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APE787Ra01 100μg Active Annexin A1 (ANXA1) 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: Met1~Asn346 Tags: N-terminal His-tag Purity: >98% 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: 6.3 Predicted Molecular Mass: 45.5kDa Accurate Molecular Mass: 45kDa 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]

MAMVSEFLKQ ACYIEKQEQE YVQAVKSYKG GPGSAVSPYP SFNPSSDVAA LHKAIMVKGV DEATIIDILT KRTNAQRQQI KAAYLQETGK PLDETLKKAL TGHLEEVVLA MLKTPAQFDA DELRAAMKGL GTDEDTLIEI LTTRSNQQIR EITRVYREEL KRDLAKDITS DTSGDFRNAL LALAKGDRCE DMSVNQDLAD TDARALYEAG ERRKGTDVNV FNTILTTRSY PHLRKVFQNY RKYSQHDMNK ALDLELKGDI EKCLTTIVKC ATSTPAFFAE KLYEAMKGAG TRHKTLIRIM VSRSEIDMNE IKVFYQKKYG IPLCQAILDE TKGDYEKILV ALCGGN

[ACTIVITY]

Annexin A1 (ANXA1), also known as lipocortin I, belongs to the annexin family of Ca^{2+} -dependent phospholipid-binding proteins that have a molecular weight of approximately 35,000 to 40,000 and are preferentially located on the cytosolic face of the plasma membrane. Annexin A1 protein has an apparent relative molecular mass of 40kDa with phospholipase A2 inhibitory activity. To test the effect of ANXA1 on cell apoptosis, A549 cells were seeded into 96-well plates at a density of 3,000 cells/well with 1% serum standard DMEM including various concentrations of recombinant rat ANXA1. 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 2h at 37°C. Proliferation of A549 cells after incubation with ANXA1 for 48h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 assay after incubation

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with recombinant rat ANXA1 for 48h. The result was shown in Figure 2. It was obvious that ANXA1 significantly inhibit cell viability of A549 cells. The ED50 is 1.75µg/mL.

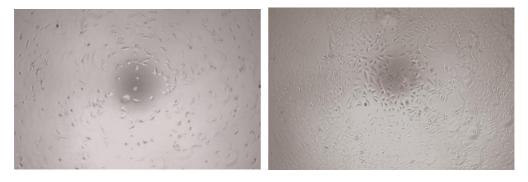


Figure 1. Inhibition of A549 cells proliferation after stimulated with ANXA1 (A) A549 cells cultured in DMEM, stimulated with 2µg/mL ANXA1 for 48h; (B) Unstimulated A549 cells cultured in DMEM for 48h.

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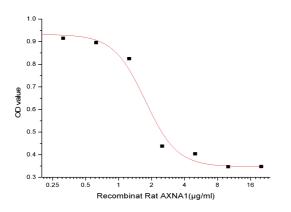


Figure 2. Inhibition of A549 cells proliferation after stimulated with ANXA1.

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[IDENTIFICATION]

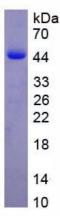


Figure 3. SDS-PAGE

Sample: Active recombinant rat, ANXA1

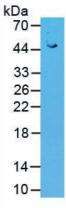


Figure 4. Western Blot

Sample: Recombinant rat, ANXA1;

Antibody: Rabbit Anti-ANXA1 rat Ab (PAE787Ra01)

[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.