

DESCRIPTION	
<b>Species Reactivity</b>	Feline
<b>Specificity</b>	Detects feline IL-12/IL-23 p40 in ELISAs and Western blots. In sandwich immunoassays, approximately 65% cross-reactivity with recombinant canine IL-12/23 p40 is observed, less than 3% cross-reactivity with recombinant mouse IL-12/23 p40 is observed, and less than 0.2% cross-reactivity with recombinant human IL-12/23 p40 is observed.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant feline IL-12/IL-23 p40 Ile23-Ser329 (Glu167Gly) Accession # O02744
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the antibody by the LAL method.
<b>Formulation</b>	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	
<b>Please Note:</b> 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.	
	<b>Recommended Concentration</b> <b>Sample</b>
<b>Western Blot</b>	0.1 µg/mL      Recombinant Feline IL-12/IL-23 p40 (Catalog # 2117-FL)
<b>Immunocytochemistry</b>	10-25 µg/mL      See Below
<b>Feline IL-12/IL-23 p40 Sandwich Immunoassay</b>	<b>Reagent</b>
<b>ELISA Capture</b>	0.2-0.8 µg/mL      Feline IL-12/IL-23 p40 Antibody (Catalog # AF1954)
<b>ELISA Detection</b>	0.1-0.4 µg/mL      Feline IL-12/IL-23 p40 Biotinylated Antibody (Catalog # BAF1954)
<b>Standard</b>	Recombinant Feline IL-12/IL-23 p40 (Catalog # 2117-FL)
<b>Neutralization</b>	Measured by its ability to neutralize IL-12-induced proliferation in PHA-activated human peripheral blood mononuclear cells (PBMC). Yokota, T. <i>et al.</i> (1986) Proc. Natl. Acad. Sci. USA <b>83</b> :5894. The Neutralization Dose (ND <sub>50</sub> ) is typically 0.5-2.0 µg/mL in the presence of 25 ng/mL Recombinant Feline IL-12.

DATA	
<p><b>Neutralization</b></p> <p><b>Cell Proliferation Induced by IL-12 and Neutralization by Feline IL-12/IL-23 p40 Antibody.</b> Recombinant Feline IL-12 (Catalog # 1954-FL) stimulates proliferation in PHA-activated human peripheral blood mononuclear cells (PBMC) in a dose-dependent manner (orange line). Proliferation elicited by Recombinant Feline IL-12 (25 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Feline IL-12/IL-23 p40 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1954). The ND<sub>50</sub> is typically 0.5-2.0 µg/mL.</p>	<p><b>Immunocytochemistry</b></p> <p><b>IL-12/IL-23 p40 in Feline PBMCs.</b> IL-12/IL-23 p40 was detected in immersion fixed feline peripheral blood mononuclear cells (PBMCs) stimulated with PMA and calcium ionomycin using Goat Anti-Feline IL-12/IL-23 p40 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1954) 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 <a href="#">Fluorescent ICC Staining of Non-adherent Cells</a>.</p>

PREPARATION AND STORAGE	
<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## BACKGROUND

Interleukin 12 (IL-12) and IL-23 are secreted heterodimeric glycoproteins belonging to the IL-12 cytokine family. The two cytokines share a common p40 (40 kDa) subunit, which is disulfide-linked with the p35 (35 kDa) subunit in IL-12, and with the p19 (19 kDa) subunit in IL-23. Feline p40 is synthesized as a 329 amino acid (aa) precursor with a 22 aa signal sequence and a 307 aa mature region. It contains a 90 aa fibronectin type III domain and a 75 aa Ig C2-like region. The expression of p40 is induced by substances such as LPS and CpG that activate antigen-presenting cells. Besides being found as a component of IL-12 or IL-23, free p40 monomers and homodimers are also secreted by cells expressing p40. Feline p40 shares 94%, 85%, 84%, 65%, and 65% aa sequence identity with canine, human, porcine, rat and mouse p40, respectively. Cells known to express p40 include macrophages, dendritic cells, monocytes, Langerhans cells, neutrophils, keratinocytes, plasmacytoid dendritic cells, and microglia. From cells that express both the p35 and p40 subunits (dendritic cells, monocytes, and CHO cells), the amount of free p40 secreted is 10-1000 fold more than the heterodimeric IL-12. The high-affinity IL-12 receptor complex that transduces IL-12 signals is composed of a 100 kDa ligand-binding subunit (IL-12 R $\beta$ 1) and a 130 kDa signal transducing subunit (IL-12 R $\beta$ 2). Similarly, the high-affinity IL-23 signaling receptor complex is composed of the shared IL-12 R $\beta$ 1 and the unique IL-23 R, a novel gp130-like protein. Both the monomeric and the dimeric free p40 can bind to the IL-12 R $\beta$ 1 and function as antagonists of IL-12 or IL-23. However, the monomeric p40 binds IL-12 R $\beta$ 1 with lower affinity and is less potent as an IL-12 antagonist. Homodimeric mouse p40 has also been shown to have agonistic functions similar to IL-12, inducing nitric oxide expression and NF $\kappa$ B activation in mouse primary microglia and peritoneal macrophages. The molecular mechanism for the agonistic effects of homodimeric p40 has not been determined (1-6).

## References:

1. Buttner, M. *et al.* (1997) *Cytokine* **10**:241.
2. Park, A.Y. and P. Scott (2001) *Scand. J. Immunol.* **53**:529.
3. Trinchieri, G. *et al.* (2003) *Immunity* **19**:641.
4. Brombacher, F. *et al.* (2003) *Trends Immunol.* **24**:207.
5. Lankford, C.S. and D.M. Frucht, 2003, *J. Leukoc. Biol.* **73**:49.
6. Abdi, K. (2002) *Scand. J. Immunol.* **5**:1.