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PRODUCT INSERT

HLA BULK MONOCLONAL ANTIBODIES

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

INTENDED USE

For use in the detection of human leukocyte antigens (HLA) on the surface of cells.

SUMMARY AND EXPLANATION

The antibodies recognize private and public determinants on HLA molecules.

PRINCIPLE(S)

These antibodies were characterized using the microcytotoxicity test. Briefly, viable lymphocytes are incubated with complement-binding antibody. If the lymphocytes express an antigen recognized by a specific antibody, the Fab portion of the antibody binds to the antigen, forming antigen-antibody complex. After these complexes have formed, rabbit complement is added. 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 with antigen-antibody complexes initiates the complement cascade, which leads to cell lysis. In a negative reaction, the lymphocytes are alive. In a positive reaction, the lymphocytes are dead.

REAGENTS

A. Identification
HLA Bulk Murine Monoclonal Antibodies are supplied in lyophilized form (100 µl). Specificity and dilution factors are determined by the microcytotoxicity test using NIH standard conditions.

B. Warning or Caution
1. For Research Use Only. Not for use in diagnostic procedures.
2. **Caution:** Avoid repeated freezing and thawing of control reagents after reconstitution.

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

D. Storage Instructions
Store at 2 - 5° C. After reconstitution, store at -20° C. Avoid high temperatures. Used before printed expiration date.

E. Purification or Treatment Required for Use
Reconstitution is required for use of lyophilized reagents. See "Directions for Use" in the box below.

F. Instability Indications
Do not use if antibody has not been stored properly.

INSTRUMENT REQUIREMENTS

- Centrifuge
- Phase contrast microscope

SPECIMEN COLLECTION AND PREPARATION

Since viable lymphocytes are required for the microcytotoxicity test, 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 and should be stored horizontally at room temperature (20 - 25° C) and processed within 48 hours for maximum T and B lymphocyte yield.

PROCEDURE

A. Materials Provided
Bulk monoclonal antibodies

B. Materials Required, But Not Provided
- Distilled water
- PBS 10X solution
- 1% bovine albumin (BSA) in PBS

C. Step-by-Step Procedure
See "Directions for Use."

DIRECTIONS FOR USE

A. Preparing Bovine Albumin
1. Measure out 10 g of BSA (protease free).
2. Dissolve BSA in 100 ml of PBS 10X solution.
3. Add 900 ml of sterile water to make a final volume of 1 liter.

B. Preparing HLA Bulk Monoclonal Antibodies for Use
1. To avoid volume loss, in a microcentrifuge, centrifuge vial for a few seconds before opening. (Powder may accumulate in cap during shipment).
2. Reconstitute with 100 µl of distilled water to obtain a 10X working dilution.
3. For a cytotoxic working dilution, add 0.9 ml of 1% BSA in PBS to 100µl working dilution.

RESULTS

See EXPECTED VALUES below.

LIMITATIONS OF THE PROCEDURE

Cell isolation difficulties and contamination of the lymphocyte preparation with red cells, monocytes, platelets, or granulocytes may cause erroneous results. In addition, erroneous results may occur when cell concentrations are above or below acceptable levels. Bacterial contamination or change in the pH of the antisera may cause false negative reactions.

EXPECTED VALUES

Cell death will occur in any test well in which the HLA cell surface antigen is recognized by its matched anti-HLA antibody. Live lymphocytes indicate a negative reaction. Dead lymphocytes indicate a positive reaction.

SPECIFIC PERFORMANCE CHARACTERISTICS

See the "Summary and Explanation" section above.