

Certificate of Analysis

Product: IgG fraction of Anti-Human Tumor Necrosis Factor (TNF α) (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 TNF α has been tested for use in neutralizations, ELISA, immunohistochemistry and immunoblotting. It recognizes the 17,000 MW TNF α . 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 TNF α from supernatants or lysates of 2×10^6 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 TNF α . This product has been assayed by ELISA against TNF α 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 TNF α and is useful in determining its presence in various assays. In general, this antibody also detects primate TNF α in the same formats using similar dilutions. The antibody does not recognize human TNF β (lymphotoxin). This IgG fraction antibody will recognize the cell-bound precursor of TNF α as a 26,000 protein in immunoblots, particularly in denatured samples. This antibody is also useful for neutralization of human and primate TNF α 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/ μ l by LAL method.

Immunogen: The whole rabbit serum used to produce this IgG fraction antibody was prepared by repeated immunizations with recombinant human TNF α 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.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.