Human CCL26/Eotaxin-3 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF653

DESCRIPTION

Species Reactivity	Human
Specificity	Detects CCL26/Eotaxin-3 in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human CCL26/Eotaxin-3 Thr24-Leu94 Accession # Q9Y258
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. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

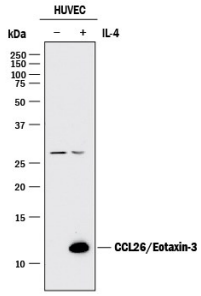
APPLICATIONS

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
Western Blot	1 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	Immersion fixed paraffin-embedded sections of human colon (vasculature)
Neutralization	Measured by its ability to neutralize CCL26/Eotaxin-3-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with mouse CCR3. The Neutralization Dose (ND_{50}) is typically 4-24 µg/mL in the presence of 1 µg/mL Recombinant Human CCL26/Eotaxin-3.	

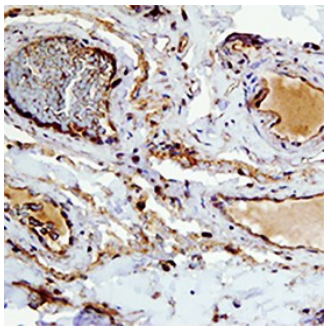
DATA

Western Blot



Detection of Human CCL26/Eotaxin-3 by Western Blot. Western blot shows lysates of HUVEC human umbilical vein endothelial cells untreated (-) or treated (+) with 100 U/mL Recombinant Human IL-4 (Catalog # Catalog # 204-IL) for 24 hours. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human CCL26/Eotaxin-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF653) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for CCL26/ Eotaxin-3 at approximately 12 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



CCL26/Eotaxin-3 in Human Colon (Vasculature). CCL26/Eotaxin-3 was detected in immersion fixed paraffin-embedded sections of human colon (vasculature) using Goat Anti-Human CCL26/Eotaxin-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF653) at 15 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell surface on endothelial cells. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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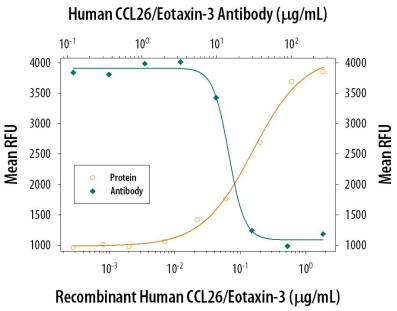
Neutralization



Chemotaxis Induced by CCL26/Eotaxin-3 and Neutralizatio by Human CCL26/Eotaxin-3 Antibody. Recombinant Human CCL26/Eotaxin-3 (Catalog # Catalog # 346-E3) 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 Human CCL26/Eotaxin-3 (1 µg/mL) is neutralized (green line) by increasing concentrations of Human CCL26/Eotaxin-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF653). The ND_{50} is typically 4-24 µg/mL.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *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.

BACKGROUND

Eotaxin-3, also named CCL26 or SCYA26, is a novel human CC chemokine that maps to chromosome 7q11.2, within 40 kilobases of the Eotaxin-2 loci. Eotaxin-3/CCL26 has been shown to be constitutively expressed in the heart and ovary. In addition, low levels of Eotaxin-3/CCL26 expression can also be detected in various tissues. The expression of Eotaxin-3/CCL26 in vascular endothelial cells has been shown to be up-regulated by IL-13 and IL-4.

Eotaxin-3/CCL26 cDNA encodes a 94 amino acid (aa) residue protein with a putative signal peptide of either 23 or 26 aa residues. Recombinant Eotaxin-3/CCL26 has been produced in insect cells using a baculovirus expression system and shown to contain 71 aa residues. Recombinant Eotaxin-3/CCL26 is chemotactic for eosinophils and PHA-activated T cells. Eotaxin-3/CCL26 induces calcium flux in eosinophils as well as in CCR3-transfected cells. Eotaxin-3/CCL26 has also been shown to cross-desensitize cells to other CCR3 ligands. Both the 71 aa residue and 68 aa residue variants of recombinant Eotaxin-3 have been expressed in *E. coli* and found to have equal potency in inducing chemotaxis of a human CCR3-transfected cell line.

References:

1. Gou, R.F. *et al.* (1999) Genomics **58**:313.
2. Kitamura, M. *et al.* (1999) J. Biol. Chem. **274**:27975.
3. Shinkai, A. *et al.* (1999) J. Immunol. **163**:1602.

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