APC008Hu01 200μg
Active Interleukin 35 (IL35)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st 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)3
Tags: N-terminal His-tag
Purity: >98%
Buffer Formulation: 10mM PBS, pH7.4, containing 5% trehalose, 0.01% sarcosyl.
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 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 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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.

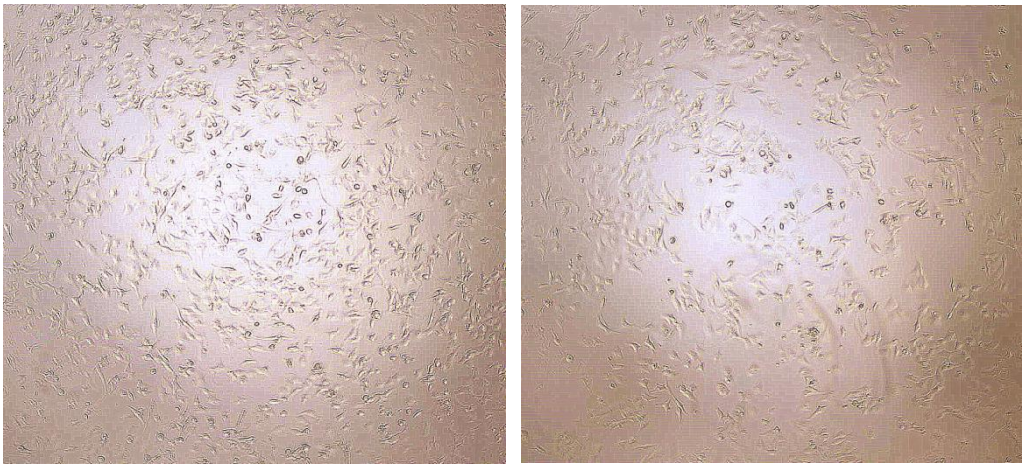
[SEQUENCE]

RKGPPAALTL PRVQCRASRY PIAVDCSWTL
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 MMALCLSSIY EDLKMYQVEF
KTMNAKLLMD PKRQIFLDQN MLAVIDELMQ ALNFNSETVP QKSSLEEPDF
YKTKIKLCIL LHAFRIRAVT IDRVMSYLNA S

[ACTIVITY]

IL35 (Interleukin 35) is an IL-12 family cytokine, which is a dimeric protein composed of IL-12α and IL-27β 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, 10μL 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.



A B

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.

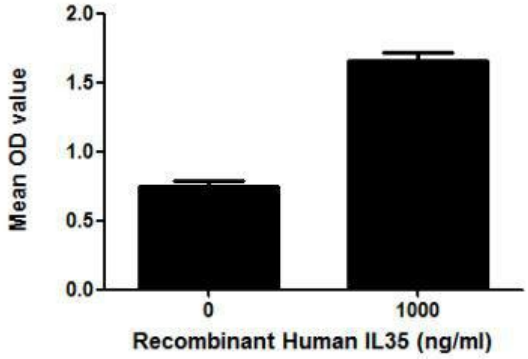


Figure 2. Cell proliferation of PANC-1 cells after stimulated with IL35.

[IDENTIFICATION]

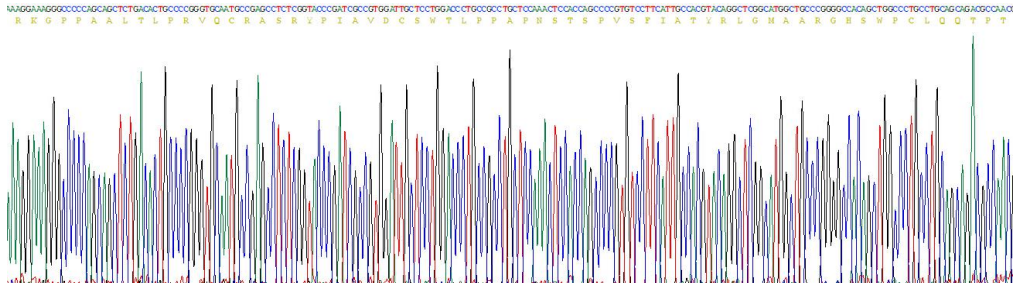


Figure 3. Gene Sequencing (extract)

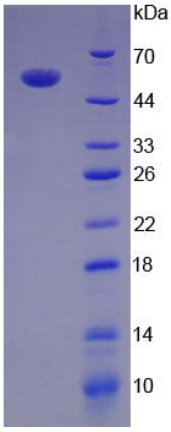


Figure 4. SDS-PAGE

Sample: Active recombinant IL35, Human

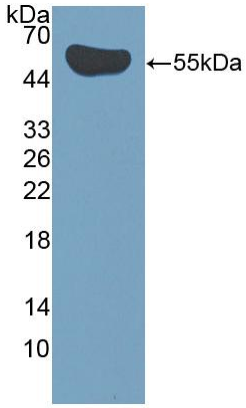


Figure 5. Western Blot

Sample: Recombinant IL35, Human;

Antibody: Rabbit Anti-Human IL35 Ab (PAC008Hu01)

[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.