Coud-Clone Corp.

APE913Hu01 50µg Active Sirtuin 3 (SIRT3) 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: Gln126~Lys399 Tags: N-terminal His-tag Purity: >92%

**Buffer Formulation:** 100mM NaHCO<sub>3</sub>, 500mM NaCl, pH8.3, containing 0.01% sarcosyl, 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: 6.0

Predicted Molecular Mass: 31.7kDa

Accurate Molecular Mass: 32kDa as determined by SDS-PAGE reducing conditions.

### [ <u>USAGE</u> ]

Reconstitute in 100mM NaHCO<sub>3</sub>, 500mM NaCl (pH8.3) 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.

### Cloud-Clone Corp.

**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]

QDVAE LIRARACQRV VVMVGAGIST PSGIPDFRSP GSGLYSNLQQ YDLPYPEAIF ELPFFFNPK PFFTLAKELY PGNYKPNVTH YFLRLLHDKG LLLRLYTQNI DGLERVSGIP ASKLVEAHGT FASATCTVCQ RPFPGEDIRA DVMADRVPRC PVCTGVVKPD IVFFGEPLPQ RFLLHVVDFP MADLLLILGT SLEVEPFASL TEAVRSSVPR LLINRDLVGP LAWHPRSRDV AQLGDVVHGV ESLVELLGWT EEMRDLVQRE TGKLDGPDK [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 humant SIRT3 and recombinant human 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 50µL 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.

# Cloud-Clone Corp.



Figure 1. The binding activity of SIRT3 with IDH2.



Figure 2. SDS-PAGE Sample: Active recombinant SIRT3, Human

## Cloud-Clone Corp.



Figure 3. Western Blot Sample: Recombinant SIRT3, Human; Antibody: Rabbit Anti-Human SIRT3 Ab (PAE913Hu01)

### [IMPORTANT NOTE]

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.