Technical Data Sheet
PE Hamster Anti-Mouse CD54

Product Information
Material Number: 553253
Alternate Name: ICAM-1
Size: 0.2 mg
Concentration: 0.2 mg/ml
Clone: 3E2
Immunogen: Not reported
Isotype: Armenian Hamster IgG1, κ
Reactivity: QC Testing: Mouse
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description
The 3E2 antibody reacts with CD54 (ICAM-1), a 95-kDa member of the Ig superfamily found on lymphocytes, vascular endothelium, high endothelial venules, epithelial cells, macrophages, and dendritic cells. ICAM-1 is a ligand for LFA1 (CD11a/CD18) and Mac-1 (CD11b/CD18). Its expression is upregulated upon stimulation by inflammatory mediators such as cytokines and LPS. Studies with mouse Icam1-transfected antigen-presenting cells, with CD54-blocking antibodies, and in CD54-deficient mice indicate that CD54 participates in inflammatory reactions and antigen-specific immune responses. In addition, there is evidence that CD54 is a receptor involved in MHC-non-restricted responses to weakly immunogenic tumor cells. The 3E2 antibody blocks in vitro and in responses.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Preparation and Storage
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed by gel filtration chromatography.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Upregulation of CD54 expression on activated splenic B lymphocytes. Left panel: Naive BALB/c splenocytes were stained with PE-conjugated 3E2 mAb (open histogram) or unstained (filled histogram). Viable resting lymphocytes were gated according to scatter profile and exclusion of 7-AAD (BD Via-Probe™, Cat. No. 555816/555819). The mean fluorescence intensity of the stained lymphocytes is about 50 times greater than that of the negative-control lymphocytes. Right panel: 2-day LPS-activated BALB/c splenocytes were stained with PE-conjugated 3E2 mAb (open histogram) or unstained (filled histogram). Viable B-cell blasts were gated according to scatter profile and exclusion of 7-AAD. The mean fluorescence intensity of the stained blasts is about 180 times greater than that of the negative-control blasts. Flow cytometry was performed on a BD FACSCan™ flow cytometry system.

Preparation and Storage
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed by gel filtration chromatography. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
Application Notes

**Application**

| Flow cytometry | Routinely Tested |

**Recommended Assay Procedure:**

This antibody conjugate is compatible with intracellular staining protocols using the BD Cytofix/Cytoperm™ Kit (Cat. No. 554714).

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>555816</td>
<td>Cell Viability Solution</td>
<td>100 tests</td>
<td>(none)</td>
</tr>
<tr>
<td>554714</td>
<td>BD Cytofix/Cytoperm Fixation/Permeabilization Kit</td>
<td>250 tests</td>
<td>(none)</td>
</tr>
<tr>
<td>553972</td>
<td>PE Hamster IgG κ Isotype Control</td>
<td>0.1 mg</td>
<td>A19-3</td>
</tr>
</tbody>
</table>

**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
4. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgGl monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster IgG mAbs, may be viewed at http://www.bdbiosciences.com/pharmingen/hamster_chart_11x17.pdf.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

**References**

- Nishio M, Podack ER. Rapid induction of tumor necrosis factor cytotoxicity in naive splenic T cells by simultaneous CD80 (B7.1) and CD54 (ICAM-1) co-stimulation. *Eur J Immunol.* 1996; 26(9):2160-2164. (Biology)