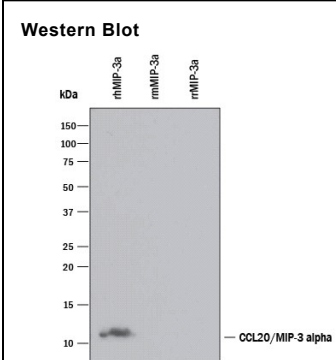
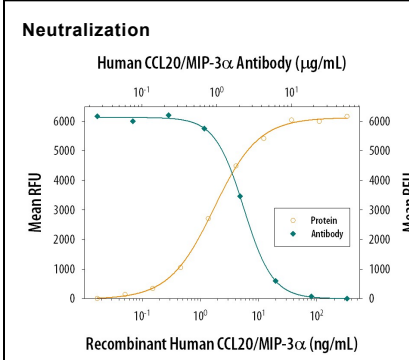


| DESCRIPTION               |  |
|---------------------------|--|
| <b>Species Reactivity</b> | Human  |
| <b>Specificity</b>        | Detects human CCL20/MIP-3 $\alpha$ in ELISAs and Western blots. In ELISAs, no cross-reactivity with recombinant human (rh) CCL19, rhCXCL1, rhCCL16, rhCCL11, recombinant mouse CCL20/MIP-3 $\alpha$ , or recombinant rat CCL20/MIP-3 $\alpha$ is observed. |
| <b>Source</b>             | Monoclonal Mouse IgG <sub>1</sub> Clone # 67310  |
| <b>Purification</b>       | Protein A or G purified from ascites   |
| <b>Immunogen</b>          | <i>E. coli</i> -derived recombinant human CCL20/MIP-3 $\alpha$<br>Ala27-Met96 (Asn95Asp)<br>Accession # P78556.1   |
| <b>Endotoxin Level</b>    | <0.10 EU per 1 $\mu$ g of the antibody by the LAL method.  |
| <b>Formulation</b>        | Lyophilized from a 0.2 $\mu$ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details.<br>*Small pack size (-SP) is supplied either lyophilized or as a 0.2 $\mu$ m filtered solution in PBS.                                    |

| APPLICATIONS   |  |
|--|--|
| <b>Please Note:</b> Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website. |  |
|  | <b>Recommended Concentration</b> <b>Sample</b>   |
| <b>Western Blot</b>  | 1 $\mu$ g/mL      See Below  |
| <b>Human CCL20/MIP-3<math>\alpha</math> Sandwich Immunoassay</b>   | <b>Reagent</b>   |
| <b>ELISA Capture</b>   | 2-8 $\mu$ g/mL      Human CCL20/MIP-3 $\alpha$ Antibody (Catalog # <a href="#">MAB360</a> )  |
| <b>ELISA Detection</b>   | 0.1-0.4 $\mu$ g/mL      Human CCL20/MIP-3 $\alpha$ Biotinylated Antibody (Catalog # <a href="#">BAF360</a> )   |
| <b>Standard</b>  | Recombinant Human CCL20/MIP-3 $\alpha$ (Catalog # <a href="#">360-MP</a> )   |
| <b>Neutralization</b>  | Measured by its ability to neutralize CCL20/MIP-3 $\alpha$ -induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CCR6. The Neutralization Dose (ND <sub>50</sub> ) is typically 1-5 $\mu$ g/mL in the presence of 10 ng/mL Recombinant Human CCL20/MIP-3 $\alpha$ . |

| DATA   |   |
|--|---|
| <p><b>Western Blot</b></p>  <p><b>Detection of Recombinant Human CCL20/MIP-3<math>\alpha</math> by Western Blot.</b> Western blot shows 100 ng of Recombinant Human CCL20/MIP-3<math>\alpha</math> (Catalog # <a href="#">360-MP</a>), Recombinant Mouse CCL20/MIP-3<math>\alpha</math> (Catalog # <a href="#">760-M3</a>) and Recombinant Rat CCL20/MIP-3<math>\alpha</math> (Catalog # <a href="#">540-RM</a>). PVDF Membrane was probed with 1 <math>\mu</math>g/mL of Mouse Anti-Human CCL20/MIP-3<math>\alpha</math> Monoclonal Antibody (Catalog # <a href="#">MAB360</a>) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # <a href="#">HAF007</a>). A specific band was detected for CCL20/MIP-3<math>\alpha</math> at approximately 11 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.</p> | <p><b>Neutralization</b></p>  <p><b>Chemotaxis Induced by CCL20/MIP-3<math>\alpha</math> and Neutralization by Human CCL20/MIP-3<math>\alpha</math> Antibody.</b> Recombinant Human CCL20/MIP-3<math>\alpha</math> (Catalog # <a href="#">360-MP</a>) chemoattracts the BaF3 mouse pro-B cell line transfected with human CCR6 in a dose-dependent manner (orange line). The amount of cells that migrated through to the lower chemotaxis chamber was measured by Resazurin (Catalog # <a href="#">AR002</a>). Chemotaxis elicited by Recombinant Human CCL20/MIP-3<math>\alpha</math> (10 ng/mL) is neutralized (green line) by increasing concentrations of Human CCL20/MIP-3<math>\alpha</math> Monoclonal Antibody (Catalog # <a href="#">MAB360</a>). The ND<sub>50</sub> is typically 1-5 <math>\mu</math>g/mL.</p> |

| PREPARATION AND STORAGE        |  |
|--------------------------------|--|
| <b>Reconstitution</b>          | Reconstitute at 0.5 mg/mL in sterile PBS.  |
| <b>Shipping</b>                | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.<br>*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C   |
| <b>Stability &amp; Storage</b> | <b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul> |

## BACKGROUND

CCL20, also known as LARC (Liver and Activation-regulated Chemokine) and as Exodus, is one of many novel  $\beta$  chemokines identified through bioinformatics. CCL20 cDNA encodes a 96 amino acid (aa) residue precursor protein with a 26 aa residue signal peptide that is predicted to be cleaved to form the 70 aa residue mature secreted protein. CCL20 is distantly related to other  $\beta$  chemokines (20 - 28% aa sequence identity) and the gene for CCL20 has been mapped to chromosome 2 rather than 17.

CCL20 has been shown to be expressed predominantly in lymph nodes, appendix, PBL, fetal liver, fetal lung and several cell lines. The expression of CCL20 is strongly up-regulated by inflammatory signals and down-regulated by the anti-inflammatory cytokine IL-10. Synthetic or recombinant CCL20 has been shown to be chemotactic for lymphocytes and to inhibit proliferation of myeloid progenitors in colony formation assays. CCL20 has now been shown to be a unique functional ligand for CCR-6 (previously referred to as GPR-CY4, CKR-L3, or STRL22 orphan receptor), a chemokine receptor that is selectively and highly expressed in human dendritic cells derived from CD34<sup>+</sup> cord blood precursors.

## References:

1. Baba, M. *et al.* (1997) J. Biol. Chem. **272**:14893.
2. Hromas, R. *et al.* (1997) Blood **89**:3315.
3. Greaves, D.R. *et al.* (1997) J. Exp. Med. **186**:837.