

DESCRIPTION	
Species Reactivity	Equine
Specificity	Detects equine IL-5 in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 50% cross-reactivity with recombinant bovine IL-5 and recombinant feline IL-5 is observed, 30% cross-reactivity with recombinant mouse IL-5 and recombinant canine IL-5 is observed, 20% cross-reactivity with recombinant rhesus macaque IL-5 is observed, and less than 10% cross-reactivity with recombinant rat IL-5 and recombinant porcine IL-5 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant equine IL-5 Leu20-Gly134 Accession # O02699
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	0.1 µg/mL	Recombinant Equine IL-5 (Catalog # 2470-EL)
Immunocytochemistry	10-25 µg/mL	See Below
Neutralization	Measured by its ability to neutralize IL-5-induced proliferation in the TF-1 human erythroleukemic cell line. Kitamura, T. <i>et al.</i> (1989) J. Cell Physiol. 140:323. The Neutralization Dose (ND ₅₀) is typically 0.5-2.0 µg/mL in the presence of 50 ng/mL Recombinant Equine IL-5.	

DATA

Neutralization

Cell Proliferation Induced by IL-5 and Neutralization by Equine IL-5 Antibody. Recombinant Equine IL-5 (Catalog # 2470-EL) stimulates proliferation in the TF-1 human erythroleukemic cell line in a dose-dependent manner (orange line). Proliferation elicited by Recombinant Equine IL-5 (50 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Equine IL-5 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2470). The ND₅₀ is typically 0.5-2.0 µg/mL.

Immunocytochemistry

IL-5 in Equine PBMCs. IL-5 was detected in immersion fixed equine peripheral blood mononuclear cells (PBMCs) treated with calcium ionomycin and PMA using Goat Anti-Equine IL-5 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2470) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

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. <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-5 (IL-5) is a 40 kDa, secreted, heparin-binding, disulfide-linked homodimeric glycoprotein that belongs to the α -helical group of cytokines (1-3). IL-5 is primarily produced by CD4⁺ Th2 cells, but other cell types such as eosinophils, endothelial cells, mast cells, visceral (airway) smooth muscle cells, bronchial epithelium, CD16⁺ NK cells and $\gamma\delta$ T cells can also produce IL-5. Equine IL-5 is synthesized as a 134 amino acid (aa) precursor that contains a 19 aa signal sequence and a 115 aa mature segment. There are four α -helices, two potential N-linked glycosylation sites, and two cysteines that form interchain disulfide bonds with a second, antiparallel IL-5 molecule (3, 4). While human and mouse IL-5 have a potential NLS in their sequence, it is unclear if equine IL-5 has such a sequence. Mature horse IL-5 shares 71%, 89%, 88%, 83%, 66% and 63% aa sequence identity with mature human, bovine, feline, canine, mouse and rat IL-5, respectively.

The receptor for IL-5 consists of a 60 kDa ligand-binding subunit (IL-5 R α) and a 120 kDa signal-transducing subunit (β_c). It is suggested that dimeric IL-5 binding to IL-5 R α recruits β_c , which subsequently covalently links with IL-5 R α . This trimeric complex then associates with another trimeric complex to form the physiologic IL-5 receptor (6). Following binding, IL-5 has targeted effects. It promotes the maturation and migration of eosinophils, partially through the effects of eotaxin. It mobilizes eosinophils and CD34⁺ progenitors from marrow. It also enhances Ig release from B cells and contributes to IL-4 production. Finally, it primes basophils for histamine and leukotriene release (1, 2, 7).

References:

1. Lalani, T. *et al.* (1999) *Ann. Allergy Asthma Immunol.* **82**:317.
2. Martinez-Moczygomba, M. and D.P. Huston (2003) *J. Allergy Clin. Immunol.* **112**:653.
3. Zabeau, L. *et al.* (2003) *Curr. Drug Targets Inflamm. Allergy* **2**:319.
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5. Geijsen, N. *et al.* (2001) *Cytokine Growth Factor Rev.* **12**:19.
6. Bagley, C.J. *et al.* (1997) *Blood* **89**:1471.
7. Mattes, J. and P.S. Foster (2003) *Curr. Drug Targets Inflamm. Allergy* **2**:169.