more than one specificity, calculate the inclusion index, the degree to which one specificity is included in the serum, as a low r value for a specificity is misleading.

Inclusion index for a given specificity =

$$\frac{\text{No. of cells with antigen being positive}}{\text{Total number of cells with antigen}} \times 100$$

SPECIFIC PERFORMANCE CHARACTERISTICS

The T lymphocytes in the cell tray are phenotyped for HLA Class I antigens using a panel of anti-HLA Class I allo and murine antibodies HLA ABC Trays. The B lymphocytes in the cell tray are phenotyped for HLA Class II antigens using a panel of anti-HLA Class II human allo and murine antibodies. Each lot of cell trays reacted correctly with 5 known antisera. Each well in the cell tray has a minimum viability of 80% cell death when tested with the positive control serum.

BIBLIOGRAPHY

Amos, D.B., Pool, P., and Grier, J.O. HLA Typing. In Manual of Clinical Immunology, Noel Rose and Herman Friedman, eds., Amer. Soc. for Microbiology, Washington, D.C., 1980.

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