

**APC008Hu01 100µg**

**Active Interleukin 35 (IL35)**

**Organism Species: Homo sapiens (Human)**

***Instruction manual***

FOR IN VITRO USE AND RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1th Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** The IL35 complex composed of IL27B subunit (Arg21~Lys229) and the mature form of human IL12A (Arg23~Ser219) linked by a polypeptide linker (GGGGS)<sub>3</sub>

**Tags:** N-terminal His-tag

**Purity:** >98%

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

**Applications:** Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

**Predicted isoelectric point:** 8.7

**Predicted Molecular Mass:** 51.7kDa

**Accurate Molecular Mass:** 55kDa as determined by SDS-PAGE reducing conditions.

**Phenomenon explanation:**

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

## [ USAGE ]

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.

**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.

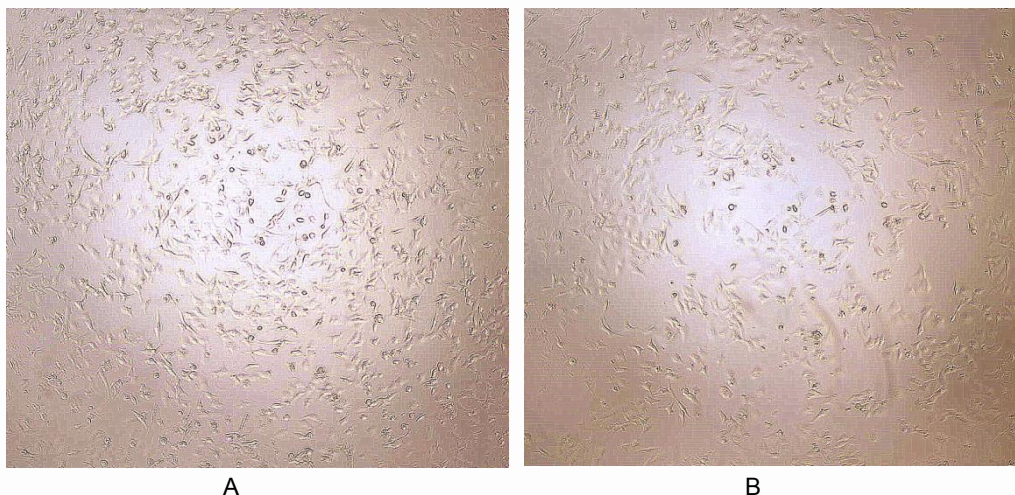
## [ SEQUENCE ]

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PPAPNSTSPV  SFIATYRLGM  AARGHSWPCL  QQTPTSTSCT  ITDVQLFSMA
PYVLNVTAVH  PWGSSSSFVP  FITEHIIKPD  PPEGVRLSPL  AERQLQVQWE
PPGSWPFPEI  FSLKYWIRYK  RQGAARFHRV  GPIEATSFIL  RAVRPRARYY
VQVAAQDLTD  YGELSDWSLP  ATATMSLGKG  GGGSRNLPVA  TPDPGMFPCL
HHSQNLLRAV  SNMLQKARQT  LEFYPCTSEE  IDHEDITKDK  TSTVEACLPL
ELTKNESCLN  SRETSFITNG  SCLASRKTSF  MMALCLSSII  EDLKMYQVEF
KTMNAKLLMD  PKRQIFLDQN  MLAVIDELMQ  ALNFNSETVP  QKSSLEEPDF
YKTKIKLCIL  LHAFRIRAVT  IDRVMSYLSA  S
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## [ ACTIVITY ]

IL35 (Interleukin 35) is an IL-12 family cytokine, which is a dimeric protein composed of IL-12 $\alpha$  and IL-27 $\beta$  chains. IL35 is thought to mediate the immune inhibitory function of regulatory T cells and has been proven to promotes pancreas cancer growth through enhancement of proliferation and inhibition of apoptosis. Thus, proliferation assay of IL35 was conducted using PANC-1 cells. Briefly,

PANC-1 cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of recombinant human IL35. After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 $\mu$ L of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37°C. Proliferation of PANC-1 cells after incubation with IIL35 for 48h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8 ) assay after incubation with human recombinant IL35 for 48h. The result was shown in Figure 2. It was obvious that IL35 significantly increased cell viability of PANC-1 cells.



**Figure 1. Cell proliferation of PANC-1 cells after stimulated with IL35.**

**(A) PANC-1 cells cultured in DMEM, stimulated with 1000ng/mL IL35 for 48h;**

**(B) Unstimulated PANC-1 cells cultured in DMEM for 48h.**

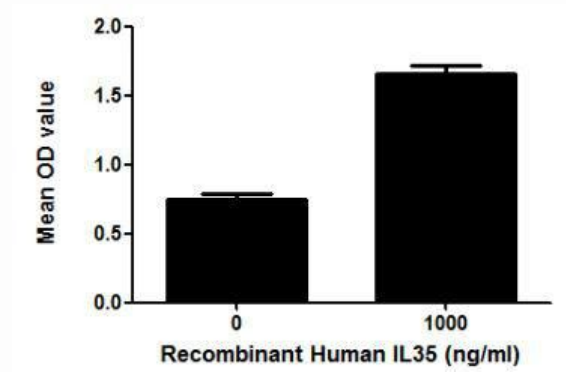


Figure 2. Cell proliferation of PANC-1 cells after stimulated with IL35.

[ IDENTIFICATION ]

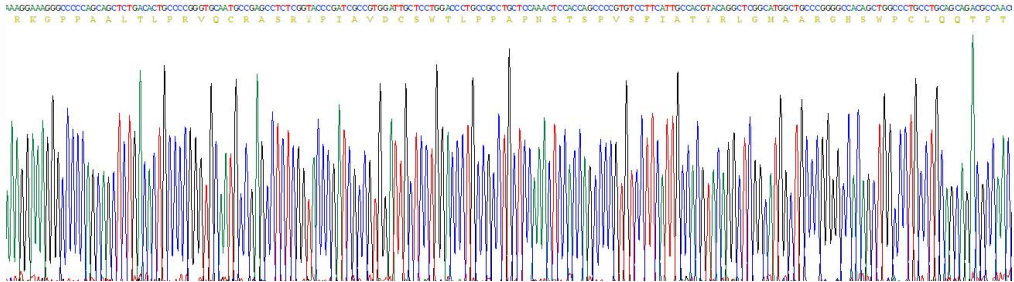


Figure 3. Gene Sequencing (extract)

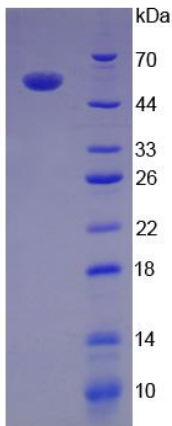
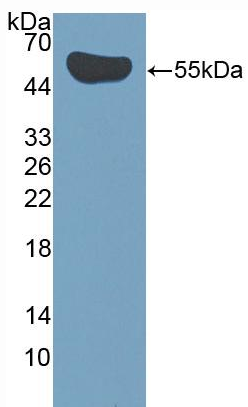


Figure 4. SDS-PAGE

Sample: Active recombinant IL35, Human



**Figure 5. Western Blot**

**Sample: Recombinant IL35, Human;**

**Antibody: Rabbit Anti-Human IL35 Ab (PAC008Hu01)**