APB353Ge01 10μg
Active Advanced Glycation End Product (AGE)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Glycosylation of extracted BSA, catalyzed by $CuSO_4$

Host: Bovine

Purity: >90% as determined by SDS-PAGE.

Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).

Purification Methods: Salt co-precipitation and ionic-Exchange chromatography.

Traits: Freeze-dried powder

Buffer Formulation: PBS, pH7.4, containing 5%Trehalose.

Original Concentration: 2000μg/mL

Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)

[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.

[ACTIVITY]

Glucose and other reducing sugars can react non-enzymatically with the amino groups of proteins to form compounds called advanced glycation end products (AGEs). AGEs exert their cellular functions via the interaction with receptor for advanced glycation end products (RAGE). It has been reported that AGE stimulates the differentiation and proliferation of 3T3, thus a proliferation assay was conducted using 3T3 cells. Briefly, 3T3 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 AGE. After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10μL of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37°C. Proliferation of 3T3 cells after incubation with AGE for 48h observed by inverted microscope was shown in Figure1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant AGE for 48h. The result was shown in Figure 2. It was obvious that AGE significantly increased cell viability of 3T3 cells.

A B

Figure 1. Cell proliferation of 3T3 cells after stimulated with AGE.

(A) 3T3 cells cultured in DMEM, stimulated with 1ng/mL AGE for 48h;

(B) Unstimulated 3T3 cells cultured in DMEM for 48h.

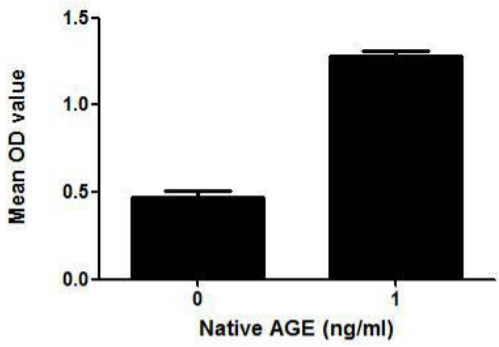

Figure 2. Cell proliferation of 3T3 cells after stimulated with AGE.

[IMPORTANT NOTE]

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.