APC300Hu01 100µg Active Active Mesencephalic Astrocyte Derived Neurotrophic Factor (MANF) 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: Gly28~Leu182 Tags: N-terminal His-tag Purity: >92% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl 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: 8.5 Predicted Molecular Mass: 21.5kDa Accurate Molecular Mass: 22kDa as determined by SDS-PAGE reducing conditions.

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

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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]

GDC EVCISYLGRF YQDLKDRDVT FSPATIENEL IKFCREARGK ENRLCYYIGA TDDAATKIIN EVSKPLAHHI PVEKICEKLK KKDSQICELK YDKQIDLSTV DLKKLRVKEL KKILDDWGET CKGCAEKSDY IRKINELMPK YAPKAASART DL

#### [ACTIVITY]

MANF (Mesencephalic astrocyte-derived neurotrophic factor) is a prosurvival protein that protects the cells when applied intracellularly in vitro or extracellularly in vivo. Recently, MANF has also been proven to inhibit cell proliferation and ER stress-induced cell death and also affects cell size and morphology under certain conditions. Thus the bioactivity of human recombinant MANF were detected using U87-MG. Briefly, U87-MG cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of MANF. After incubated for 48h, cells were observed by inverted microscope and were shown in Figure 1. It was obvious that MANF inhibited cell proliferation of U87-MG and affected cell size and morphology.

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Figure 1. Inhibition of U87-MG cell proliferation after stimulated with MANF.

- (A) U7-MG cells cultured in DMEM, stimulated with 0.01ng/mL MANF for 48h;
- (B) Unstimulated U87-MG cells cultured in DMEM for 48h.

#### [IDENTIFICATION]



Figure 2. Gene Sequencing (extract)

| and the second second | kDa |
|-----------------------|-----|
| -                     | 70  |
| -                     | 44  |
| -                     | 33  |
| -                     | 26  |
|                       | 22  |
| -                     | 18  |
| -                     | 14  |
| -                     | 10  |
|                       |     |

Figure 3. SDS-PAGE Sample: Active recombinant MANF, Human

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Figure 4. Western Blot

Sample: Recombinant MANF, Human;

Antibody: Rabbit Anti-Human MANF Ab (PAC300Hu01)

### [<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.