ROCKLAND
Immunochemicals for Research

800-656-7625

fax 610-367-7825

Certificate of Analysis

Product: IgG fraction of Anti-Human Tumor Necrosis Factor (TNFα) (Rabbit)

Code: 209-401-306 **Lot #:** 14092

Size: 1.0 mg **Physical State:** Liquid (sterile filtered)

Protein Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is six (6) months from date of opening.

Application Note(s): This IgG fraction antibody of anti-Human TNFα has been tested for use in neutralizations, ELISA, immunohistochemistry and immunoblotting. It recognizes the 17,000 MW TNFα. Reactivity in other immunoassays is unknown.

Recommended Dilutions: This product has been assayed by immunoblot using HRP Goat-anti-Rabbit IgG [H&L] (code # 611-1302) and TMB as a substrate. A working dilution range of 1:100 to 1:200 is suggested for this application to detect TNFα from supernatants or lysates of 2 x 10^6 endotoxin-stimulated human peripheral blood mononuclear cells (PBMC). PBMC are stimulated for 24 hours with 1% (v/v) human serum plus 10 ng/mL E.coli LPS. This product has been assayed by immunohistochemistry. A dilution range of 1:100 to 1:200 is suggested for this immunoassay. Either paraffin fixation or cryofixation can be used for immunohistochemistry using a dilution of 1:200 for staining of TNFα. This product has been assayed by ELISA against TNFα using HRP Conjugated Anti-Rabbit IgG [H&L] (Goat) (code # 611-1302) and ABTS as a substrate for 30 minutes at room temperature. A working dilution range of 1:200 to 1:1,000 is suggested for this product. For use in ELISA formats, this antibody is best used as the second antibody in combination with a monoclonal antibody as a capture antibody. Optimal titers for other applications should be determined by the researcher. See below for use in neutralizations.

Purity and Specificity: This is an IgG preparation of whole rabbit serum purified by Protein G chromatography. This antibody is primarily directed against mature 17,000 MW human TNFα and is useful in determining its presence in various assays. In general, this antibody also detects primate TNFα in the same formats using similar dilutions. The antibody does not recognize human TNGβ (lymphotoxin). This IgG fraction antibody will recognize the cell-bound precursor of TNFα as a 26,000 protein in immunoblots, particularly in denatured samples. This antibody is also useful for neutralization of human and primate TNFα activity in bioassays. It does not neutralize the biological activity of lymphotoxin. For neutralization, it is recommended to incubate the sample with a 1:200 dilution of the antibody for at least 4 hours before being tested. A control of similarly diluted normal rabbit IgG is recommended.

Endotoxin Content: <10 pg/μl by LAL method.

Immunogen: The whole rabbit serum used to produce this IgG fraction antibody was prepared by repeated immunizations with recombinant human TNFα produced in *E.coli.*

Reference(s):

Cseh, K. and B. Beutler (1989) Alternative cleavage of the cachectin/tumor necrosis factor propeptide results in a larger, inactive form of secreted protein. *J Biol Chem* **264** (27): 16256-60.