Species Reactivity: Human

Specificity: Detects human CCL5/RANTES in direct ELISAs. Detects human CCL5/RANTES and mouse CCL5/RANTES Western blots. In direct ELISAs, approximately 40% cross-reactivity with recombinant canine CCL5/RANTES is observed and approximately 20% cross-reactivity with recombinant cotton rat CCL5/RANTES is observed. In Western blots, no cross-reactivity with recombinant human (rh) CCL3 and rhCCL4 is observed.

Source: Polyclonal Goat IgG

Purification: Antigen Affinity-purified

Immunogen: E. coli-derived recombinant human CCL5/RANTES Ser24-Ser91 Accession # P13501

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.

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

- Western Blot: 0.1 µg/mL See Below
- Immunocytochemistry: 5-15 µg/mL See Below
- Immunohistochemistry: 5-15 µg/mL See Below
- Neutralization: Measured by its ability to neutralize CCL5/RANTES-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CCR5. The Neutralization Dose (ND50) is typically 0.1-0.4 µg/mL in the presence of 0.01 µg/mL Recombinant Human CCL5/RANTES.

DATA

Western Blot

Detection of Recombinant Human and Mouse CCL5/RANTES by Western Blot. Western blot shows 25 ng of Recombinant Human CCL5/RANTES (Catalog # 278-RN), Recombinant Mouse CCL5/RANTES (Catalog # 478-MR), Recombinant Human CCL3/MIP-1α isoform LD78a (Catalog # 270-LD), and Recombinant Human CCL4/MIP-1β (Catalog # 271-BME). PVDF Membrane was probed with 0.1 µg/mL of Goat Anti-Human CCL5/RANTES Antibody (Catalog # AF-278-NA) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for CCL5/RANTES at approximately 10 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.

Immunocytochemistry

CCL5/RANTES in Human PBMCs. CCL5/RANTES was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Goat Anti-Human CCL5/RANTES Antibody (Catalog # AF-278-NA) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.
**Immunohistochemistry**

CCL5/RANTES was detected in immersion fixed paraffin-embedded sections of human tonsil using Goat Anti-Human CCL5/RANTES Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-278-NA) at 10 µg/mL overnight at 4 °C. Tissue was stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

**Neutralization**

Chemotaxis Induced by CCL5/RANTES and Neutralization by Human CCL5/RANTES Antibody.

Recombinant Human CCL5/RANTES (Catalog # 278-RN) chemoattracts the BaF3 mouse pro-B cell line transfected with human CCR5 in a dose-dependent manner (orange line). The amount of cells that migrated through to the lower chemotaxis chamber was measured by Resazurin (Catalog # AR002). Chemotaxis elicited by Recombinant Human CCL5/RANTES (0.01 µg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human CCL5/RANTES Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-278-NA). The ND50 is typically 0.1-0.4 µg/mL.

**PREPARATION AND STORAGE**

<table>
<thead>
<tr>
<th>Reconstitution</th>
<th>Reconstitute at 0.2 mg/mL in sterile PBS.</th>
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</thead>
<tbody>
<tr>
<td>Shipping</td>
<td>The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.</td>
</tr>
<tr>
<td>*Small pack size (SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C</td>
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</tbody>
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**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.

**BACKGROUND**

RANTES (Regulated upon Activation, Normal T cell Expressed and presumably Secreted) is a member of the β (C-C) chemokine subfamily and is now designated CCL5. It binds and activates the chemokine receptors CCR1, 3 and 5.