

**APA111Hu62 100µg**

**Active Interleukin 12 (IL12)**

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

***Instruction manual***

**FOR RESEARCH USE ONLY**

**NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES**

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13th Edition (Revised in Aug, 2023)

## **[ PROPERTIES ]**

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** Arg23~Ser219+GGGGS\*3+Ile23~Ser328

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

**Purity:** >95%

**Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method).

**Buffer Formulation:** PBS, pH7.4, containing 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:** 5.7

**Predicted Molecular Mass:** 59.8kDa

**Accurate Molecular Mass:** 72kDa 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 2-8°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.

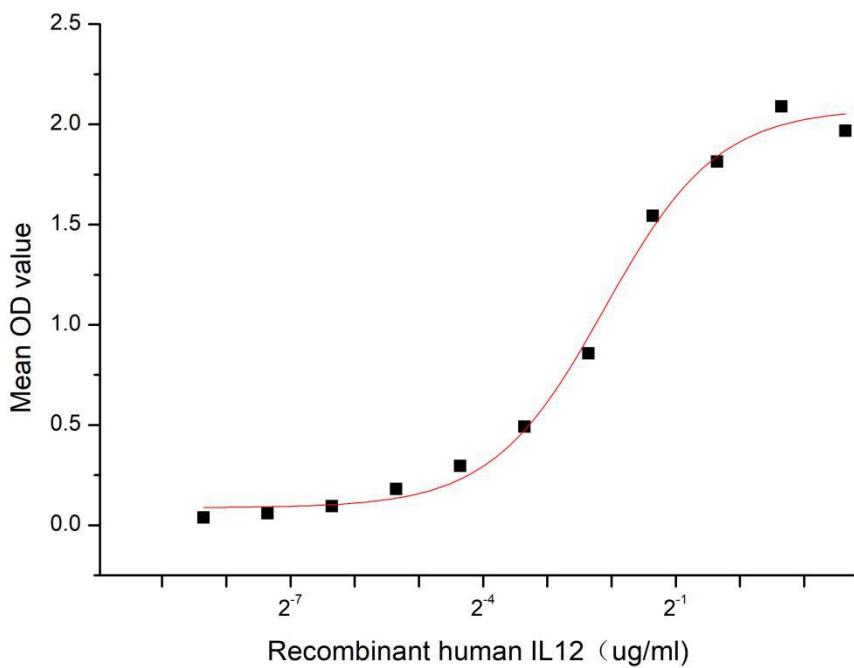
## **[ SEQUENCE ]**

RNLPVATPDPMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITDKTSTVEACL  
PLELTKNESCLNSRETTSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKR  
QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYTKIKLCILLHAFRIRAVTIDRVMWSYLN  
AS  
GGGGSGGGGGGGGG  
IWEKKDVYVVELDWYPDAPGEMVVLCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDA  
GQYTCHKGGEVLSHSSLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSRFTCWWLTTIST  
DLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYVECQEDSACPAAEESLPIEVMVDA  
VHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQG  
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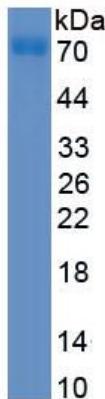
## **[ ACTIVITY ]**

Interleukin 12 (IL12) is a heterodimeric cytokine composed of p35 (IL12A) and p40 (IL12B) subunits, primarily produced by dendritic cells, macrophages, and B cells. It plays a critical role in bridging innate and adaptive immunity by promoting Th1 differentiation, enhancing IFN- $\gamma$  production, and stimulating NK and T cell cytotoxicity. IL12 is pivotal in anti-tumor and anti-infectious responses but dysregulation can contribute to autoimmunity. IL12 binds IL12R $\beta$ 1 (a subunit of IL12 receptor) with high affinity, initiating JAK-STAT signaling. Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human IL12 and recombinant mouse IL12R $\beta$ 1. Briefly, biotin-linked IL12 were

diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$  l were then transferred to IL12Rb1-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50 $\mu$ l stop solution to the wells and read at 450nm immediately. The binding activity of IL12 and IL12Rb1 was shown in Figure 1, the EC50 for this effect is 0.23ug/mL.



**Figure 1. The binding activity of recombinant human IL12 and recombinant mouse IL12Rb1**

**[ IDENTIFICATION ]**

**Figure 2. SDS-PAGE**

**Sample: Active recombinant IL12, Human**

**[ 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.