APA820Ra02 100µg Active Coagulation Factor II (F2) Organism Species: Homo sapiens (Human) *Instruction manual*

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

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Ser201~Arg323

 Tags:
 N-terminal
 His-tag

Purity: >95%

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: 4.9

Predicted Molecular Mass: 14.4kDa

Accurate Molecular Mass: 20kDa 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.

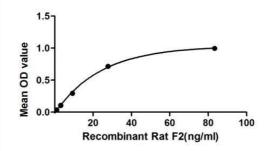
[<u>SEQUENCE</u>]

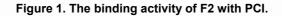
SRGSKENLSP PLGECLLERG RLYQGNLAVT TLGSPCLAWD SLPTKTLSKY QNFDPEVKLV QNFCRNPDRD EEGAWCFVAQ QPGFEYCSLN YCDEAVGEEN HDGDESIAGR TTDAEFHTFF DER

[ACTIVITY]

Coagulation Factor II (F2) also commonly called pro-thrombin is a coagulation protein in the blood stream that has many effects in the coagulation cascade. It is a serine protease that converts fibrinogen to fibrin and activates factors V, VII, VIII, XIII, and, in complex with thrombomodulin, protein C. Besides, Protein C Inhibitor (PCI) has been identified as an interactor of F2, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat F2 and recombinant rat PCI. Briefly, F2 were diluted serially in PBS, with 0.01%BSA (pH 7.4). Duplicate samples of 100uL were then transferred to PCI-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-F2 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 50μ L stop solution to the wells and read at 450nm immediately. The binding activity of of F2 and PCI was shown in Figure 1, and this effect was in a dose dependent manner.





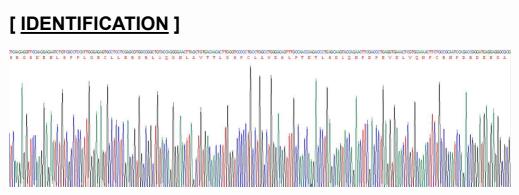


Figure 2. Gene Sequencing (extract)

	kDa 70
	44
1	33
100	26
	22
	18
1.134	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant F2, Human

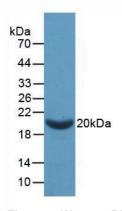


Figure 4. Western Blot

Sample: Recombinant F2, Human;

Antibody: Rabbit Anti-Human F2 Ab (PAA820Ra02)