Human CCL11/Eotaxin Antibody

Monoclonal Mouse IgG_1 Clone # 43911

Catalog Number: MAB320

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CCL11/Eotaxin in ELISAs and Western blots. In Western blots, this antibody does not cross-react with recombinant human CCL1, 2, 3, 4, 5, 7, 8, 9/10/MIP-1γ, 14, 17, 19, 20, 21, 25, recombinant mouse CCL2, 3 , 4 , 5 ,6 , 7, 9/10/MIP-1γ, 11, 21, or 25.
Source	Monoclonal Mouse IgG_1 Clone # 43911
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human CCL11/Eotaxin Gly24-Pro97 Accession # P51671
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS and NaCl with Trehalose. See Certificate of Analysis for details. *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
Dual RNAscope ISH-IHC Compatible	3-25 µg/mL	Immersion fixed paraffin-embedded sections of human duodenum
Western Blot	1 µg/mL	Recombinant Human CCL11/Eotaxin (Catalog # 320-EO) under non-reducing conditions only
Immunohistochemistry	8-25 µg/mL	See Below
Human CCL11/Eotaxin Sandwich Immunoassay		**Reagent**
ELISA Capture	2-8 µg/mL	Human CCL11/Eotaxin Antibody (Catalog # MAB320)
ELISA Detection	0.1-0.4 µg/mL	Human CCL11/Eotaxin Biotinylated Antibody (Catalog # BAF320)
Standard		Recombinant Human CCL11/Eotaxin (Catalog # 320-EO)
Neutralization	Measured by its ability to neutralize CCL11/Eotaxin-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with mouse CCR3. The Neutralization Dose (ND_{50}) is typically 1-5 µg/mL in the presence of 5 ng/mL Recombinant Human CCL11/Eotaxin.	

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China | info.cn@bio-techne.com TEL: 400.821.3475

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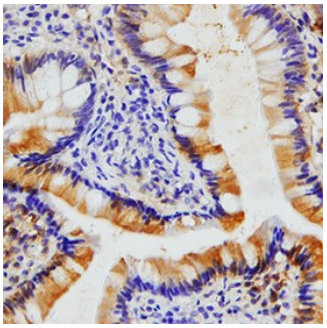
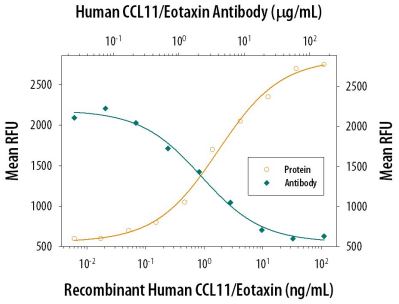
DATA

Immunohistochemistry



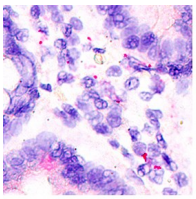
CCL11/Eotaxin in Human Colon. CCL11/Eotaxin was detected in immersion fixed paraffin-embedded sections of human colon using Mouse Anti-Human CCL11/Eotaxin Monoclonal Antibody (Catalog # MAB320) at 15 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm of epithelial and stromal cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Neutralization

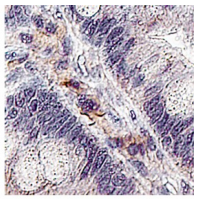


Chemotaxis Induced by CCL11/Eotaxin and Neutralization by Human CCL11/Eotaxin Antibody. Recombinant Human CCL11/Eotaxin (Catalog # Catalog # 320-EO) chemoattracts the BaF3 mouse pro-B cell line transfected with mouse CCR3 in a dose-dependent manner (orange line). The amount of cells that migrated through to the lower chemotaxis chamber was measured by Resazurin (Catalog # Catalog # AR002). Chemotaxis elicited by Recombinant Goat Anti-Human CCL11/Eotaxin (5 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human CCL11/Eotaxin Monoclonal Antibody (Catalog # MAB320). The ND_{50} is typically 1-5 µg/mL.

In-situ Hybridization



In Situ Hybridization (ISH)



Immunohistochemistry (IHC)

Detection of CCL11/Eotaxin in Human Duodenum. Formalin-fixed paraffin-embedded tissue sections of human duodenum were probed for Eotaxin mRNA (ACD RNAScope Probe, catalog # 438468; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using mouse anti-human Eotaxin monoclonal antibody (R&D Systems catalog # Catalog # MAB320) at 20ug/mL with overnight incubation at 4 degrees Celsius followed by incubation with anti-mouse IgG VisUCyte HRP Polymer Antibody (Catalog # Catalog # VC001) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm of epithelial and stromal cells.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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.

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BACKGROUND

CCL11 is a potent eosinophil chemoattractant that was originally purified from bronchoalveolar lavage fluid of guinea pigs sensitized by aerosol challenge with ovalbumin. Microsequencing of the purified protein revealed the guinea pig CCL11 to be a member of the beta (CC) chemokine family of inflammatory and immunoregulatory cytokines. cDNA clones for guinea pig, mouse, and human CCL11 have been isolated. Human CCL11 cDNA encodes a 97 amino acid residue precursor protein from which the amino-terminal 23 amino acid residues are cleaved to generate the 74 amino acid residue mature human CCL11. At the protein sequence level, mature human CCL11 is approximately 60% identical to mature mouse and guinea pig CCL11. In addition, human CCL11 also shows high amino acid sequence identity to human MCP-1, 2, and 3. Human CCL11 is chemotactic for eosinophils, but not mononuclear cells or neutrophils. The CC chemokine receptor 3 (CCR3) has now been identified to be a specific human CCL11 receptor (1-3). CCR3 has also been shown to serve as a cofactor for a restricted subset of primary HIV viruses and binding of CCL11 to CCR3 inhibited infection by the HIV isolates (4).

References:

1. Kitamura, M. *et al.* (1996) J. Biol. Chem **271**:7725.
2. Garcia-Zepeda, E.A. *et al.* (1996) Nature Medicine **2**:449.
3. Ponath, P.D. *et al.* (1996) J. Clin. Invest. **97**:604.
4. Choe, H. *et al.* (1996) Cell **85**:1135.

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