Paraformaldehyde gives off formaldehyde gas when heated. Formaldehyde is toxic, allergenic, and is known to cause cancer. If eye or skin contact occurs, flush with plenty of water and contact a physician. Inhalation or ingestion is harmful and may be fatal. If swallowed, induce vomiting and contact a physician immediately. Dispose of according to federal, state, and local regulations.

Procedure

Specimen collection and handling
Collect blood specimens by venipuncture (18) into evacuated tubes containing an appropriate anticoagulant, completely expelling the vacuum in the tubes. Mix the blood well with the anticoagulant to prevent clotting. At least 100 µL of whole blood is required for each test. Ideally, blood samples should be processed immediately after collection. When this is not possible, each laboratory should validate that its collection and handling methods maintain specimen integrity comparable to freshly processed material. When using ACD or heparin anticoagulants, blood samples may be stored at 18-22 °C before testing for up to 30 hours after venipuncture (19). When using EDTA, blood samples should be held at room temperature (18-22 °C) and processed within 24 hours (19, 20).

Sample preparation
Optimum concentration of whole blood for each test is 1 ± 0.3 x 10^7 cells/mL. For samples with a high white blood cell count, dilute samples with 1% phosphate buffered saline (PBS). For samples containing a low white blood cell count (less than 0.7 x 10^7 cells/mL), more blood may be used or a separation procedure may be used to concentrate cells.

Procedures for sample handling

Precautions

1. For in vitro diagnostic use.
2. Wear protective clothing when handling any human material.
3. Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.
4. The sodium azide (NaN₃) in the reagents is intended to stabilize the reagents.
5. Never pipette reagents by mouth and avoid their contact with skin and mucous membranes.
6. Avoid microbial contamination of reagents or inoculum results may occur.
7. Incubation times or temperatures other than those specified may give erroneous results; any such change must be validated by the user.
8. Do not use reagents beyond expiration date for prescribed storage method. If reagents are stored under any conditions other than those specified in the specification sheet, the conditions must be validated by the user.

Monoclonal Mouse Anti-Human CD3/FITC, Clone UCHT1 Monoclonal Mouse Anti-Human CD19/RPE, Clone HD37

Code FR686

Intended use

For in vitro diagnostic use.

Monoclonal Mouse Anti-Human-CD3/CD19/UCHT1, and Monoclonal Mouse Anti-Human-CD19/RPE. Clone HD37, (Dako Anti-CD3 FITC and Anti-CD19/RPE) has been developed for use in flow for cytometry for the analysis of T-cells and B-cells. This reagent allows simultaneous detection and quantification of total T-cells and B-cells in peripheral blood of normal and pathological conditions such as immunodeficiency disorders. It is one component of the suggested monoclonal antibody (mAb) combinations for routine immunophenotyping of lymphocytes in peripheral blood using flow cytometry (13).

Summary and explanation

The lymphocyte population of white blood cells (WBC) consists essentially of three subpopulations: T-cells (thymus derived), B-cells (bone marrow derived), and natural killer cells. These subpopulations are identified by cell surface antigens and biological function.

CD3 consists of at least three polypeptide chains (designated gamma, delta and epsilon) with MWs of 21, 23 and 20 kDa respectively. CD3 is closely associated with the T-cell receptor (TcR) on the lymphocyte cell surface (14-16). The CD3 antigen is detectable in early thymocytes and probably represents one of the earliest signs of commitment to the T-lymphocyte lineage (17).

Some CD3 monoclonal antibodies react with the T-cell associated antigen (4, 5), which is expressed on human T-cells. Dako Anti-CD3, UCHT1, reacts with the non-glycosylated and transmembrane oriented CD3 epsilon chain (4).

The CD91 antigen appears early during B-lymphocyte maturation, probably at the pro-B-lymphocyte stage (7, 8). It then persists throughout B-lymphocyte maturation and is lost shortly before the terminal plasma cell stage 9. The antigen has a possible role in the regulation of B-lymphocyte proliferation and differentiation (9-11). Cross-blocking studies indicate that all anti-CD91 in the workshop behave define a single epitope (12).

The monoclonal staining is widely applied for monitoring the immunostatic component of the immunoperoxidase technique in solid tissue preparation (15), and is an aid in the diagnosis and monitoring of immunodeficiency (16, 17). Dako Anti-CD20/CD20a dual antibody reagent is one of the reagents utilized when performing immunophenotyping of lymphocytes.

Primary antibodies

This dual antibody contains purified mouse anti human CD3, conjugated with fluorescein isothiocyanate (FITC) and purified mouse anti human CD19, conjugated with phycoerythrin (PE). The CD3 and CD19 antibodies were produced in mouse by hybridization of spleen cells from immunized BALB/c mice with the myelomas P3-X63-A.515-138-86 and X63-A.515-2-2-13 respectively.

Other considerations

The T-lymphocyte count and the B-lymphocyte count are usually expressed as a percentage of lymphocytes. When a sample is analyzed on a flow cytometer, percent lymphocytes can be determined directly by excluding non-lymphocytes from the analysis gate on the basis of size and internal complexity. Because each cytometer has different operating characteristics, each laboratory must determine its optimal operating procedure.

Reagent provided


Amount per vial: 0.5 mL for 50 tests (10 µL antibody to 10^7 cells)

Storage

Store in the dark at 2-8 °C. Do not freeze.

Alteration in the appearance of the reagent, such as precipitation, indicates instability or deterioration. In such cases, the reagent is not to be used.

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A test of Anti-CD19/RPE, HD37 monoclonal antibody was performed to determine the linearity of the binding to the CD19 cluster determinant. Raji cell line suspended cells (positive epitope for the CD19 antibody) were mixed with JM cell line suspended cells (negative for the CD19 antibody) at several concentrations to test for binding linearity. Anti-CD19/RPE, HD37 bound with the Raji cells on a linear basis ($y = 0.02 + 0.98x$, $r^2 = 1.0$) with the slope approaching 1.0.

**Expected values**

Blood samples were collected from 150 apparently healthy males and females at three geographically separate locations. The population included adults from a variety of races ranging in age from 19 to 62 years. Samples were stained with Dako Anti-CD3/FITC and Anti-CD19/RPE monoclonal antibodies. Normal CD3 positive T-cell and CD19 B-cell values were measured by flow cytometry using the whole blood method, and are presented in the following table. Values are expressed as a percentage of the total lymphocyte count due to unacceptable variation in absolute counts (24), and are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.

<table>
<thead>
<tr>
<th>% CD3$^+$ lymphocytes</th>
<th>150</th>
<th>52.5</th>
<th>84.0</th>
<th>70.4 ± 7.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>% CD19$^+$ lymphocytes</td>
<td>150</td>
<td>5.4</td>
<td>27.6</td>
<td>12.8 ± 5.3</td>
</tr>
</tbody>
</table>

**Quality Assurance**

Peripheral blood cells should be run as a positive control to ensure proper working conditions. Normal ranges should be established within a local population of normal, apparently healthy donors.

**Inferences**

In the counts of percent positive cells by monocytes and granulocytes with Fc receptor bound antibody may be reduced by proper flow cytometric gating on lymphocytes.

An appropriate Dako isotype negative control that is non-human reactive (IgG1-FITC/IgG1-RPE, Dako Code X0932) is used to control for nonspecific antibody binding to lymphocytes in each patient sample. The brightly-stained lymphocyte population is measured within markers set to exclude the low level of nonspecific fluorescence.

Nonspecific fluorescence above the background marker is usually limited to 1-2% in normal individuals. If the background level above the marker for any control sample is greater than 1-2%, the test results may be erroneous.

**Limitations**

Blood samples should be stained within 30 hours of collection for optimal results. Retain samples in the original sample tube at room temperature prior to staining and analysis. Do not refrigerate. Refrigerated samples or samples stored longer than 30 hours may give erroneous results. To ensure maximum validity, analyze stained cells promptly.

Samples from certain patients may present special problems due to abnormal erythrocyte or lymphocyte populations that are a result of illness or drug usage.

Blood samples from abnormal donors may not show abnormal values for the percent of lymphocytes stained with a monoclonal antibody. Results obtained from flow cytometric analysis should be reviewed with results from other diagnostic procedures.

Accurate values obtained by flow cytometric procedures depend upon correct alignment and calibration of the laser, as well as proper gating and compensation processes.

**Linearity**

A test of the Dako Anti-CD3/FITC, UCHT1 monoclonal antibody was performed to determine the linearity of the binding to the CD3 cluster determinant. JM cell line suspended cells (positive epitope for the CD3 antibody) were mixed with suspended Raji cell line suspended cells (negative for the CD3 antibody) at several dilutions to test for binding linearity. The Dako Anti-CD3/FITC bound with the JM cells on a linear basis ($y = 0.02 + 0.98x$, $r^2 = 1.0$) with the slope approaching 1.0.
References


