APE787Mu01 10μg Active Annexin A1 (ANXA1) Organism Species: *Mus musculus (Mouse) 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: Glu6~Asn346

Tags: N-terminal His Tag

Purity: >92%

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

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% skl and 5% trehalose.

Original Concentration: 100µg/mL

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: 44.9kDa

Accurate Molecular Mass: 40kDa as determined by SDS-PAGE reducing conditions. **Phenomenon explanation:**

The possible reasons that the actual band size differs from the predicted are as follows:

- 1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
- 2. Relative charge: The composition of amino acids may affects the charge of the protein.
- 3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
- 4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
- 5. Polymerization of the target protein: Dimerization, multimerization etc.

[<u>USAGE</u>]

Reconstitute in ddH_2O to a concentration of 0-0.1mg/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.

[SEQUENCE]

EFLKQ ARFLENQEQE YVQAVKSYKG GPGSAVSPYP SFNVSSDVAA LHKAIMVKGV DEATIIDILT KRTNAQRQQI KAAYLQENGK PLDEVLRKAL TGHLEEVVLA MLKTPAQFDA DELRGAMKGL GTDEDTLIEI LTTRSNEQIR EINRVYREEL KRDLAKDITS DTSGDFRKAL LALAKGDRCQ DLSVNQDLAD TDARALYEAG ERRKGTDVNV FTTILTSRSF PHLRRVFQNY GKYSQHDMNK ALDLELKGDI EKCLTTIVKC ATSTPAFFAE KLYEAMKGAG TRHKALIRIM VSRSEIDMNE IKVFYQKKYG ISLCQAILDE TKGDYEKILV ALCGGN

[ACTIVITY]

Annexin A1 (ANXA1), also known as lipocortin I, belongs to the annexin family of Ca²⁺-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. Besides, S100 Calcium Binding Protein A11 (S100A11) has been identified as an interactor of ANXA1, thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse ANXA1 and recombinant mouse S100A11. Briefly, ANXA1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate

samples of 100µL were then transferred to S100A11-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-ANXA1 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 ANXA1 and S100A11 was shown in Figure 1, and this effect was in a dose dependent manner.

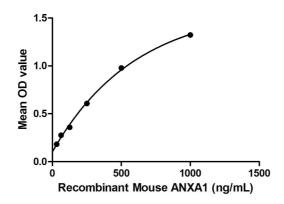
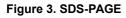


Figure 1. The binding activity of ANXA1 with S100A11.

Figure 2. Gene Sequencing (extract)

kDa 70	-
44	
33	-
26	-
22	-
18	-
14	-
10	-



Sample: Active recombinant ANXA1, Mouse

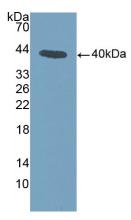


Figure 4. Western Blot

Sample: Recombinant ANXA1, Mouse;

Antibody: Rabbit Anti-Mouse ANXA1 Ab (PAE787Mu01)

[<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.