Flow Cytometry

Detection of NKp30/NCR3 in Human Blood Lymphocytes by Flow Cytometry.

Human peripheral blood lymphocytes were stained with either (A) Mouse Anti-Human NKp30/NCR3 Monoclonal Antibody (Catalog # MAB1849) or (B) Mouse IgG2A Isotype Control (Catalog # MAB003) followed by anti-Mouse IgG APC-conjugated secondary antibody (Catalog # F0101B) and Mouse Anti-Human NCAM-1/CD56 PE-conjugated Monoclonal Antibody (Catalog # FAB2408P). Staining was performed using our Staining Membrane-associated Proteins protocol.

**Species Reactivity**: Human

**Specificity**: Detects human NKp30/NCR3 on cell transfectants and peripheral blood NK cells.

**Source**: Monoclonal Mouse IgG2A Clone # 210845

**Purification**: Protein A or G purified from hybridoma culture supernatant

**Immunogen**: Mouse T cell hybridoma transfected with human NKp30/NCR3 and Mouse myeloma cell line NS0-derived recombinant human NKp30 Fc Chimera

**Endotoxin Level**: <0.10 EU per 1 µg of the antibody by the LAL method.

**Formulation**: Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

*A small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

**APPLICATIONS**

**Please Note**: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

**Recommended Concentration**

**Sample**

<table>
<thead>
<tr>
<th>Agonist Activity</th>
<th>0.2-1.2 µg/mL</th>
<th>See Below</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Cytometry</td>
<td>0.25 µg/10⁶ cells</td>
<td>Human whole blood lymphocytes</td>
</tr>
<tr>
<td>CyTOF-ready</td>
<td>Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.</td>
<td></td>
</tr>
</tbody>
</table>

**DATA**

**Agonist Activity**

Human NKp30 Antibody induces IFN-γ secretion in NK-92 Cells. Human NKp30 Monoclonal Antibody induces IFN-γ secretion in the NK-92 human natural killer lymphoma cell line in a dose-dependent manner, as measured using the Quantikine Human IFN-γ ELISA Kit (Catalog # DIF50). The ED₅₀ for this effect is typically 0.2-1.2 µg/mL.

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**PREPARATION AND STORAGE**

**Reconstitution**

Reconstitute at 0.5 mg/mL in sterile PBS.

**Shipping**

The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

*A small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C.

**Stability & Storage**

- Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
  - 12 months from date of receipt, -20 to -70 °C as supplied.
  - 1 month, 2 to 8 °C under sterile conditions after reconstitution.
  - 6 months, -20 to -70 °C under sterile conditions after reconstitution.
NKp30, along with NKp44 and NKp46, constitute a group of receptors termed “Natural Cytotoxicity Receptors” (1). These receptors play a major role in triggering NK-mediated killing of most tumor cell lines. NKp30 is a type I transmembrane protein having a single extracellular V-like immunoglobulin domain (2). A physical association with the ITAM-bearing accessory protein, CD3ζ, occurs via a charged residue in the NKp30 transmembrane domain. Ligation of NKp30 with a specific antibody results in phosphorylation of CD3ζ (3). NKp30 is expressed on both resting and activated NK cells of the CD56<sup>dim</sup>, CD16<sup>+</sup> subset that account for more that 85% of NK cells found in peripheral blood and spleen (4). NKp30 is absent from the CD56<sup>bright</sup>, CD16<sup>+</sup> subset that constitutes the majority of NK cells in lymph node and tonsil, however, its expression is up-regulated in these cells upon IL-2 activation (4). Studies with neutralizing antibodies reveal that NKp30 is partially responsible for triggering lytic activity against several tumor cell types and that it is the main receptor responsible for NK-mediated lysis of immature dendritic cells (2, 5). The ligand(s) recognized by NKp30 has not been described.

References: