APA223Hu61 100µg Active Interleukin 1 Receptor Antagonist (IL1RA) Organism Species: *Homo sapiens (Human) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: Arg26~Glu177 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 10mM PBS, pH7.6, containing 5% trehalose. Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 5.4 Predicted Molecular Mass: 18.7kDa Accurate Molecular Mass: 22/25/27kDa 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.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.6) 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.

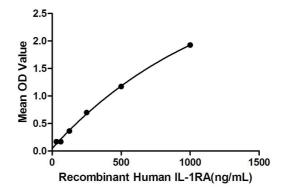
[<u>SEQUENCE</u>]

RPSGR KSSKMQAFRI WDVNQKTFYL RNNQLVAGYL QGPNVNLEEK IDVVPIEPHA LFLGIHGGKM CLSCVKSGDE TRLQLEAVNI TDLSENRKQD KRFAFIRSDS GPTTSFESAA CPGWFLCTAM EADQPVSLTN MPDEGVMVTK FYFQEDE [ACTIVITY]

IL-1RA is an agent that binds non-productively to the cell surface interleukin-1 receptor (IL-1R), the same receptor that binds interleukin 1 (IL-1), preventing IL-1 from sending a signal to that cell. Besides, Interleukin 1 Alpha (IL-1a) has been identified as an interactor of IL-1RA, thus a binding ELISA assay was conducted to detect the interaction of recombinant human IL-1RA and recombinant human IL-1a. Briefly, IL1RA were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µL were then transferred to IL-1a-coated microtiter wells and incubated for 2 h at 37 °C. Wells were washed with PBST and incubated for 1 h with anti-IL-1RA pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With

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the addition of substrate solution, wells were incubated 15-25 minutes at 37° C. Finally, add 50μ L stop solution to the wells and read at 450nm immediately. The binding activity of IL-1RA and IL-1a was shown in Figure 1, and this effect was in a dose dependent manner.





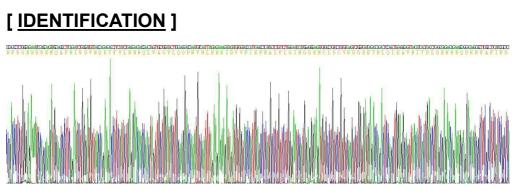


Figure 2. Gene Sequencing (extract)

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kDa 70
44
33
26
22
18
14

Figure 3. SDS-PAGE

Sample: Active recombinant IL1RA, Human

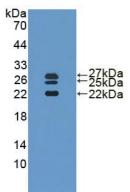


Figure 4. Western Blot

Sample: Recombinant IL1RA, Human;

Antibody: Rabbit Anti-Human IL1RA Ab (PAA223Hu06)

[<u>IMPORTANT NOTE</u>]

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