

DESCRIPTION	
Species Reactivity	Canine
Specificity	Detects canine CXCL8/IL-8 in direct ELISAs and Western blots. Does not cross-react with recombinant human CXCL1, 2, 3, 5, 6, 7, 8, 9, 10, 11, CXCL12/SDF-1 α , 12/SDF-1 β , 13, 16, recombinant mouse CXCL1, 6, 9, 10, CXCL12/SDF-1 α , 13, recombinant rat (rr) CXCL1, CXCL3/CINC-2 α , rrCXCL3/CINC-2 β , or recombinant porcine CXCL8.
Source	Monoclonal Mouse IgG ₁ Clone # 258911
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant canine CXCL8/IL-8 Ala23-Pro101 Accession # P41324
Endotoxin Level	<0.10 EU per 1 μ g of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 μ m 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 μ m filtered solution in PBS.

APPLICATIONS		
Please Note: 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.		
	Recommended Concentration	Sample
Western Blot	1 μ g/mL	Recombinant Canine IL-8/CXCL8 (Catalog # 1608-CL)
Immunocytochemistry	8-25 μ g/mL	See Below
Neutralization	Measured by its ability to neutralize IL-8/CXCL8-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CXCR2. The Neutralization Dose (ND ₅₀) is typically 0.3-1.2 μ g/mL in the presence of 10 ng/mL Recombinant Canine IL-8/CXCL8.	

DATA		
<p>Neutralization</p> <p>Chemotaxis Induced by IL-8/CXCL8 and Neutralization by Canine IL-8/CXCL8 Antibody. Recombinant Canine IL-8/CXCL8 (Catalog # 1608-CL) 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 Canine IL-8/CXCL8 (10 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Canine IL-8/CXCL8 Monoclonal Antibody (Catalog # MAB1608). The ND₅₀ is typically 0.3-1.2 μg/mL.</p>	<p>Immunocytochemistry</p> <p>IL-8/CXCL8 in Canine PBMCs. IL-8/CXCL8 was detected in immersion fixed canine peripheral blood mononuclear cells (PBMCs) treated with calcium ionomycin and PMA using Mouse Anti-Canine IL-8/CXCL8 Monoclonal Antibody (Catalog # MAB1608) at 25 μg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.</p>	

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. <ul style="list-style-type: none"> ● 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

Interleukin 8 (IL-8), also named monocyte-derived neutrophil chemotactic factor (MDNCF), neutrophil-activating protein 1 (NAP-1), neutrophil-activating factor (NAF) and granulocyte chemotactic peptide (GCP), belongs to the Glu-Leu-Arg motif containing (ELR*) CXC chemokine family and has been designated CXCL8. IL-8 is a potent neutrophil chemoattractant that recruits neutrophils to sites of inflammation. IL-8 also activates neutrophil functions and through a poorly understood mechanism, promotes angiogenesis. The biological activities of IL-8 is mediated by two types of G protein-coupled chemokine receptors, CXCR1 and CXCR2. In normal tissues, IL-8 expression and secretion is barely detectable. Upon stimulation by a wide range of pro-inflammatory signals including exposure to IL-1, TNF, bacterial or viral products, IL-8 production is rapidly induced in many different cell types. Secreted IL-8 is not glycosylated but has N-terminal sequence heterogeneity due to proteolytic processing. In human, two major forms, the 72 amino acid (aa) monocyte-derived IL-8 and the 77 aa endothelial IL-8 have been identified. Whereas the 72 aa isoform is a more potent chemoattractant, only the 77 aa isoform can induce apoptosis in leukemic cells. The N-terminal pentapeptide in the 77 aa isoform has been identified as the active site for the IL-8 apoptotic activity. Canine IL-8 encodes a 101 aa precursor protein with a putative 22 aa signal peptide. It shares 77% and 87% aa sequence identity with human and porcine IL-8, respectively. Similar to human IL-8, recombinant canine IL-8 also undergoes N-terminal processing. Two major peptides (the 79 aa and 74 aa variants that differ by an analogous N-terminal pentapeptide) are present in the recombinant canine IL-8 preparations.

References:

1. Van Damme, J. *et al.* (1998) in *The Cytokine Handbook*, A.W. Thomson, ed., Academic Press, New York., p. 271.
2. Terui, Y. *et al.* (1998) *Blood* **92**:2672.
3. Terui, Y. *et al.* (1999) *Cancer Research* **59**:5651.