3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Product No. I 5018
Anti-Human Interleukin-1α
Developed in Rabbit
IgG Fraction of Antiserum

Lot 103H4815

Product Description
Anti-Human Interleukin-1α (IL-1α) is developed in rabbit using purified human recombinant IL-1α produced in *E. coli* as the immunogen. Whole antiserum is fractionated and then further purified by ion exchange chromatography to provide the IgG fraction of antiserum. This fraction is essentially free of other rabbit serum proteins.

Reagents
The product is supplied as a sterile-filtered liquid in 0.01 M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide as a preservative.

Specificity
Rabbit Anti-Human IL-1α detects natural and recombinant human IL-1α by dot blot immunoassay and RIA. No reaction is observed versus recombinant mouse GM-CSF, recombinant human IL-1β, recombinant human TNF-α, recombinant human IL-2, recombinant human TNF-β, recombinant human IL-3 and recombinant human IL-6.

Description
Interleukin-1, originally known as Lymphocyte Activating Factor (LAF), activates T cell lymphocytes which then proliferate and secrete Interleukin-2 (IL-2).[1] IL-1 is released primarily from stimulated macrophages and monocytes, but is also released from several other cell types[2] and plays a key role in inflammatory and immune responses.[3] Other synonyms for IL-1 include: endogenous pyrogen (EP), mitogenic protein (MP), helper peak-1 (HP-1), T cell replacing factor III (TRF III or TRF_M), B cell activating factor (BAF) and B cell differentiation factor (BDF).[4] The two closely related agents Interleukin-1α (IL-1α) and Interleukin-1β (IL-1β) share 62% homology in amino acid sequence and elicit nearly identical biological responses. IL-1α and IL-1β are both approximately 17 kD with some heterogeneity in the amount of glycosylation. Biological activity of IL-1α and IL-1β is measured using the murine T cell line EL4-NOB1 which produces IL-2 in response to IL-1.[5] The amount of IL-2 produced is proportional to the IL-1 concentration and is determined by ^{3}H-thymidine uptake into an IL-2 dependent T cell line such as CTLL-2.

Uses
Rabbit Anti-Human IL-1α may be used to study human IL-1α using immunoblotting, dot blot, RIA, ELISA, or selective neutralization of human IL-1α bioactivity in cell culture.

Bioactivity
One ml of Rabbit Anti-Human IL-1α neutralizes a minimum of 300,000 International units of recombinant human IL-1α. Neutralization of proliferative activity is tested in culture using CTLL/EL4-NOB1 cells.[5] One unit is defined as the amount of IL-1α required to induce a half-maximal incorporation of ^{3}H-thymidine. Activity is expressed in International units (NIBSC reference preparation for IL-1α code 86/632).

Dot Blot
A 1:2,000 dilution was determined using 100 ng recombinant human IL-1α/dot on nitrocellulose membranes.

Protein Concentration: 7.8 mg/ml by extinction.
$E_{280}^{1\%} = 14.0$.

RIA SYSTEM

RIA Characterization
The antiserum is characterized utilizing a second antibody-polyethylene glycol (PEG) RIA protocol, where 0.1 ml of a 1:120,000 dilution of antiserum at the working dilution has been found to bind at least 40% of 15 picograms of iodinated IL-1α.

It is recommended that the antiserum be evaluated in the particular assay system chosen due to differences in systems and procedures.

RIA Reagents

1. Standards: Prepare and freeze aliquots of a stock standard solution of 50 ng/ml human IL-1α in dilution buffer. Thaw one aliquot for each assay and dilute in dilution buffer to the following concentrations: 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8 and 0.4 ng/ml.
2. Dilution buffer: 0.01 M phosphate buffered saline, pH 7.8 containing 0.5% BSA and 0.1% sodium azide.
3. Normal rabbit serum (Sigma Product No. R-9133): 2% in dilution buffer without BSA.
4. EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt (Sigma Stock No. ED2SS), 0.1 M, pH 7.8 in distilled water. Adjust pH with 10 N NaOH.
5. Second antibody: Goat Anti-Rabbit IgG (Sigma Product No. R 0881) reconstituted in dilution buffer. Dilute reconstituted antiserum 1:5 in dilution buffer for use.
6. PEG solution: 6% PEG (Sigma Product No. P 2139, approximate molecular weight 8,000) in dilution buffer without BSA.

RIA Protocol

1. In polypropylene test tubes, add 0.2 ml sample or standard and 0.1 ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 1 hour at 37 °C.
4. Add 0.1 ml I^{125} radioactive tracer diluted in dilution buffer.
5. Vortex the tubes.
6. Incubate for 2 hours at 37 °C followed by an incubation of 18-20 hours at 4 °C.
7. Add 0.1 ml EDTA solution and 0.1 ml 2% rabbit serum to all tubes.
8. Vortex the tubes.
9. Add 0.1 ml second antibody to all tubes.
10. Add 0.7 ml PEG solution to all tubes.
11. Vortex the tubes.
12. Incubate for 5 minutes at room temperature.
13. Centrifuge at 2000 x g for 15 minutes at 4 °C.
14. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. In the above system the sensitivity has been found to be 15 pg.

RIA Specificity

Specificity of the antiserum is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross-reactivity data obtained in the second antibody-PEG I^{125} RIA system is as follows:

Cross-Reactant	**%Cross-Reactivity**
Human IL-1α, recombinant	100
Human IL-6, recombinant	< 0.01
Human IL-1β, recombinant	< 0.01
Human TNFα, recombinant	< 0.01
Human TNFβ, recombinant	< 0.01
Human IL-2	< 0.01
Human IL-3	< 0.01
Mouse GM-CSF	< 0.01

(Dilutions for standard curve: 50.0-0.4 ng/ml; dilutions for crossreactants: 100-25 ng/ml)

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using this RIA system.
$K_a = 1.3 \times 10^{10}$ L/mole.

Dilution and Use

Dilute antibody in tissue culture media containing 10% serum or buffered saline containing 1% BSA, according to the planned application.

Storage

Store undiluted antibody at –20 °C. The product should be stored frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended.

References

1. Gery, I., et al., J. Exp. Med., **136**, 128 (1972).
2. Oppenheim, J., et al., Immunol. Today, **7**, 45 (1986).
3. Durum, S., et al., Ann. Rev. Immunol., **3**, 263 (1985).
4. Aarden, L., et al., J. Immunol., **123**, 2928 (1979).
5. Gearing, A., et al., J. Immunol. Meth., **99**, 7 (1987).