INTENDED USE
For In Vitro Diagnostic Use
Monoclonal Mouse Anti-Human, CD19, RPE-Cy5 conjugated, has been developed for use in flow cytometry for the analysis of B-cells. This reagent allows simultaneous detection and quantification of CD19-positive cells (B-cells) in normal and pathological conditions such as immunodeficiency disorders. It is one of the suggested monoclonal antibody (mAb) combinations for routine immunophenotyping of lymphocytes in peripheral blood (10).

SUMMARY AND EXPLANATION
The lymphocyte population of white blood cells (WBC's) 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.

- Anti-CD19, HD37, reacts with a 90kDa polypeptide and was designated CD19 at the Second International Workshop on Human Leucocyte Differentiation Antigens (Boston, 1984). The CD19 gene has been cloned. This antigen has been shown to be a transmembrane glycoprotein with at least two extracellular immunoglobulin-like domains. The intracytoplasmic domain bears some resemblance to proteins encoded by Epstein-Barr virus and by the RFT-1 oncogene (5).

- The CD19 antigen appears early during B lymphocyte maturation, probably at the pre-B-lymphocyte stage (17, 18). It then persists throughout B-lymphocyte maturation and is lost shortly before the terminal plasma cell stage (7). The antigen has a possible role in the regulation of B-lymphocyte proliferation and differentiation (8-10). Cross-blocking studies indicate that all antibodies in the CD19 cluster studied at the above workshop define a single epitope (11).

- Immunophenotyping of lymphocytes is widely applied for diagnosis of immunodeficiency (12,15). Anti-CD19/RPE-Cy5 is one of the reagents utilized when performing immunophenotyping of lymphocytes.

PRINCIPLE OF PROCEDURE
Anti-CD19/RPE-Cy5, HD37, is a monoclonal antibody to the CD19 epitope that has been conjugated with a fluorochrome, R-phycoerythrin (RPE) covalently coupled with cyanin 5 (Cy5). Cells bound with the conjugated antibody can be detected by light excitation of the fluorochrome. When bound to the specific B-cell epitope, that light emission allows measurement of the lymphocyte subset by flow cytometry. Cells that have bound antibody conjugate with RPE-Cy5 emit fluorescence read at 670nm, which displays red.

Subpopulations of lymphocytes may be stained with fluorochrome-conjugated antibody and evaluated in peripheral blood specimens. WBC's are mixed with the fluorochrome-conjugated antibody and contaminating red blood cells (RBC's) are used, prior to analysis. A subpopulation of WBC's is selected for assessment based upon cell morphology. The B-cell count is usually expressed as a percent of lymphocytes. When the sample is analysed on a flow cytometer, percent positive lymphocytes can be determined directly based upon size and internal complexity. Because each flow cytometer has different operating characteristics, each laboratory must determine its optimal operating procedure.

REAGENTS PROVIDED
PRIMARY ANTIBODY
Purified mouse anti-human CD19, clone HD37, conjugated with R-phycocerythrin, covalently coupled with cyanin 5 (Cy5), is present in 0.05 M Tris-HCl buffer, pH 7.2, 0.1 M NaCl, 0.015 M sodium azide, stabilized with 1% carrier protein.

- Immunogen: Hairy cell leukemia cells
- Source: Tissue culture supernatant
- Clone/Ref: HD37
- Isotype: IgG1, kappa
- Antibody: 90 kDa
- Purification: Affinity chromatography
- Conjugation: Anti-CD19/Cy5 - R-phycoerythrin + Cyanin 5
- Protein concentration: 10 mg conjugate/L
- Fluorescence (RPE-Cy5): RPE excites at 488 nm, emits at 575 nm. The photons emitted at 575 nm excite Cy5 (Red) that emits around 670 nm.

AMOUNT per VIAL: 100 tests (10 µl antibody to 10^6 cells).

Reagents are not considered sterile.

STORAGE
1. Store in the dark at 2-8°C. Do not freeze.
2. Do not use after expiration date stamped on vial.
3. Alteration in the appearance of the reagent, such as precipitation, indicates instability or deterioration. In such cases, the reagent is not to be used.

PRECAUTIONS
For In Vitro Diagnostic Use
- Biological 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. Wear appropriate personal protective equipment including disposable gloves. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, flush with a copious amount of water for at least 15 minutes.
- Paraffin melting may give off formaldehyde gas when heated. Formaldehyde is toxic, allergic, and is known to cause cancer. Use only in well-ventilated areas or in a fume hood. Avoid contact with eyes and skin or clothing. If eye or skin contact occurs, immediately flush with plenty of water and contact a physician if exposure symptoms develop. Inhalation or ingestion is harmful. If swallowed, induce vomiting and contact a physician immediately.
- This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, build-up of sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent acid build-up in plumbing.
- Avoid microbial contamination of reagents or incorrect results may occur.
- Incubation times or temperatures other than those specified may give erroneous results, any such change must be used by the user.
- 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.

FLOW CYTOMETRIC ANALYSIS
1. Analyze cells on a flow cytometer properly standardized and gated on lymphocytes according to the instrument operating manual.
2. Verify gate purity with the control tube (CD19/44).
3. Set the cursor using the isotypic control so that 99±1% events are in the negative cell population.

REPRESENTATIVE HISTOGRAMS
Lymphocytes from peripheral blood (healthy individual) labeled with RPE-Cy5-Conjugated Anti-CD19, HD37, Dako Cytomx C7066 (3.8 labeled lymphocytes)
EXPECTED VALUES
Blood samples were collected from 153 apparently healthy males and females at three geographically separate locations. The population included adults from a variety of races ranging in age from 20 to 65 years. Samples were stained with Anti-CD19/RPE-Cy5, HD37. Normal CD19-positive B-lymphocyte values were measured by flow cytometry using the White Blood method, and are presented in the following table. Values are expressed as a percentage of the total lymphocyte count and are intended as representative values only. Due to unacceptable variance among the different laboratory methods for determining absolute lymphocyte counts, an assessment of the accuracy of the method used is necessary. [Each laboratory should establish its own expected values from the local population of normal donors.]

<table>
<thead>
<tr>
<th>% Positive</th>
<th>% Positive</th>
<th>% Positive</th>
<th>% Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ave. (n=5)</td>
<td>(range)</td>
<td>(range)</td>
<td>(range)</td>
<td>(range)</td>
</tr>
<tr>
<td>0.04</td>
<td>(0.0-0.2)</td>
<td>1.10</td>
<td>(0.3-1.7)</td>
<td>7.66</td>
</tr>
<tr>
<td>13.34</td>
<td>(10.1-17.6)</td>
<td>0.22</td>
<td>(0.0-0.5)</td>
<td></td>
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</tbody>
</table>

QUALITY ASSURANCE
Peripheral blood from a normal, apparently healthy donor 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.

Interference 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 isotype negative control that is non-human reactive (IgG1/RPE-Cy5, Dako Code X0955) is used to control for nonspecific antibody binding to lymphocytes in each patient sample. The positively-stained lymphocyte population is measured within a window cell to exclude the low level of nonspecific fluorescence.

Nonspecific fluorescence above the background is usually limited to 1.2% in normal individuals with higher values found with some disease states. If the background level above the lower control sample is greater than 1.0, 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. Do not refrigerate. Refrigerated samples or samples stored longer than 30 hours may give erroneous results. To ensure maximum viability, analyze stained cells promptly.

Samples from certain patients may present special problems due to abnormal erythrocyte or leukocyte 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.

Accuracy 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 linearity test of the Anti-CD19/RPE-Cy5, HD37, was performed to determine the linearity of the binding to the CD19 cluster determinant. Raji cell line suspended cells (positive control for the CD19 antibody) were mixed with JU cell line suspended cells (negative for the CD19 antibody) at several concentrations to test for binding linearity. Anti-CD19/RPE-Cy5, HD37, bound with the Raji cells on a linear basis (y = 0.01 + 0.38x, Pearson product moment correlation coefficient = 0.999) with the slope approaching 1.0.

REPRODUCIBILITY
Ten replicate measurements of each of three levels (normal, high and low) of CD19-positive lymphocyte levels were analyzed on a Becton-Dickinson FACScan flow cytometer and a Coulter Profile II. Different levels of CD19-positive lymphocytes were selected from a population of normal and abnormal peripheral blood samples. Values are expressed as a percent of the total lymphocyte count.

<table>
<thead>
<tr>
<th>FACScan</th>
<th>Mean % CD19</th>
<th>± 1 SD</th>
<th>±CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0.06</td>
<td>±0.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Medium</td>
<td>51.28</td>
<td>±1.47</td>
<td>2.87</td>
</tr>
<tr>
<td>Low</td>
<td>26.77</td>
<td>±1.02</td>
<td>3.81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Profile II</th>
<th>Mean % CD19</th>
<th>± 1 SD</th>
<th>±CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>87.16</td>
<td>±2.02</td>
<td>2.40</td>
</tr>
<tr>
<td>Medium</td>
<td>49.75</td>
<td>±1.54</td>
<td>3.09</td>
</tr>
<tr>
<td>Low</td>
<td>26.13</td>
<td>±1.08</td>
<td>4.11</td>
</tr>
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</table>

SPECIFICITY
Background (non-specific staining) can be subtracted from flow cytometry measurements by using test samples stained with appropriate mouse isotypic control antibodies. The population of normal cells can be set to exclude the low level of fluorescence. Antibody binding to non-lymphocyte cells may be excluded by proper gating on the flow cytometer. Specificity of Anti-CD19/RPE-Cy5, HD37, has been verified by tests performed on five apparently healthy adult donors of various races at Dako Corporation. Cell populations tested were PBMCs, granulocytes, monocytes, lymphocytes and platelets. The results indicate antibody binding of Anti-CD19/RPE-Cy5, HD37, is specific for lymphocytes. However, non-specific binding cannot be excluded from the lymphocyte analysis by proper gating on lymphocytes.