APA037Hu02 100µg Active Fibronectin (FN) Organism Species: Homo sapiens (Human) *Instruction manual*

FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Ser1998~Ser2106

Tags: N-terminal His-tag

Purity: >92%

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: 9.1

Predicted Molecular Mass: 13.2kDa

Accurate Molecular Mass: 26kDa 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 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.

[<u>SEQUENCE</u>]

SNL

RFLATTPNSL LVSWQPPRAR ITGYIIKYEK PGSPPREVVP RPRPGVTEAT ITGLEPGTEY TIYVIALKNN QKSEPLIGRK KTDELPQLVT LPHPNLHGPE ILDVPS

[ACTIVITY]

Fibronectin (FN) is a high-molecular weight (~440kDa) glycoprotein of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins. Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. Fibronectin has numerous functions. For example, it involved in cell adhesion, cell motility, opsonization, wound healing, maintenance of cell shape, and so on. Besides, Connective Tissue Growth Factor (CTGF) has been identified as an interactor of FN, thus a binding ELISA assay was conducted to detect the interaction of recombinant human FN and recombinant human CTGF. Briefly, FN were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to CTGF-coated

microtiter wells and incubated for 2h at 37 $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti-FN 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 $^{\circ}$ C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of of FN and CTGF was shown in Figure 1, and this effect was in a dose dependent manner.



Figure 1. The binding activity of FN with CTGF.



Figure 2. Gene Sequencing (extract)

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Sample: Active recombinant Fibronectin, Human



Figure 4. Western Blot

Sample: Recombinant Fibronectin, Human;

Antibody: Rabbit Anti-Human Fibronectin Ab (PAA037Hu02)