Lambda Monoclonal HLA Class I and Class II Tissue Typing Trays

REF **Catalog # LM144, LM144A, LM144B, LM172, LM272, LM372, LM72OR, LM96, LMB2703, MDR172, MDR272**

IVD For In Vitro Diagnostic Use

INTENDED USE

For use in determining HLA Class I and Class II cell surface antigens with a complement dependent microlymphocytotoxic technique.

SUMMARY AND EXPLANATION

The HLA Class I and Class II Lambda Monoclonal Typing (LMT™) Trays contain a mixture of known monoclonal reagent and rabbit complement which are used to determine the presence of HLA Class I or II antigens on T and B lymphocytes. Each well contains 1 microliter (1 µl) of a specific monoclonal and complement mixture, as well as 5 microliters (5 µl) of heavy mineral oil. Each tray contains a positive and negative control. The Class II typing trays also include an anti-B and anti-T lymphocyte monoclonal antibody.

PRINCIPLE(S)

Viable lymphocytes are incubated with a mixture of complement-binding monoclonal antibodies and complement. If the lymphocytes express an antigen recognized by a specific antibody, the Fab portion of the antibody binds to the antigen, forming an antigen-antibody complex. After these complexes have formed, the C1q and Ca++ from the complement binds to the FC portion of the antibody. One IgM antibody is required to bind one molecule of C1q or two IgG antibodies are required to bind one molecule of C1q. Binding of C1q initiates the complement cascade, which leads to cell lysis with antigen antibody complexes. In a negative reaction, the lymphocytes remain viable. In a positive reaction, the lymphocytes are dead.

REAGENTS

A. Identification

The HLA Class I or Class II Lambda Monoclonal Typing Trays contain a mixture of anti-Class I or Class II monoclonal antibody and rabbit complement. Each well contains 1µl of a specific monoclonal and complement mixture and 5µl of heavy mineral oil.

Note: *Sources of monoclonal may be mouse or human.*

IVD B. Warning or Caution

1. FDA Designation: IVD
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 method can offer assurance that products derived from human blood will not transmit infectious agents.
3. **Caution**: Ethidium bromide is a known carcinogen. Handle with appropriate caution. Can be harmful if absorbed through skin. Avoid splashing in eyes or on skin or clothing. Keep tightly sealed. Wash thoroughly after handling.
4. Refer to the Material Safety Data Sheet for detailed information.

One Lambda, Inc.
22801 Roscoe Blvd. West Hills, CA 91304 U.S.A
T: 747.494.1000 F: 747-494-1001

C. Preparing Reagents for Use
   1. See "Directions for Use".

D. Storage Instructions

   Store reagents at temperature indicated on package. Use before printed expiration date.

E. Purification or Treatment Required for Use

   See "Directions for Use".

F. Instability Indications

   Bacterial contamination and/or exposure to carbon dioxide will cause the sera to change pH. This pH change is indicated by a change in color from pink to yellow. If such a change occurs, discard trays.

SPECIMEN COLLECTION AND PREPARATION

A. Since viable lymphocytes are required for serological typing, blood should be received and processed immediately following procurement. Lymphocyte yield decreases with time and extreme temperature. Blood should be collected in acid citrate dextrose (ACD) or sodium heparin, stored horizontally at room temperature (20 - 25º C), and processed within 48 hours for maximum T and B lymphocyte yield.

PROCEDURE

A. Materials Provided

1. HLA Class I or Class II typing tray(s), 72 or 96 well.
2. Worksheets identifying the specificity of each monoclonal antibody.

B. Materials Required, But Not Provided

1. Microsyringes
2. Insta-Seal™ (OLI Cat. # TIS250U) cover slides or glass slides and petrolatum (Vaseline™).
3. Stain and Fix reagents:
   a. For dye exclusion testing: Eosin-Y (sodium base) and Formaldehyde.
   b. For fluorescence testing: FluoroQuench™ AO/EB (OLI Cat. # FQAE-100/500), FQZAE-100,or For CFDA Testing (part “B” below), add 1ml EB stock solution to 9 ml hemoglobin or 1% ink (see Materials #s 4 - 7 below).
4. Hemoglobin Quench
   a. From lyophilized bovine serum:
      (1) Dissolve 10gm hemoglobin in 100ml 5% EDTA PBS. Add 1ml of 1% Sodium Azide.
      (2) Centrifuge for 45 minutes at 1000g. Store supernatant at -20°C.
   b. From whole red cells:
      (1) Stock solution: Wash packed RBC 3 times with saline. Make a 70% hematocrit and freeze and thaw. Ultracentrifuge for 45 minutes at 20,000g. Dialyze 3 days against saline.
      (2) Working solution: Add 10ml 5% EDTA PBS. Add 1ml 1% sodium azide to 89ml hemoglobin. Store at -20º C.
5. 1% Ink Working Quench
   a. Dissolve 1gm Bovine Serum Albumin (BSA) in 10ml 5% EDTA PBS.
   b. To 9.8ml BSA/5% EDTA/PBS add:
      (1) 100µl 1% Sodium Azide

(2) 100µl Higgins Black Calligraphy Ink

6. 5% EDTA (disodium) PBS
   a. Dissolve 5gm EDTA in 90ml PBS.
   b. Adjust pH to 7.2 with 10M NaOH.
   c. Bring final volume to 100ml with PBS.
7. Ethidium Bromide (EB) Stock Solution: Dissolve 50mg in 1ml distilled water. Add 49ml PBS. Heat in water bath at 56º C for 30 minutes. Store at -20ºC.
8. Carboxyfluorescein Diacetate (CFDA)
   a. Stock solution: Dissolve 10mg CFDA in 1ml acetone in a glass tube. Store at -20º C in Beckman tubes.
   b. Working solution: Use either of the following:
      (1) Prepared in PBS at pH 7.2: Add 30µl CFDA stock solution to 5ml PBS (pH 7.2). Store at 2 - 5ºC for up to 1 week.
      (2) Prepared in PBS at pH 5.5: Add 30µl CFDA stock solution to 5ml PBS (pH 5.5). Store at 2 - 5ºC for up to 1 week.

C. Directions for Use

Note: *For lymphocyte isolation methods, refer to the ASHI Laboratory Procedure Manual[5] or the product insert for One Lambda FluoroBeads™ or LymphoKwik™ for lymphocyte isolation methods.*

1. Testing
   a. Thaw trays at room temperature (20 - 25ºC) for 15 minutes and use within 1 hour of thawing. ***Caution:*** *Do not refreeze.*
   b. To each well, add 1µl of a 2 X 10^6 cells/ml suspension of T lymphocytes to the Class I tray or B lymphocytes to the Class II tray. For fluorescence testing using CFDA, see part "2" below.
   c. Mix the microdroplets together using an electrostatic mixer or a wire.
   d. Incubate the trays at room temperature (20 - 25ºC) for 1 hour.
   e. After incubation, stain and fix the cells:
      (1) For dye exclusion testing, add to each well:
         - 5µl of eosin dye, followed 2 minutes later by 5µl per well of formaldehyde.
      (2) For fluorescence testing, add 5µl of FluoroQuench™ AO/EB (OLI Cat. # FQAE-100/500), FQZAE-100. For CFDA Testing (part "2" below), add 5µl per well of the hemoglobin or 1% ink quench with Ethidium Bromide.
   f. Cover the trays with Terasaki Insta-Seal™ (OLI Cat. # TIS250U). If a glass slide is used instead, seal with melted petrolatum. Let trays stand at 20 - 25°C 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.
2. Fluorescence: CFDA Labeling of Lymphocyte Preparation
   a. Incubate lymphocytes in 500µl CFDA pH 7.2 at 37ºC for 15 minutes or CFDA pH 5.5 for 5 minutes at 20 - 25ºC. For lymphocytes isolated with magnetic beads, incubate in 500µl CFDA pH5.5 at 20 - 25º C for 10 minutes.
   b. Centrifuge for 1 minute at 1000g. Remove supernatant. For lymphocytes isolated with magnetic beads, place on magnet for 1 minute. Remove supernatant.
   c. Resuspend in PBS.
   d. Repeat Steps b and b twice.

e. Resuspend cells in McCoy's with 0.5% FCS and adjust the cells to a concentration of 2 x 10^6 cells/ml. Protect from light.

f. Follow Steps b - f of "Testing" above.

RESULTS

A. Data Acquisition

1. Cell death will occur in any test well where the HLA cell surface antigen is recognized by its matched anti-HLA antibody. When dye exclusion is used, negative (live) lymphocytes appear small, bright, and refractile. Positive (dead) lymphocytes appear dark and non-refractile with eosin dye. The reactions are scored by estimating the percentage of cell death.

B. Data Analysis

1. For fluorescence testing using carboxyfluorescein diacetate (CFDA) or Acridine Orange (AO), the negative (live) lymphocytes appear green. Using Ethidium Bromide (EB) or Propidium Iodide (PI), the positive (dead) lymphocytes appear red.

LIMITATIONS OF THE PROCEDURE

- Cell isolation difficulties and contamination of the lymphocyte preparation with red cells, yeast, monocytes, platelets or granulocytes may cause erroneous results. Additionally, erroneous results may occur when cell concentrations are above or below the acceptable level. Bacterial contamination or change in pH of the monoclonal reagents may cause false negative reactions. Certain HLA antigens often exhibit weak specificities. These are called cross-reacting antigens and are detailed by antigens and antibodies from each tray in the enclosed reaction pattern guide sheet.
- On Class II typing trays, the anti-B lymphocyte control must be positive in order to validate that the cell preparation is B lymphocyte-enriched. If this control is less than a reaction score of **6**, the test is not valid.

EXPECTED VALUES

Microscopic Evaluation Of Tests

The reactions are scored by estimating the percentage of cell death. If the negative control contains dead lymphocytes, the percentage of cell death in the remaining wells have to be adjusted accordingly.

The ASHI reading standard is shown in the following table:

Score	Cell Death	Interpretation
1	0-10%	Negative
2	11-20%	Doubtful negative
4	21-50%	Weak positive
6	51-80%	Positive
8	81-100%	Strong positive
0	Not readable	

The phenotype frequencies for HLA Class I and Class II will vary among different populations (*i.e.*, Caucasians, American Blacks, Orientals, etc.).[4]

SPECIFIC PERFORMANCE CHARACTERISTICS

A. Potency and Specificity

Test reagents have been precisely characterized by separate sequential serological screenings. Reference panels of frozen lymphocyte samples are used in two separate screenings.

Two thirds of all reagents selected are strong with clearly defined specific HLA reactivity (with 70% Strength Index), allowing no more than 10% false positive and 15% false negative reactions. The remaining one third of the reagents do not meet this criteria, but are investigatively useful when used to support other well-defined antibodies. Multispecific antibodies are used only if no monospecific antibodies are available for a particular specificity. Multispecific antibodies were chosen with the same performance characteristics for all specificities as the monospecific antibodies. Screening against a panel of freshly prepared lymphocytes is used to confirm and validate serum strength and specificity. Analysis is performed using computing techniques of the Eighth International Histocompatibility Workshop in 1980.[4]

B. Negative Control

The negative control antiserum is from a healthy male of blood type AB, which has no cytotoxic reactivity in tests with random lymphocyte donors. This control is used to determine lymphocyte viability.

C. Positive Control

The positive control is a monoclonal antibody and is strongly cytotoxic to human lymphocytes. This control is used to determine complement activity.

D. Anti-B Lymphocyte Control

The anti-B lymphocyte control is a monoclonal antibody and is strongly cytotoxic to B lymphocytes and non-reactive against platelets, monocytes, and red blood cells. This control is used to determine the purity of B lymphocytes.

BIBLIOGRAPHY

1. Terasaki PI, Bernoco F, Park MS, Ozturk G, and Iwaki Y. Microdroplet testing for HLA A, B, C and D antigens. Am J. Clin Pathol 69: 103-120, 1978.
2. Danilovs J, Terasaki PI, Park MS, Ayoub G. B lymphocyte isolation by thrombin nylon wool. In Histocompatibility Testing. Terasaki PI, Ed., UCLA Tissue Typing Laboratory, Los Angeles, CA 287-288, 1980.
3. ASHI Laboratory Manual, 2nd ed. Edited by Zachary, Andrea A. and Teresi, Gary, p. 199, 1990.
4. Terasaki PI, Ed., Histocompatibility Testing. Los Angeles, CA, 1980.
5. Nikaein A, Ed., ASHI Procedure Manual, 3rd Edition, ASHI, Lenexa, KS.

TRADEMARKS

™FluoroBeads, FluoroQuench, Insta-Seal, Lambda Monoclonal Trays (LMT), and LymphoKwik are trademarks of One Lambda, Inc.

EUROPEAN AUTHORIZED REPRESENTATIVE

EC REP MDSS GmbH, Schiffgraben 41, 30175, Hannover, Germany

EXPLANATION OF SYMBOLS *(reference EN ISO 15223-1: Medical devices – Symbols to be used with medical device labels, labeling and information to be supplied)*

Symbol	Description
 *ISO 7000 Reg No. 1641*	Consult instructions for use
 *ISO 7000 Reg No. 2493*	Catalog number
	In vitro diagnostic medical device
 *ISO 7000 Reg No. 0434A*	Caution, consult accompanying documents
 *ISO 7000 Reg No. 0659*	Biological risks
 *ISO 7000 Reg No. 0633*	Upper limit of Temperature
	Batch Code
 *ISO 7000 Reg No. 3082*	Manufacturer
	Authorized representative in the European Community
 *ISO 7000 Reg No 2497*	Date of Manufacture
 *ISO 7000 Reg No 2607*	Use By Date

Batch field on the label is for traceability of manufacturing event

REVISION HISTORY

Revision	Date of Issue	Revision Description
01	08 Apr 2019	Upgraded Internal Document Control System. No changes to the document content.
02	20 Sep 2019	Updated contact information and address to reflect change in legal manufacture site.
03	Current	Removed Stain-Fix™ (OLI Cat. #SF-500), FluoroQuench EB™ (OLI Cat. #FQEB-500). Removed Catalog# 2LM72, LMB2701, LMBL72, MDR160. Deleted under Directions for Use line C.1.b. “either” “B”.

CE 0197