

**APC321Mu01 1mg**  
**Active Asporin (ASP)**  
**Organism Species: *Mus musculus (Mouse)***  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1th Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Lys16~Lys373

**Tags:** N-terminal His Tag

**Purity:** >90%

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

**Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

**Original Concentration:** 1200µg/mL

**Applications:** Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

**Predicted isoelectric point:** 8.8

**Predicted Molecular Mass:** 44.6kDa

**Accurate Molecular Mass:** 45kDa 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 2-8°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 ]**

```
      KPFFS PSHTALKNMM LKDMEDTDDD DNDDDDNSLF
PTKEPVNPF PFDLFPTCPF GCQCYSRVVH CSDLGLTSVP NNIPFDTRMV
DLQNNKIKEI KENDFKGLTS LYALILNNNK LTKIHPKTF L TTKKLRRRLYL
SHNQLSEIPL NLPKSLAELR IHDNKVKKIQ KDTFKGMNAL HVLEMSANPL
ENNGIEPGAF EGVTVFHIRI AEAKLTSIPK GLPPTLLELH LDFNKISTVE
LEDLKRYREL QRLGLGNRI TDIENGT FAN IPRVREIHLE HNKLKKIPSG
LQELKYLQII FLHYNSIAKV GVNDFCPTVP KMKKSLYSAI SLFNNPMKYW
EIQPATFRCV LGRMSVQLGN VGK
```

## **[ ACTIVITY ]**

Asporin (ASPIN) is a protein belongs to a family of leucine-rich repeat (LRR) proteins implicated in cancer, osteoarthritis, and periodontal ligament mineralization. The protein negatively regulates chondrogenesis in the articular cartilage and periodontal ligament (PDL) differentiation, inhibits BMP2-induced cytodifferentiation of PDL cells and also inhibits the interaction between TGFB1 and TGF-beta receptor type II in the presence of heparin/heparan sulfate in vitro. In addition Lumican (LUM) and ASPIN can contribute to the fibrinogenesis of cardiac remodeling of ICM, thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse ASPIN and recombinant mouse LUM. Briefly, ASPIN were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$ l were then transferred to LUM-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-ASPIN 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 50  $\mu$ L stop solution to the wells and read at 450 nm immediately. The binding activity of recombinant mouse ASPN and recombinant mouse LUM was shown in Figure 1, the EC50 for this effect is 2.6  $\mu$ g/mL.

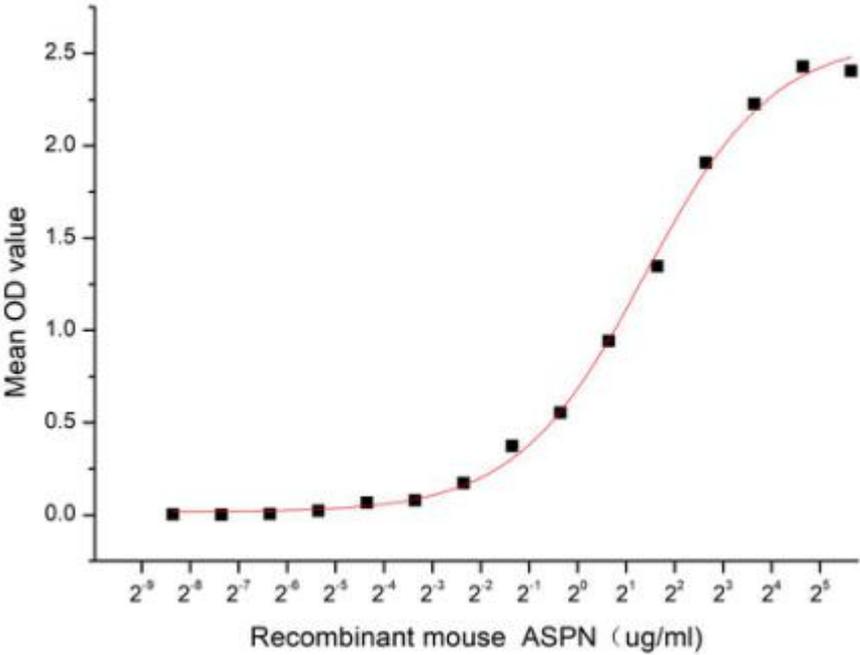


Figure 1. The binding activity of recombinant mouse ASPN and recombinant mouse LUM

[ IDENTIFICATION ]

ATG G C ACG G TG G TGGCT GG G TATG AGGG A GGGGG TCGTGGATATCGGGGATCCAAACCTTCTTTAGGOCCTTCCGACAGCACTGAMGAAATAT GATGTTGAGGGATATGGAGACACAGATGATGACGATAAACGATGATGACGACAACTCTCTTTTTCACGAAAGAGCCAGTGAACT

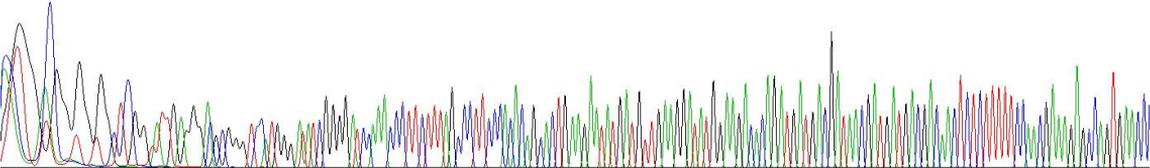
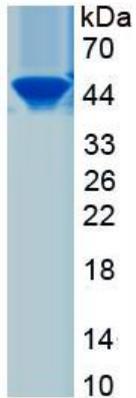


Figure2. Gene Sequencing (Extract)



**Figure 3. SDS-PAGE**

**Sample: Active recombinant ASPN, Mouse**

**[ 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.