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APA677Mu61 100µg Active Coagulation Factor XII (F12) Organism Species: *Mus musculus (Mouse) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Ala20~Ser597

Tags: N-terminal His-tag

Purity: >95%

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

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

Original Concentration: 200µg/mL

Applications: Activity Assays.

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

Predicted isoelectric point: 6.9

Predicted Molecular Mass: 65.3kDa

Accurate Molecular Mass: 110kDa 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.

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[<u>USAGE</u>]

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]

A	PPWKDSKKFK	DAPDGPTVVL	TVDGRLCHFP
CIHKRRPGSR	PWCATTPNFD	EDQQWGYCLE	PKKVKDHCSK
INTPNGPHCL	CPEHLTGKHC	QKEKCFEPQL	LKFFHENELW
CECKGSEAHC	KPVASQACSI	NPCLNGGSCL	LVEDHPLCRC
LDLWATCYEG	RGLSYRGQAG	TTQSGAPCQR	WTVEATYRNM
GHHAFCRNPD	NDTRPWCFVW	SGDRLSWDYC	GLEQCQTPTF
ESPSQAPSLS	HAPNDSTDHQ	TSLSKTNTMG	CGQRFRKGLS
ALPGSHPYIA	ALYWGNNFCA	GSLIAPCWVL	TAAHCLQNRP
QDRHNQSCEW	CQTLAVRSYR	LHEGFSSITY	QHDLALLRLQ
SPHVQPVCLP	SGAAPPSETV	LCEVAGWGHQ	FEGAEEYSTF
LDRCSNSNVH	GDAILPGMLC	AGFLEGGTDA	CQGDSGGPLV
TLRGVISWGS	GCGDRNKPGV	YTDVANYLAW	IQKHIAS
	A CIHKRRPGSR INTPNGPHCL CECKGSEAHC LDLWATCYEG GHHAFCRNPD ESPSQAPSLS ALPGSHPYIA QDRHNQSCEW SPHVQPVCLP LDRCSNSNVH TLRGVISWGS	A PPWKDSKKFK CIHKRRPGSR PWCATTPNFD INTPNGPHCL CPEHLTGKHC CECKGSEAHC KPVASQACSI LDLWATCYEG RGLSYRGQAG GHHAFCRNPD NDTRPWCFVW ESPSQAPSLS HAPNDSTDHQ ALPGSHPYIA ALYWGNNFCA QDRHNQSCEW CQTLAVRSYR SPHVQPVCLP SGAAPPSETV LDRCSNSNVH GDAILPGMLC TLRGVISWGS GCGDRNKPGV	A PPWKDSKKFK DAPDGPTVVL CIHKRRPGSR PWCATTPNFD EDQQWGYCLE INTPNGPHCL CPEHLTGKHC QKEKCFEPQL CECKGSEAHC KPVASQACSI NPCLNGGSCL LDLWATCYEG RGLSYRGQAG TTQSGAPCQR GHHAFCRNPD NDTRPWCFVW SGDRLSWDYC ESPSQAPSLS HAPNDSTDHQ TSLSKTNTMG ALPGSHPYIA ALYWGNNFCA GSLIAPCWVL QDRHNQSCEW CQTLAVRSYR LHEGFSSITY SPHVQPVCLP SGAAPPSETV LCEVAGWGHQ LDRCSNSNVH GDAILPGMLC AGFLEGGTDA TLRGVISWGS GCGDRNKPGV YTDVANYLAW

[ACTIVITY]

Coagulation factor XII (FXII), a S1A serine protease, is mainly produced in the liver and circulates in plasma as a single chain zymogen. F12 plays a role in initiating the contact activation pathway during clotting. When blood comes into contact with a foreign body, F12 is activated, initiating a series of reactions that eventually lead to the production of thrombin and the formation of fibrin, which promotes blood clotting. In addition, the combination of Plasminogen (Plg) with F12 can promote plasminogen activation and thus increase plasminogen

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production, which plays an important role in thrombolysis and tissue repair. Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse F12 and recombinant human Plg. Briefly, biotin-linked F12 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to Plg-coated microtiter wells and incubated for 1h at 37 $^\circ$ C . Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^\circ$ C. Finally, add 50 μ l stop solution to the wells and read at 450 nm immediately. The binding activity of recombinant mouse F12 and recombinant human Plg was shown in Figure 1, and this effect was in a dose dependent manner.



Figure 1. The binding activity of recombinant mouse F12 and recombinant human PIg

[IDENTIFICATION]





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Figure 3. SDS-PAGE

Sample: Active recombinant F12, Mouse

[<u>IMPORTANT NOTE</u>]

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.