Monoclonal Antibodies Detecting Human Antigens

CD154 (89-76)

Research applications include studies of:

- T-, B-lymphocyte interactions\(^1\)–\(^3\)
- B-lymphocyte maturation, isotype switching, and antibody production\(^4\)–\(^7\)
- CD4\(^+\) T-lymphocytes\(^8\),\(^9\)
- T-lymphocyte development and clonal deletion\(^6\),\(^10\)
- Reactive T lymphocytes in malignancies\(^11\)
- Hyper-IgM syndrome\(^12\),\(^13\)
- Regulatory T cell (Treg) function\(^14\),\(^15\)

Description

Specificity

The CD154 (CD40 ligand or CD40L) antibody recognizes a 39-kilodalton (kDa) antigen that is a member of the tumor necrosis factor (TNF) receptor family.\(^3\),\(^9\) CD154 is transiently expressed on activated CD4 T lymphocytes.\(^16\),\(^17\) The expression of CD154 by activated T-helper cells triggers B-cell cycling through binding to CD40.\(^18\)–\(^20\) This co-receptor interaction is required for B-lymphocyte maturation, response to T-dependent ligands, and isotype switching.\(^4\),\(^6\),\(^20\),\(^21\) T-lymphocyte proliferation and generation of cytotoxic T lymphocytes (CTLs) is associated with the presence of CD154.\(^8\) Defects in the CD154 gene are associated with the X-linked hyper IgM syndrome.\(^12\),\(^13\)

Antigen distribution

The CD154 antigen (CD40L) is found predominately on activated CD4\(^+\) peripheral T lymphocytes,\(^6\),\(^8\),\(^9\) as well as on a small fraction of CD8\(^+\) lymphocytes,\(^2\) certain malignant cells,\(^11\) mast cells, and basophils.\(^4\) Mature CD4\(^+\)8\(^–\) thymocytes also express CD154 following stimulation.\(^10\),\(^21\)

Clone

The CD154 antibody, clone 89-76,\(^*\) is derived from hybridization of NS-1 mouse cells with spleen cells from BALB/c mice immunized with sgp39-CD8 and activated human peripheral blood lymphocytes (PBLs).

Composition

The CD154 antibody is composed of mouse IgG\(_1\) heavy chains and kappa light chains.

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

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

The following reagents 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)</th>
<th>Amount provided (µg)</th>
<th>Total volume (mL)</th>
<th>Concentration (µg/mL)</th>
<th>Stabilizer</th>
<th>Preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>50</td>
<td>20</td>
<td>12.5</td>
<td>1.0</td>
<td>12.5</td>
<td>Gelatin</td>
<td>0.1% Sodium azide</td>
</tr>
<tr>
<td>APC</td>
<td>100</td>
<td>5</td>
<td>50</td>
<td>0.5</td>
<td>100</td>
<td>Gelatin</td>
<td>0.1% Sodium azide</td>
</tr>
</tbody>
</table>

a. Volume required to stain 10⁶ cells.

PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Perform the following steps:

1. Add 20 µL of CD154 PE or 5 µL of CD154 APC to activated peripheral blood mononuclear cells (PBMCs).

2. Incubate for 15 to 30 minutes in the dark at room temperature.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on phorbol 12-myristate 13-acetate (PMA) and ionomycin-activated PBMCs and then gated on lymphocytes. Laser excitation was at 488 nm or 635 nm.

Figure 1  Representative data analyzed with a BD FACS™ brand 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.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection²²,²³ 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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**REFERENCES**


