

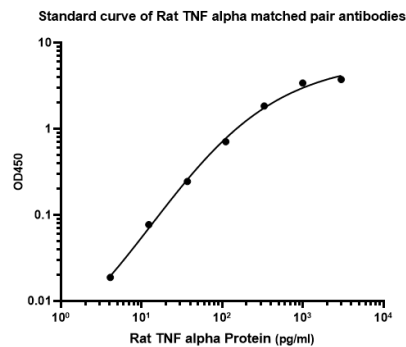


<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Mouse, Rat
<b>Applications:</b>	ELISA(Det)
<b>Molecular Wt:</b>	Predicted band size: 25.8 kDa
<b>Clone number:</b>	PSH03-30

<b>Description:</b>	Tumor necrosis factor alpha (TNF-alpha ), also known as cachectin and TNFSF1A, is the prototypic ligand of the TNF superfamily. It is a pleiotropic molecule that plays a central role in inflammation, immune system development, apoptosis, and lipid metabolism. TNF-alpha is also involved in a number of pathological conditions including asthma, Crohn's disease, rheumatoid arthritis, neuropathic pain, obesity, type 2 diabetes, septic shock, autoimmunity, and cancer. Mouse TNF-alpha is synthesized as a 26 kDa type II transmembrane protein that consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 179 aa extracellular domain (ECD). Within the ECD, mouse TNF-alpha shares 95% aa identity with rat, and 80% aa identity with canine, equine, feline, human, rabbit, and porcine TNF-alpha. It is produced by a wide variety of immune, epithelial, endothelial, and tumor cells. TNF-alpha is assembled intracellularly to form a noncovalently linked homotrimer which is expressed on the cell surface. Cell surface TNF-alpha can both induce the lysis of tumor cells and virus infected cells, and generate its own downstream cell signaling following ligation by soluble TNF RI. Shedding of membrane bound TNF-alpha by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa soluble trimer containing the TNF-alpha extracellular domain. TNF-alpha binds the ubiquitous 55-60 kDa TNF RI and the hematopoietic cell-restricted 78-80 kDa TNF RII, both of which are also expressed as homotrimers. Both type I and type II receptors bind TNF-alpha with comparable affinity and can promote NFkB activation. Only TNF RI, however, contains a cytoplasmic death domain which triggers the activation of apoptosis. Soluble forms of both types of receptors are released into human serum and urine, and can neutralize the biological activity of TNF.
<b>Immunogen:</b>	Recombinant protein within Rat TNF alpha aa 80-235 (P16599).
<b>Positive control:</b>	Recombinant rat TNF alpha protein, Recombinant mouse TNF alpha protein.
<b>Subcellular location:</b>	Secreted.
<b>Database links:</b>	SwissProt: P06804 Mouse   P16599 Rat
<b>Recommended Dilutions:</b>	
<b>ELISA(Det)</b>	Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH03-29] to Mouse/Rat TNF alpha (Capture) (HA721974) and recombinant standard Mouse TNF alpha (HA210527) and Rat TNF alpha (HA210538) as the standard. The reference range value is 12.3-3000 pg/ml.
<b>Storage Buffer:</b>	PBS (pH7.4).
<b>Storage Instruction:</b>	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
<b>Purity:</b>	Protein A affinity purified.

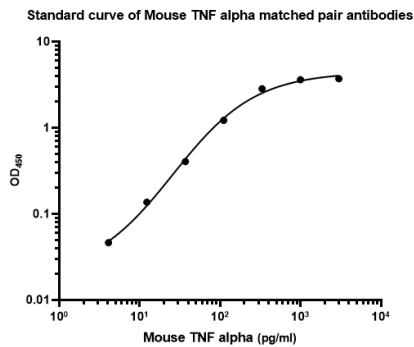
Images

**Fig1:** Sandwich ELISA analysis of rat TNF alpha matched pair antibodies

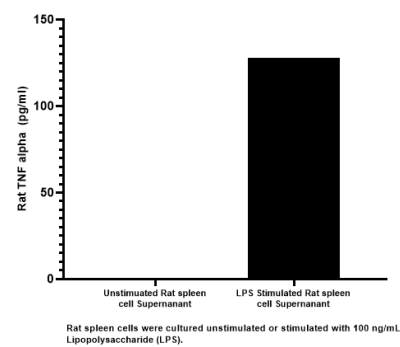


Elisa assay was performed by coating wells of a 96-well plate with 100  $\mu$ l per well of capture antibody (HA721974) diluted in carbonate/bicarbonate buffer, at a concentration of 2  $\mu$ g/ml overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150  $\mu$ l 0.05% tween-20 1%BSA blocking buffer, and incubated with serial diluted rat TNF alpha protein (HA210538) starting from 3000 pg/ml to 0 pg/ml and detect antibody (HA721975) -Biotin (0.2  $\mu$ g/ml) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 100  $\mu$ l per well of SA-HRP for 0.5 hour at 30 $^{\circ}$ C with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

**Fig2:** Sandwich ELISA analysis of mouse TNF alpha matched pair antibodies



Elisa assay was performed by coating wells of a 96-well plate with 100  $\mu$ l per well of capture antibody (HA721974) diluted in carbonate/bicarbonate buffer, at a concentration of 2  $\mu$ g/ml overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150  $\mu$ l 0.05% tween-20 1%BSA blocking buffer, and incubated with serial diluted mouse TNF alpha protein (HA210527) starting from 3000 pg/ml to 0 pg/ml and detect antibody (HA721975)-Biotin (0.2  $\mu$ g/ml) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 100  $\mu$ l per well of SA-HRP for 0.5 hour at 30 $^{\circ}$ C with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.



**Fig3:** Conditioned media was harvested after 72 hours. TNF- $\alpha$  was measured in 100% unstimulated and LPS stimulated rat spleen cell supernatant. The concentrations of TNF- $\alpha$  were interpolated from the TNF- $\alpha$  standard curves. The mean TNF- $\alpha$  concentration was determined to be 128 pg/mL in LPS stimulated rat spleen cell supernatant. There was no detectable signal in unstimulated supernatant.