DESCRIPTION

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Species Reactivity	Canine
Specificity	Detects canine IFN-γ in direct ELISAs and Western blots. In direct ELISAs, less than 40% cross-reactivity with recombinant porcine IFN-γ is observed and less than 5% cross-reactivity with recombinant human IFN-γ, recombinant rhesus macaque IFN-γ, recombinant mouse IFN-γ, and recombinant rat IFN-γ is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant canine IFN-γ Gln24-Lys166 Accession # P42161
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 Canine IFN-γ (Catalog # 781-CG)
Immunocytochemistry	5-15 µg/mL	See Below
Neutralization	Measured by its ability to neutralize IFN-γ inhibition of VSV-induced cytopathy in the A-72 canine fibroma cell line. The Neutralization Dose (ND$_{50}$) is typically <8 µg/mL in the presence of 1.5 ng/mL Recombinant Canine IFN-γ.	

DATA

Neutralization
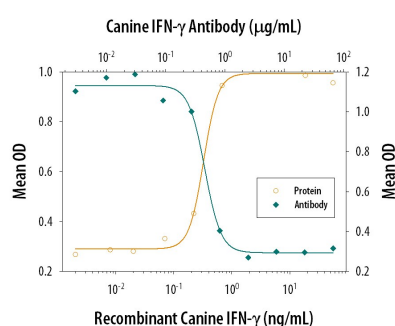
IFN-γ Inhibition of VSV-induced Cytopathy and Neutralization by Canine IFN-γ Antibody. Recombinant Canine IFN-gamma (Catalog # Catalog # 781-CG) reduces the Vesticular Stomatitis Virus (VSV)-induced cytopathy in the A-72 canine fibroma cell line in a dose-dependent manner (orange line), as measured by crystal violet staining. Inhibition of VSV activity elicited by Recombinant Canine IFN-gamma (1.5 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Canine IFN-gamma Antigen Affinity-purified Polyclonal Antibody (Catalog # AF781). The ND$_{50}$ is typically <8 µg/mL.

Immunocytochemistry
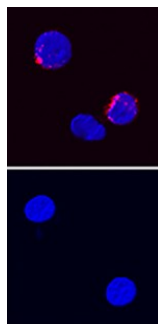
IFN-γ in Canine PBMCs. IFN-γ was detected in immersion fixed canine peripheral blood mononuclear cells (PBMCs) untreated (lower panel) or treated with PMA and calcium ionomycin (upper panel) using Goat Anti-Canine IFN-γ Antigen Affinity-purified Polyclonal Antibody (Catalog # AF781) at 15 µg/mL for 3 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 cytoplasmic. 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.** • 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

Interferon-gamma (IFN-γ), also known as type II or immune interferon, exerts a wide range of immunoregulatory activities and is considered to be the prototype proinflammatory cytokine (1, 2). Mature canine IFN-γ exists as a noncovalently linked homodimer of 20-25 kDa variably glycosylated subunits (3). It shares 79-88% amino acid sequence identity with bovine, equine, and feline IFN-γ, 62-73% with human, porcine, and rhesus IFN-γ, and 40-47% with cotton rat, mouse, and rat IFN-γ. IFN-γ dimers bind to IFN-γ RI (alpha subunits) which then interact with IFN-γ RII (beta subunits) to form the functional receptor complex of two α and two β subunits. Inclusion of IFN-γ RII increases the binding affinity for ligand and the efficiency of signal transduction (4, 5). IFN-γ is produced by a variety of immune cells under inflammatory conditions, notably by T cells and NK cells (6). It plays a key role in host defense by promoting the development and activation of Th1 cells, chemoattraction and activation of monocytes and macrophages, up regulation of antigen presentation molecules, and immunoglobulin class switching in B cells. It also exhibits antiviral, antiproliferative, and apoptotic effects (6, 7). In addition, IFN-γ functions as an anti-inflammatory mediator by promoting the development of regulatory T cells and inhibiting Th17 cell differentiation (8, 9). The pleiotropic effects of IFN-γ contribute to the development of multiple aspects of atherosclerosis (7).

References:

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2. Pestka, S. *et al.* (2004) Immunol. Rev. **202**:8.
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8. Muhl, H. and J. Pfeilschifter (2003) Int. Immunopharmacol. **3**:1247.
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