

Product Specification Sheet

Product: IgG fraction of Anti-Human IL-1 α (Rabbit)

Code: 209-401-302

Lot #: 16051

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

Preservative: 0.01% (w/v) Sodium Azide

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 IL-1 α has been tested for use in neutralizations, ELISA, immunohistochemistry, flow cytometry and immunoblotting. It recognizes the 17,000 MW mature IL-1 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 IL-1 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 1:200 is suggested for this immunoassay. Either paraffin fixation or cryofixation can be used for immunohistochemistry using a dilution of 1:200 for staining of intracellular IL-1 α . This product has been assayed by ELISA against IL-1 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 DEAE fractionation. This antibody is primarily directed against the 17,000 MW human IL-1 α and is useful in determining its presence in various assays. In general, this antibody also detects primate IL-1 α in the same formats using similar dilutions. The antiserum does not recognize human IL-1 β or Mouse or Rabbit IL-1 α . In ELISA formats and other immunoreactive assays, this antibody will recognize both the mature 17,000 MW IL-1 α as well as the 31,000 MW IL-1 α precursor in either non-denatured (native) or denatured samples. Unlike the IL-1 β precursor, the native precursor of IL-1 α is recognized by the antibody produced to the 17,000 MW form. The 31,000 precursor of IL-1 α is biologically active and is found primarily intracellularly. The precursor of IL-1 α , unlike that of IL-1 β , is biologically active when applied to cells and is thought to have a role as a functional molecule intracellularly and can be found constitutively expressed in various cell. This antibody is also useful for neutralization of human and primate IL-1 α activity in bioassays. It does not neutralize the biological activity IL-1 β . It does not neutralize the biological activity of mouse, rat or rabbit IL-1 α . It will neutralize primate IL- α . For neutralization, it is recommended to incubate the sample with a 1:100 dilution of the antibody for at least 4 hours before being tested. A control of similarly diluted normal rabbit IgG is recommended. This antibody can be used for FACS analysis. Caution should be exhibited as the F(c) domain of the rabbit IgG molecule may interact with cells non-specifically.

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 IL-1 α produced in *E.coli*. The MW of the recombinant IL-1 α was 17,000. This is the cleavage site generated by the IL-1 β converting enzyme (ICE, capase-1).

Reference(s):

Cerretti, D.P., et al. (1992) Molecular cloning of the IL-1 β processing enzyme. *Science* **256**: 97-100.

Thornberry, N.A., et al. (1992) A novel heterodimeric cysteine protease is required for interleukin-1 β processing in monocytes. *Nature* **356** (6372) 768-74.

Lonnemann, G., et al. (1995) Cytokines in human renal interstitial fibrosis. II. Intrinsic Interleukin (IL)-1 synthesis and IL-1-dependent production of IL-6 and IL-8 by cultured kidney fibroblasts. *Kidney Int* **47**: 845-854.

Lonnemann, G., et al. (1995) Cytokines in human renal interstitial fibrosis. I Interleukin-1 is a paracrine growth factor for cultured fibrosis-derived kidney fibroblasts. *Kidney Int* **47**: 837-844.

March, C.J., et al. (1985) Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* **315**: 641-645.

Mosley, B., et al (1987) The interleukin-1 receptor binds the human interleukin 1 α precursor, but not the interleukin 1- β precursor. *J. Biol Chem* **262**: 2941-2944.

Stevenson, F.T., et al. (1993) The 31-kDa precursor of interleukin-1 α is myristoylated on specific lysines within the 16-kDa N-terminal propiece. *Proc Natl Acad Sci USA* **90**: 7245-7249.

Stevenson, F.T., et al. (1997) The N-terminal propiece of interleukin 1 α is a transforming nuclear oncoprotein. *Proc Natl Acad Sci USA* **94**: 508-513.

USDA Certification: 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.

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.