Coud-Clone Corp.

APA056Hu61 100µg Active Interleukin 10 (IL10) Organism Species: Homo sapiens (Human) *Instruction manual*

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1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: Ser19~Asn178 Tags: N-terminal His-tag Purity: >98% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300. Predicted isoelectric point: 7.6 Predicted Molecular Mass: 20.3kDa Accurate Molecular Mass: 18kDa as determined by SDS-PAGE reducing conditions. Applications: Cell culture; Activity Assays; In vivo assays. (May be suitable for use in other assays to be determined by the end user.)

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

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Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[<u>SEQUENCE</u>]

SP GQGTQSENSC THFPGNLPNM LRDLRDAFSR VKTFFQMKDQ LDNLLLKESL LEDFKGYLGC QALSEMIQFY LEEVMPQAEN QDPDIKAHVN SLGENLKTLR LRLRRCHRFL PCENKSKAVE QVKNAFNKLQ EKGIYKAMSE FDIFINYIEA YMTMKIRN

[ACTIVITY]

Mechanism: Interleukin 10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. IL-10 was initially reported to suppress cytokine secretion, antigen presentation and CD4⁺ T cell activation. Further investigation has shown that IL-10 predominantly inhibits lipopolysaccharide (LPS) mediated induction of the pro-inflammatory cytokines TNF α , IL-1 β , IL-12, and IFN γ made by cells such as macrophages and Th1 T cells. Therefore, THP1 cells were cultured in 24 well plates at a concentration of 10⁶ cells/mL and activated by LPS (1ug/mL) in the absence and presence of IL-10 (5ng/mL, 10ng/mL) for 24h and production of IL-1 β was determined in the supernatants by cytokine specific ELISA.

Result 1: Activation of THP1 cells with LPS (1ug/mL) resulted in production of high levels of IL-1 β and strong inhibitory effects of IL-10 (5ng/mL, 10ng/mL) were observed on the production of IL-1 β as shown in Table 1.

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Sample (cell supernatant of THP1 cells)	O.D.value	Corrected	Concentration of IL-1β (ng/mL)
Stimulated with LPS (1ug/mL) and	0.160	0.000	<2.343
IL-10 (5ng/mL)			
Stimulated with LPS (1ug/mL) and	0.150	-0.010	<2.343
IL-10 (10ng/mL)			
Stimulated with LPS (1ug/mL)	0.676	0.516	19.9
Unstimulated	0.151	-0.009	<2.343

Table 1. Effects of IL-10 on IL-1 β production by THP1 cells

[IDENTIFICATION]



Figure 1. SDS-PAGE

Sample: Active recombinant IL10, Human



Sample: Recombinant IL10, Human;

Antibody: Rabbit Anti-Human IL10 Ab (PAA056Hu06)