Monoclonal Antibodies Detecting Human Antigens

CD95 (DX2)

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

Monoclonal Antibodies

Detecting Human Antigens

Research applications include studies of:
- Apoptosis and clonal deletion\(^1\)\(^-\)\(^3\)
- Expression in various malignant cell populations\(^4\)\(^-\)\(^6\)
- Autoimmune disease\(^7\)
- Human immunodeficiency virus (HIV) and other viral infections\(^3\)

DESCRIPTION

Specificity

The CD95 antibody recognizes a 48-kilodalton (kDa) antigen (Fas/APO-1) that is a member of the tumor necrosis factor/nerve growth factor (TNF/NGF) superfamily.\(^8\)\(^,\)\(^9\) It is associated with activated cells.\(^1\) Cross linking of CD95 antigen on activated cells can elicit apoptosis.\(^2\)

Antigen distribution

The CD95 antigen is found on some normal T and B lymphocytes, natural killer (NK) lymphocytes, and monocytes.\(^8\) CD95 antigen is preferentially expressed on CD45RO\(^{\text{high}}\) memory T lymphocytes and γ\(^\delta\) T lymphocytes.\(^1\)\(^,\)\(^8\) Some myeloma and T-lymphocyte lines express the antigen.\(^4\) Surface expression of CD95 antigen may be reduced in certain carcinomas\(^5\) and upregulated in some autoimmune diseases\(^7\) and viral infections, including those associated with acquired immune deficiency syndrome (AIDS).\(^3\)

Clone

The CD95 antibody, clone DX2,\(^*\) is derived from hybridization of Sp2/0 mouse myeloma cells with spleen cells from C3H/He mice immunized with L cells transfected with CD95 antigen.\(^10\)

Composition

The CD95 antibody is composed of mouse IgG\(_1\) heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

<table>
<thead>
<tr>
<th>Form</th>
<th>Number of tests</th>
<th>Volume per test (µL)(^a)</th>
<th>Amount provided (µg)</th>
<th>Total volume (mL)</th>
<th>Concentration (µg/mL)</th>
<th>Stabilizer</th>
<th>Preservative</th>
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<tbody>
<tr>
<td>FITC</td>
<td>50</td>
<td>20</td>
<td>12.5</td>
<td>1.0</td>
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<td>Gelatin</td>
<td>0.1% Sodium azide</td>
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<tr>
<td>PE</td>
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<td>6.3</td>
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<tr>
<td>APC</td>
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<td>5</td>
<td>2.5</td>
<td>0.5</td>
<td>50</td>
<td>Gelatin</td>
<td>0.1% Sodium azide</td>
</tr>
</tbody>
</table>

\(^a\)Volume required to stain 10\(^6\) cells.

* This clone has not been submitted to any previous Workshop on Human Leucocyte Differentiation Antigens.

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PROCEDURE

Method for direct immunofluorescence for CD95 FITC/CD95 PE

Add 20 µL of reagent to 100 µL of whole blood (resting or activated) in a staining tube. Mix thoroughly and incubate for 15 to 30 minutes in the dark at room temperature (20°C–25°C). Add 2 mL of 1X BD FACS™ lysing solution (Cat. No. 349202) at room temperature and vortex tube thoroughly. Incubate for 10 to 12 minutes at room temperature in the dark. Wash cells with 1X PBS with 0.1% sodium azide, add 0.5 mL of 1% paraformaldehyde, mix thoroughly, and analyze. If samples will not be analyzed immediately, mix thoroughly just prior to analysis. Refer to the BD FACS Lysing Solution package insert for more information.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on activated peripheral blood with an FL3 threshold set and gated on the CD3+ lymphocyte fraction. Laser excitation was at 488 nm.

Figure 1 PMA + ionomycin-activated lysed whole blood analyzed with a BD FACScan™ flow cytometer

HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Method for direct immunofluorescence for CD95 APC

Add 5 µL of reagent (plus additional reagents when appropriate) to 100 µL of whole blood (resting or activated) in a staining tube. Mix thoroughly and incubate for 15 to 30 minutes in the dark at room temperature (20°C–25°C). Add 2 mL of 1X BD FACS lysing solution (Cat. No. 349202) and vortex tube thoroughly. Incubate for 10 to 12 minutes in the dark. Wash cells with 2 mL of 1X PBS with 0.1% sodium azide, add 0.5 mL of 1% paraformaldehyde, mix thoroughly, and analyze. If samples will not be analyzed immediately, mix thoroughly just prior to analysis. Refer to the BD FACS Lysing Solution package insert for more information.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on peripheral blood with a scatter gate set on the lymphocyte fraction. Laser excitation was at 635 nm.

Figure 2 Lysed whole blood analyzed with a BD FACS Calibur™ flow cytometer
**WARNING**

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

**CHARACTERIZATION**

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

**WARRANTY**

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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