Coud-Clone Corp.

APC008Mu01 100µg Active Interleukin 35 (IL35) Organism Species: *Mus musculus (Mouse) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Arg23~Ala215 Tags: N-terminal His-tag Purity: >90% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 200µg/mL Applications: Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.2 Predicted Molecular Mass: 25.4kDa Accurate Molecular Mass: 25kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) 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.

Cloud-Clone Corp.

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.

[SEQUENCE]

RVIPVSGP ARCLSQSRNL LKTTDDMVKT AREKLKHYSC TAEDIDHEDI TRDQTSTLKT CLPLELHKNE SCLATRETSS TTRGSCLPPQ KTSLMMTLCL GSIYEDLKMY QTEFQAINAA LQNHNHQQII LDKGMLVAID ELMQSLNHNG ETLRQKPPVG EADPYRVKMK LCILLHAFST RVVTINRVMG YLSSA

[ACTIVITY]

IL-35, also known as Interleukin-35, is a heterodimeric cytokine composed of two subunits, p35 and Epstein-Barr virus-induced gene 3 (EBI3). It belongs to the IL-12 family of cytokines and is primarily associated with the regulation of immune responses. IL-35 is produced by regulatory T cells (Tregs) and has been implicated in the suppression of immune responses, playing a role in maintaining immune tolerance and preventing autoimmune diseases. It is also produced by B regulatory cells and can contribute to the suppression of immune reactions in certain contexts.Besides,Interleukin 23 Subunit Alpha (IL23a) has been identified as an interactor of IL-35, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse IL-35 and recombinant human IL23a. Briefly, IL-35 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ I were then transferred to IL23a-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-IL-35 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 $^\circ$ C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^\circ$ C. Finally, add 50 μ L stop solution to the wells and read at 450/630nm

Cloud-Clone Corp.

immediately. The binding activity of recombinant mouse IL-35 and recombinant human IL23a was shown in Figure 1, the EC50 for this effect is 0.049ug/mL.

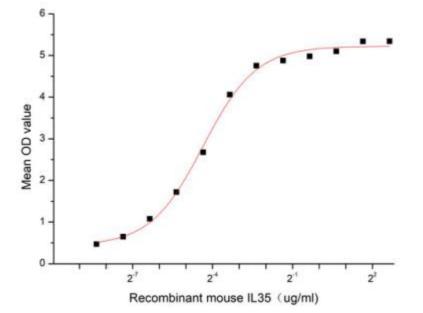


Figure 1. The binding activity of recombinant mouse IL-35 and recombinant human

IL23a

[IDENTIFICATION]





Cloud-Clone Corp.

	kDa 70
	44
	33
-	26
	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant IL35, Mouse

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.