Species Reactivity: Porcine
Specificity: Detects porcine TNF-α in direct ELISAs and Western blots.
Source: Polyclonal Goat IgG
Purification: Antigen Affinity-purified
Immunogen: E. coli-derived recombinant porcine TNF-α
  Arg78-Leu232
  Accession # P23563
Endotoxin Level: <0.01 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.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Western Blot</th>
<th>0.1 μg/mL</th>
<th>Recombinant Porcine TNF-α (Catalog # 690-PT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunocytochemistry</td>
<td></td>
<td>5-15 μg/mL</td>
<td>See Below</td>
</tr>
<tr>
<td>Neutralization</td>
<td></td>
<td></td>
<td>Measured by its ability to neutralize TNF-α-induced cytotoxicity in the PK-15 porcine kidney epithelial cell line. Matthews, N. and M. L. Neale (1987) in Lymphokines and Interferons, A Practical Approach. Clemens, M. J. et al. (eds): IRL Press. 221; Bertoni et al. (1993) J. Immunol. Meth. 160:267. The Neutralization Dose (ND₅₀) is typically 0.2-0.8 μg/mL in the presence of 0.05 ng/mL Recombinant Porcine TNF-α and 1 μg/mL actinomycin D.</td>
</tr>
</tbody>
</table>

DATA

Neutralization

Cytotoxicity induced by TNF-α and Neutralization by Porcine TNF-α Antibody. Recombinant Porcine TNF-α (Catalog # 690-PT) induces cytotoxicity in the the PK-15 porcine kidney epithelial cell line in a dose-dependent manner (orange line), as measured by crystal violet staining. Cytotoxicity elicited by Recombinant Porcine TNF-α (0.05 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Porcine TNF-α Antibody (Catalog # AF690). The ND₅₀ is typically 0.2-0.8 μg/mL in the presence of the metabolic inhibitor actinomycin D (1 μg/mL).

Immunocytochemistry

TNF-α in Porcine PBMCs. TNF-α was detected in immersion fixed porcine peripheral blood mononuclear cells (PBMCs) treated with calcium ionomycin and PMA using Goat Anti-Porcine TNF-α Antibody Affinity-purified Polyclonal Antibody (Catalog # AF690) 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.
  - 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.
Tumor necrosis factor alpha (TNF-α), also known as cachectin and TNFSF2, is the prototypic ligand of the TNF superfamily. It is a pleiotropic molecule that plays a central role in inflammation, apoptosis, and immune system development. TNF-α is produced by a wide variety of immune and epithelial cell types (1, 2). Porcine TNF-α consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 176 aa extracellular domain (ECD) (3). Within the ECD, porcine TNF-α shares 69%-86% aa sequence identity with bovine, canine, cotton rat, equine, feline, human, mouse, rat, and rhesus TNF-α. The 26 kDa type 2 transmembrane protein is assembled intracellularly to form a noncovalently linked homotrimer (4). Ligation of this complex induces reverse signaling that promotes lymphocyte costimulation but diminishes monocyte responsiveness (5). Cleavage of membrane bound TNF-α by TACE/ADAM17 releases a 55 kDa soluble trimeric form of TNF-α (6, 7). TNF-α trimers bind the ubiquitous TNF RI and the hematopoietic cell-restricted TNF RII, both of which are also expressed as homotrimers (1, 8). TNF-α regulates lymphoid tissue development through control of apoptosis (2). It also promotes inflammatory responses by inducing the activation of vascular endothelial cells and macrophages (2). TNF-α is a key cytokine in the development of several inflammatory disorders (9). It contributes to the development of type 2 diabetes through its effects on insulin resistance and fatty acid metabolism (10, 11).

References: