#### APA223Ra01 100µg Active Interleukin 1 Receptor Antagonist (IL1RA) Organism Species: *Rattus norvegicus (Rat) 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: His27~Gln178 Tags: N-terminal His-tag Purity: >92% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 6.7 Predicted Molecular Mass: 21.1kDa Accurate Molecular Mass: 21kDa 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.

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**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.

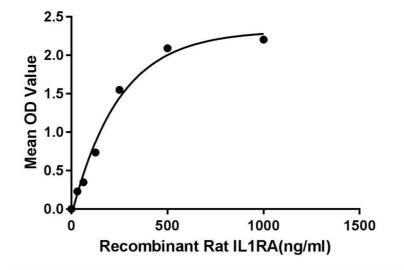
### [SEQUENCE]

HPAGKRPCKMQAFRIWDTNQKTFYLRNNQLIAGYLQGPNTKLEEKIDMVPIDFRNVFLGIHGGKLCLSCVKSGDDTKLQLEEVNITDL NKNKEEDKRFTFIRSETGPTTSFESLACPGWFLCTTLEADHPVSLTNTPKEPCTVTKFYFQEDQ

## [ACTIVITY]

The interleukin-1 receptor antagonist (IL-1RA) is a member of the interleukin 1 cytokine family. IL1Ra is secreted by various types of cells including immune cells, epithelial cells, and adipocytes, and is a natural inhibitor of the pro-inflammatory effect of IL1 $\beta$ . IL-1RN is an agent that binds to the cell surface interleukin-1 receptor (IL-1R), which would prevent IL-1 from intracellular signal transduction. Thus, a binding ELISA assay was conducted to detect the interaction of recombinant rat IL-1RN and recombinant rat IL-1R1. Briefly, IL-1RA were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µl were then transferred to IL-1R1-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-IL-1RN pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were incubated 15-25 minutes at 37 °C. Finally, add 50µl stop solution to the wells and read at 450nm immediately. The binding activity of IL-1RA and IL-1R1 was shown in Figure 1, and this effect was in a dose dependent manner.

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### [IDENTIFICATION]

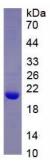


Figure 2. SDS-PAGE

Sample: Active recombinant IL1RA, Rat

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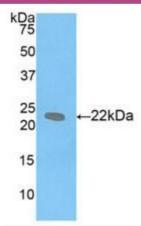


Figure 3. Western Blot

Sample: Recombinant IL1RA, Rat;

Antibody: Rabbit Anti-Rat IL1RA Ab (PAA223Ra01)

# [<u>IMPORTANT NOTE</u>]

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.