

**APC321Mu01 100µg
Active Asporin (ASP)**

**Organism Species: *Mus musculus (Mouse)*
Instruction manual**

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Lys16~Lys373

Tags: Two N-terminal Tags, His-tag and GST-tag

Purity: >95%

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

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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: 8.8

Predicted Molecular Mass: 70.9kDa

Accurate Molecular Mass: 71kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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 LYALILNNK LTKIHPKTF LTKLRLRYL
SHNQLSEIPL NLPKSLAELR IHDNKVKKIQ KDTFKGMNAL HVLEMSANPL
ENNGIEPGAF EGVTVFHIRI AEAKLTSIPK GLPPTLLELH LDFNKISTVE
LEDLKRYREL QRLGLGNRI TDIENGTAN IPRVREIHLE HNKLKKIPSG
LQELKYLQII FLHYSIAKV GVNDFCPTVP KMKKSLYSAL SLFNNPMKYW
EIQPATFRCV LGRMSVQLGN VGK
```

[ACTIVITY]

Asporin (ASPN) is a protein belongs to a family of leucine-rich repeat (LRR) proteins associated with the cartilage matrix. 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. Besides, Peptidylprolyl Isomerase E (PPIE) has been identified as an interactor of ASPN, thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse ASPN and recombinant mouse PPIE. Briefly, ASPN were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to PPIE-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-ASPN 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 ASPN and PPIE was shown in Figure 1, and this effect was in a dose dependent manner.

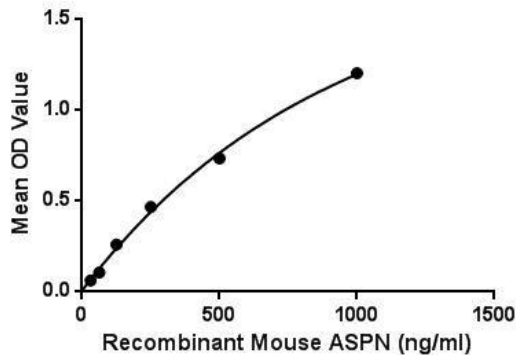


Figure 1. The binding activity of ASPN with PPIE.

[IDENTIFICATION]

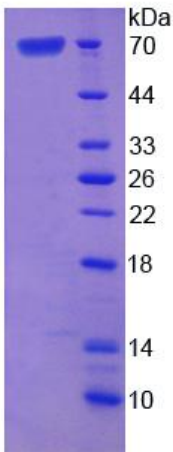


Figure 2. SDS-PAGE

Sample: Active recombinant ASPN, Mouse

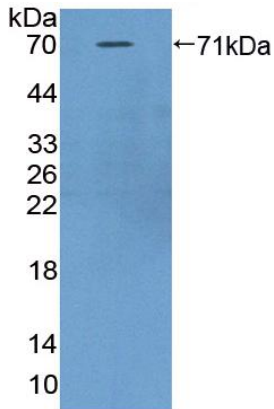


Figure 3. Western Blot

Sample: Recombinant ASPN, Mouse;

Antibody: Rabbit Anti-Mouse ASPN Ab (PAC321Mu01)

[IMPORTANT NOTE]

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.