Human/Primate CXCL1/GROα/KC/CINC-1 Antibody

Monoclonal Mouse IgG_{2B} Clone # 20326

Catalog Number: MAB275

DESCRIPTION

Species Reactivity	Human/Primate
Specificity	Detects human CXCL1/GROα/KC/CINC-1 in ELISAs and Western blots. In Western blots, this antibody shows approximately 20% cross-reactivity with recombinant human (rh) CXCL2/GROβ and rhCXCL3/GROγ and no cross-reactivity with recombinant rat (rr)CINC-1, rrCINC-2α, rrCINC-2β, rrCINC-3, rhMIP-1α, recombinant mouse (rm)MIP-1α, rmMIP-1β, rmMIP-1β, rhMIP-1δ, rmMIP-1γ, rmMIP-2, rhMIP-3α, rmMIP-3α, rrMIP-3α, rhMIP-3β, or rmMIP-3β.
Source	Monoclonal Mouse IgG_{2B} Clone # 20326
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human CXCL1/GROα/KC/CINC-1
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 as a 0.2 µm filtered solution in PBS.

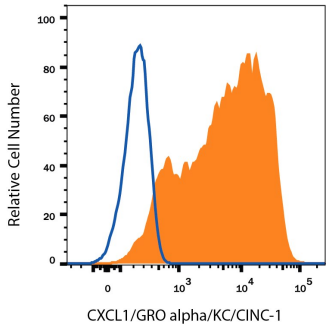
APPLICATIONS

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	Recombinant Human CXCL1/GROα/KC/CINC-1 (Catalog # 275-GR)
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Human/Primate CXCL1/GROα Sandwich Immunoassay		**Reagent**
ELISA Capture	2-8 µg/mL	Human/Primate CXCL1/GROα/KC/CINC-1 Antibody (Catalog # MAB275)
ELISA Detection	0.1-0.4 µg/mL	Human/Primate CXCL1/GROα/KC/CINC-1 Biotinylated Antibody (Catalog # BAF275)
Standard		Recombinant Human CXCL1/GROα/KC/CINC-1 (Catalog # 275-GR)
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize CXCL1/GROα/KC/CINC-1-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CXCR2. The Neutralization Dose (ND_{50}) is typically 3-15 µg/mL in the presence of 10 ng/mL Recombinant Human CXCL1/GROα/KC/CINC-1.	

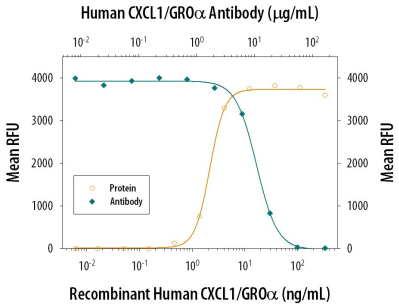
DATA

Intracellular Staining by Flow Cytometry



Detection of CXCL1/GROα/KC/CINC-1 in Human PBMCs Treated with LPS and Monensin by Flow Cytometry. Human peripheral blood mononuclear cell (PBMCs) treated with 1 µg/mL LPS for 24 hours and 3 µM monensin for 2 hours was stained with Mouse Anti-Human/Primate CXCL1/GROα/KC/CINC-1 Monoclonal Antibody (Catalog # MAB275, filled histogram) or isotype control antibody (Catalog # MAB004, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).

Neutralization



Chemotaxis Induced by CXCL1/GROα and Neutralization by Human CXCL1/GROα Antibody. Recombinant Human CXCL1/GROα/KC/CINC-1 (Catalog # 275-GR) chemoattracts the BaF3 mouse pro-B cell line transfected with human CXCR2 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/Primate CXCL1/GROα/KC/CINC-1 (10 ng/mL) is neutralized (green line) by increasing concentrations of Human CXCL1/GROα/KC/CINC-1 Monoclonal Antibody (Catalog # MAB275). The ND_{50} is typically 3-15 µg/mL.

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. *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.

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Catalog Number: MAB275

BACKGROUND

The gene for CXCL1/GROα was initially discovered in hamster cells, using subtractive hybridization techniques, as a message that is over-expressed in tumorigenic cells and in normal cells during growth stimulation. The hamster cDNA was cloned and used as a probe for the subsequent cloning of the human GRO cDNA. Independently, a cDNA encoding a secreted protein with melanoma growth stimulating activity (MGSA) was also cloned from a human melanoma cell line and found to be identical to GRO. In addition to the initially cloned GRO gene, now designated CXCL1, two additional GRO genes, GROβ or MIP-2α and GROγ or MIP-2β, which shared 90% and 86% amino acid sequence homology, respectively, with CXCL1, have been identified. All three human GROs are members of the alpha (C-X-C) subfamily of chemokines.

The three GRO cDNAs encode 107 amino acid precursor proteins from which the N-terminal 34 amino acid residues are cleaved to generate the mature GROs. There are no potential N-linked glycosylation sites in the amino acid sequences. GRO expression is inducible by serum or PDGF and/or by a variety of inflammatory mediators, such as IL-1 and TNF, in monocytes, fibroblasts, melanocytes, and epithelial cells. In certain tumor cell lines, GRO is expressed constitutively.

Similar to other alpha chemokines, the three GRO proteins are potent neutrophil attractants and activators. In addition, these chemokines are also active toward basophils. All three GROs can bind with high affinity to the IL-8 receptor type B. It remains to be seen if a unique GRO receptor(s) also exist. The rat homolog of human CXCL1, CINC, is much more active than human CXCL1 on rat neutrophils, suggesting that this cytokine may have selective species specificity.

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