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PRODUCT INSERT

Alkaline Phosphatase-Conjugated Anti-Human IgM for LAT™

Catalog # LATIGM

For Research Use Only. Not for Use in Diagnostic Procedures.

INTENDED USE

For use with the Lambda Antigen Tray™ (LAT) in the detection of HLA Class I and II IgM antibodies.

SUMMARY AND EXPLANATION

Recently, an ELISA technique was introduced as an alternative to the lymphocytotoxicity assay for detection of anti-HLA antibody (1). The Lambda Antigen Tray (LAT™) is based on this principle of presenting purified HLA antigens, instead of cells, as targets for the binding of the patient's antibody. The increased specificity of the assay offers the advantages of eliminating non-HLA false positive reactions and distinguishing Class I and II reactions (2). The Class I and II Mixed (LAT™ M) assay is intended for prescreening of sera to identify positive samples (3). The LAT™ assay presents a panel of HLA antigens, rather than pooled antigen, thus permitting the evaluation of percent PRA and of the specificity of the test sample. Furthermore, like flow cytometry, the LAT™ can detect non-complement binding HLA-specific antibody that is missed by lymphocytotoxicity (4, 5).

Finally, it is desirable to distinguish IgG from IgM antibody, because the latter appears to be non-detrimental to the transplant (6, 7). However, this data was based on the lymphocytotoxicity assay, which would not be able to eliminate certain non-HLA false positive reactions and to distinguish Class I and Class II reactions. The lymphocytotoxicity test is often performed on dithiothreitol-treated sera in order to eliminate the IgM reactivity (8, 9). In contrast, the LAT™ ELISA assay uses a gamma chain-specific second antibody to positively identify IgG antibody in the test sample (1, 2, 10). The IgM antibody specific for HLA Class I or Class II antigens can be detected using the reagent provided here--Alkalkine Phosphatase-Conjugated Anti-Human IgM--as the secondary antibody together with the LAT™ trays.

PRINCIPLE

The LATIGM provides pre-calibrated reagents for use with the LAT™ in the detection of IgM antibody to HLA Class I or Class II antigens in human serum. Defined amounts of affinity-purified HLA antigens are presented in different wells of an LAT™. The specific binding of antibody from the test sample with any of these antigens is detected by a subsequent incubation with this alkaline phosphatase-conjugated antibody that recognizes only human IgM. A quantitative measure of the extent of reaction is obtained by spectrophotometric determination following the addition of the appropriate enzyme substrate for the development of color. Qualitative assessment of antibody specificity is performed by analysis of the LAT™ reactivity pattern using a lot-specific LAT™ code sheet.

REAGENTS

A. Identification

1. Alkaline Phosphatase-Conjugated Anti-Human IgM, $Fc_{5\mu}$ fragment specific (minimum X Bovine Serum Protein)
2. Antibody diluent

B. Warning or Caution

1. For Research Use Only. Not for Use in Diagnostic Procedures.
2. Refer to the Material Safety Data Sheet for detailed information.

C. Instructions for Use

See DIRECTIONS FOR USE section.

D. Storage Instructions

1. Store reagents at 2 - 5° C upon receipt.
2. Do not use reagents if microbial or cross-contamination is suspected. Be sure to use clean pipette tips, or wash channel pipettors thoroughly between dispensing of different reagents. Do not interchange vial caps on reagents.
3. Do not use past the expiration date on the package.

INSTRUMENT REQUIREMENTS

ELISA reader (and printer) with ability to read Terasaki trays.

A. Single wavelength reading at 630nm for colorimetric (BCIP) assay.
B. Computer software (optional)

Follow manufacturer's procedure for instrument calibration.

SPECIMEN COLLECTION AND PREPARATION

1. Serum from aseptically drawn blood specimens can be tested immediately, after brief storage at 2-5°C, or after long-term storage at approximately -20°C.
2. Test samples should be diluted with the antibody diluent provided. Do not use buffers containing EDTA for sample dilution.
3. Do not use heat-inactivated serum, as it will give a high background in this assay.

PROCEDURE

A. Materials Provided

1. 1 vial - 0.5 ml 100X alkaline phosphatase-conjugated anti-human IgM
2. 1 bottle – 50 ml antibody diluent

B. Other Materials Required, but Not provided

- Microcentrifuge tubes (1.5 ml) for serum dilution
- Plastic bottle for wash buffer
- Deionized water (900 ml)
- Manual, electronic, or 6-channel pipettor
- Disposable pipette tips
- Lambda Antigen Tray(s)™ of your choice

C. Step-by-Step Procedure.
See DIRECTIONS FOR USE section.

Note: *A separate IgM serum control is not included; use the serum control with the alkaline phosphatase-conjugated anti-IgG, which is supplied with the LAT product, as the control for the integrity of the antigens on the tray. For specific information on LAT™ product configurations, see the* ***Lambda Antigen Tray (LAT™) for ELISA*** *product insert (Document ID: LAT_PI.DOC).*

DIRECTIONS FOR USE:

Reagent Preparation

1. Reconstitute 10X serum control (OLI Cat. #LAT-SC) with **0.1** or **0.2** ml of sterile deionized water (OLI Cat. #LAT-DW) at least 20 minutes prior to the first assay. Keep the reagent at room temperature and tap the vial occasionally until the antibody is solubilized.
2. Just prior to assay, dilute each test sample with 1X antibody diluent (OLI Cat. #LAT-AD) (1:3 for LAT and 1:2 for LATM).
   Note*: If prescreen indicated a strong antibody, then you may wish to dilute further to assign specificity on LAT.*
3. Take 8 µl (per tray) of reconstituted 10X serum control and dilute with 72 µl antibody diluent.
4. a) Just before the first wash step, dilute 3 µl (for three rows) of 100X alkaline phosphatase-conjugated anti-human **IgG** (OLI Cat. #LAT-APIGG) with 297 µl of 1X antibody diluent.
   Note: *It is necessary to use the IgG, because the positive control serum detects IgG and not IgM.*
   b) Then, dilute 10 µl (per tray) of 100X alkaline phosphatase-conjugated anti-human **IgM** (OLI Cat. #LAT-APIGM) with 990 µl of 1X antibody diluent.
5. Take 5 ml (per tray) of 10X wash buffer (OLI Cat. #LAT-WB) and dilute with 45 ml deionized water (enough for 4 washes).
6. Combine equal volumes of the colorimetric substrate Blue-Phos reagents **A** (OLI Cat. #LAT-BCIPA) and **B** (OLI Cat. #LAT-BCIPB)—500 µl of each per tray—just before second wash step. Take care to change pipette tips to avoid cross contamination of stock solutions.

Test Procedure

1. Add 10 µl of (diluted) test sample, 1X serum control, and 1X antibody diluent to the appropriate wells of the LAT™ (see table on page 1 of the LAT™ product insert). ***Note:*** *To avoid cross-contamination of reagents, change pipette tips regularly.*
2. Replace tray lid, and incubate for **1 hour** at **20 - 25°C** (Optional: Incubate on rotary platform at low speed).
3. Remove all reagents from wells by hand flicking of trays. Strike inverted tray into paper toweling to blot. Approximately one lambda (1 µl of liquid should remain in the wells. Keep trays inverted or covered until addition of the next reagent. Do not let trays dry out.
4. Add 15-20 µl of 1X wash buffer with a six-channel pipettor or bottle reservoir/dispenser, or pour buffer into trays to fill all wells. Swirl gently, and pour off excess buffer. Then, remove wash buffer from wells as above (step 3). Repeat wash and buffer removal.
5. Dispense 10 µl 1X LAT-APIGG on row 1 for LAT1288; rows 1 and 12 for LAT140, LAT240, and LAT1240; and rows 1 and 2 for LATM10X5 and LATM20X5. Dispense 10 µl 1X LAT-APIGM to the remaining rows. Discard leftover diluted reagent.
   Note: *As an option, on trays that contain multiple tests, you may run the test(s) for IgM antibodies on the same tray as the test(s) for IgG. For example, on the LAT1240, LAT240 trays (2 tests per tray), or LAT™ M tray, you can test for IgG on the top half, including the control wells, and IgM on the bottom half of each tray.*
6. Replace tray lid, and incubate for **40 minutes** at **20 - 25°C**. (Optional: Incubate on rotary platform at low speed).
7. Remove all reagents from wells, as described in step 3 above. Do not let trays dry out.
8. Add and remove 1X wash buffer twice as in step 4. Approximately one lambda (1 µl) of liquid should remain in the wells.
9. Dispense 10 µl of colorimetric substrate reagents A and B to each well of the LAT™. Discard leftover reagent.
10. Replace tray lid, and incubate at **37° C** for **10-15** minutes protected from light. (Optional: Incubate on rotary platform at low speed.)
    Note: *Do not let the reaction proceed longer than 15 minutes unless it is carefully monitored. The positive control wells should be dark blue and the negative wells should be clear. It is important to stop the color development before non-specific background arises.*
11. Stop the reaction by adding 5 µl of 1X stop reagent (OLI Cat. #LAT-SR) to each well of the LAT™ tray. Replace tray lid.
12. Read the results within 1 hour with an ELISA reader (OLI Cat. #ELX800NB) adapted for the Terasaki tray format. Remove lid for tray reading. Trays (with lids) can be stored at 2 - 5°C for up to 3 days after stopping the reactions and can be read again, if needed to confirm reaction pattern. However, O.D. values will decrease slightly during storage, and weak reactions may be lost.

Note: *If pNPP is substituted for BCIP as the colorimetric substrate, incubation time (step 10 of* ***Test Procedure****) should be for 30 minutes at 37°C.*

PRECAUTIONS AND LIMITATIONS

Determination of percent PRA is considered a first screen diagnostic test, but not the sole basis for a clinical decision affecting the patient's treatment. A final crossmatch test is routinely required prior to transplant.

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