APA259Ra01 50μg
Active Annexin A5 (ANXA5)
Organism Species: *Rattus norvegicus (Rat)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Met1~Asp319
Tags: N-terminal His Tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.2
Predicted Molecular Mass: 37.0kDa
Accurate Molecular Mass: 35kDa as determined by SDS-PAGE reducing conditions.

[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 2-8ºC for one month.
Aliquot and store at -80ºC 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 37ºC 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]

MALRGTVTDF SGFDGRADAE VLRKAMKGLG TDEDSILNLL TARSNAQRQQ
IAEEFKTLFG RDLVNDMKSE LTGKFEKLIV ALMKPSRLYD AYELKHALKG
AGTDEKVLTE IIASRTPEEL RAIKQAYEEE YGSNLEDDVV GDTSGYYQRM
LVVLLQANRD PDTAIDDAQV ELDAQALFQA GELKWGTDEE KFITILGTRS
VSHLRRVFDK YMTISGFQIE ETIDRETSGN LENLLLAVVK SIRSIPAYLA
ETLYYAMKGA GTDDHTLIRV IVSRSEIDLF NIRKEFRKNF ATSLYSMIKG
DTSGDYKKAL LLLCGGEDD

[ACTIVITY]

Annexin V (ANXA5) is a multifunctional protein that is highly expressed on the apical surfaces of syncytiotrophoblasts, and plays an important role in haemostatic regulations, maintaining blood fluidity of the placenta. Lower ANXA5 levels have been observed in M2/ANXA5 haplotype carrying chorion. The association found between the maternal carriage of the M2/ANXA5 haplotype and an elevated risk of IUGR and/or PE supports the hypothesis that carrier status of this haplotype and the consequently reduced placental ANXA5 expression might be responsible, at least partially, for the onset of these gestational vascular complications. ANXA5 could be used as a biomarker for the early detection of PE and for the prediction of its severity. ANXA5 as an embryonic anticoagulant that appears deficient in contiguous specter of thrombophilia-related pregnancy complications culminating more frequently in miscarriage in a maternal M2 carrier background. Besides, Gamma Actin (ACTG) has been identified as an interactor of ANXA5, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat ANXA5 and recombinant human ACTG.

Briefly, ANXA5 were diluted serially in PBS, with 0.01% BSA (pH7.4). Duplicate samples of 100 μl were then transferred to ACTG-coated microtiter wells and incubated for 1h at 37℃. Wells were washed with PBST and incubated for 1h with anti-ANXA5 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μL stop solution to the wells and read at 450 nm immediately. The binding activity of ANXA5 and ACTG was shown in Figure 1, and this effect was in a dose dependent manner, the EC50 was 0.78 ug/ml.

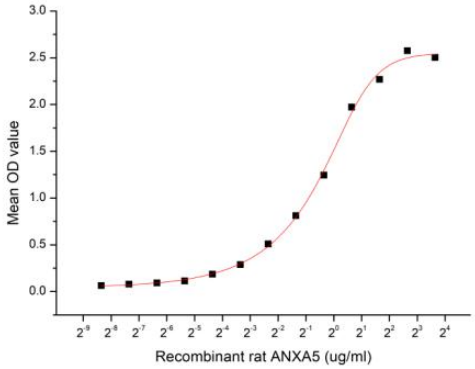

Figure 1. The binding activity of recombinant rat ANXA5 and recombinant human ACTG

Annexin V is a calcium-dependent phospholipid binding protein that can be used to bind Phosphatidylserine (PS) during an early apoptosis event where the PS becomes exposed at the cell surface. Jurkat cells were treated with 10 uM camptothecin for 4h, 2*105 cells which were resuspended in binding buffer were stained with 5 ug recombinant rat Annexin V-FITC and 10 ul Propidium iodide (PI) for 20min in dark room temperature. The flow cytometry was used to detect the early apoptotic and late apoptotic of camptothecin-treated Jurkat cells (Figure 2), the combination of Annexin V-FITC and propidium iodide allows for the distinction between early apoptotic cells (Annexin V-FITC positive and propidium iodide negative), late apoptotic and/or necrotic cells (Annexin V-FITC and propidium iodide positive), and viable cells (unstained). Thus, the recombinant rat Annexin V-FITC can bind Phosphatidylserine (PS) at early apoptosis of Jurkat.

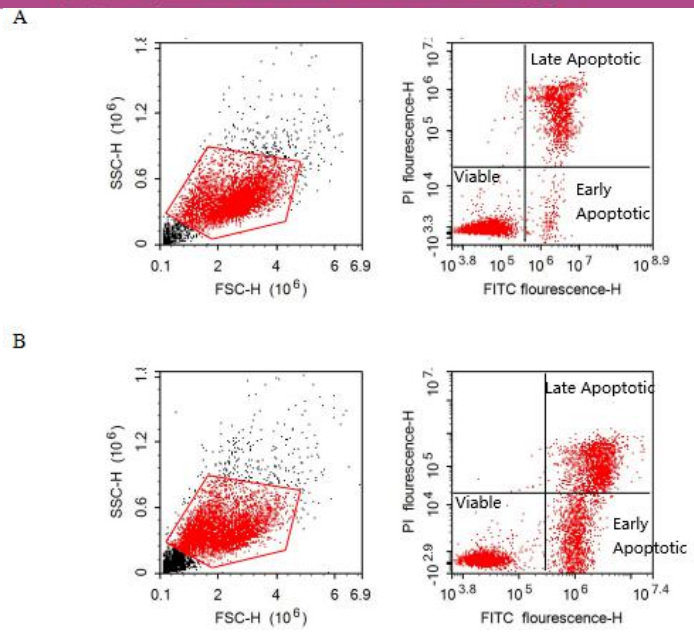

Figure 2. Flow cytometry of apoptotic Camptothecin-treated Jurkat by annexin V staining

(A) Jurkat were untreated with 10 uM camptothecin

(B) Jurkat were treated with 10 uM camptothecin

[IDENTIFICATION]

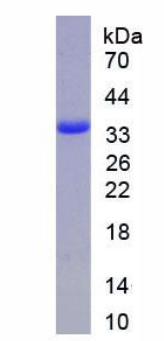

Figure 3. SDS-PAGE

Sample: Active recombinant ANXA5, Rat

[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.