APE913Ra01 50µg Active Sirtuin 3 (SIRT3) 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: Pro127~Glu303 Tags: N-terminal His-tag **Purity: >95%** Buffer Formulation: 10mM PBS, pH7.4, containing 1mM DTT and 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: 6.2 Predicted Molecular Mass: 20.8kDa Accurate Molecular Mass: 22kDa as determined by SDS-PAGE reducing conditions.

### [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.

#### [<u>SEQUENCE</u>]

PNVA HYFLRLLHDK ELLLRLYTQN IDGLERASGI PASKLVEAHG SFVSATCTVC RRSFPGEDIR ADVMADRVPR CPVCTGVVKP DIVFFGEQLP ARFLLHVADF ALADLLLILG TSLEVEPFAS LSESVQKSVP RLLINRDLVG SFALSPRRKD VVQLGDVVQG VERLVDLLGW TQE

### [ACTIVITY]

Sirtuin 3 (SIRT3), the NAD-dependent deacetylaseis is member of the mammalian sirtuin family of proteins. In human, sirtuins have a range of molecular functions and have emerged as important proteins in aging, stress resistance and metabolic regulation. It also can regulate epigenetic gene silencing and suppress recombination of rDNA in yeast. SIRT3 expression in white and brown adipose tissue. Besides, Isocitrate Dehydrogenase 2, mitochondrial (IDH2) has been identified as an interactor of SIRT3, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat SIRT3 and recombinant rat IDH2. Briefly, SIRT3 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to IDH2-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-SIRT3 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50 µL stop solution to the wells and read at 450nm immediately. The binding activity of SIRT3 and IDH2 was shown in Figure 1, and this effect was in a dose dependent manner.

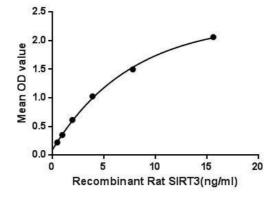


Figure 1. The binding activity of SIRT3 with IDH2.

#### [IDENTIFICATION]

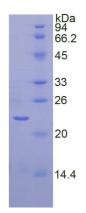


Figure 2. SDS-PAGE

Sample: Active recombinant SIRT3, Rat

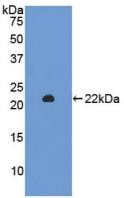


Figure 3. Western Blot Sample: Recombinant SIRT3, Rat; Antibody: Rabbit Anti-Rat SIRT3 Ab (PAE913Ra01)

## [<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.