

APA840Rb03 100µg

Active Coagulation Factor IX (F9)

Organism Species: *Oryctolagus cuniculus* (Rabbit)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Met1~Thr462

Tags: N-terminal His-tag

Purity: >80%

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

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

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 5.4

Predicted Molecular Mass: 55.8kDa

Accurate Molecular Mass: 56kDa 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.

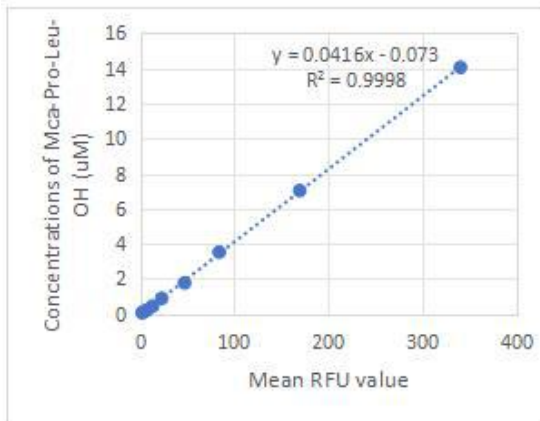
[SEQUENCE]

```
MQFLNTIMAE SPGLITICLL GYLLSAECTV FLDHENATKI LNRKRYNSG
KLEEFVSGNL ERECIEERCS FEEAREVFEN TEKTTEFWKQ YVDGDQCESN
PCLNGGSKCD DINAYECWCQ YGFEGKNCCL DSTCSIKNGR CEQFCRKNRN
NKIICSCTEG YRLAENQKSC EPAVPFPCGR VSVSHASKKI TRATTIFSNT
EYENFTEAET IRGNVTQDAQ SSDDFTRIVG GENAKPGQFP WQVLLNGKVE
AFCGGSINE KVVVTAHCI KPDDNITVVA GEYNIQETEN TEQKRVIRI
IPYHKYNATI NKYNHDIALL ELDKPLTLNS YVTPICIANR EYTNIPLNFG
SGYVSGWGRV FNRGRQASIL QYLRVPFVDR ATCLRSTKFT IYNNMFCAGF
DVGKDSCEG DSGGPHVTEV EGTSFLTGII SWGEECAIKG KYGVYTRVSW
YVNIKEKTK LT
```

[ACTIVITY]

Coagulation Factor IX, also known as Christmas Factor, is secreted by the liver and plays a key role in the activation of the intrinsic clotting cascade. Factor IX consists of a Gla domain, two tandem EGF-like domains, an activation peptide, and an S1 serine protease domain. Mature human Factor IX shares approximately 81% amino acid sequence identity with mouse and rat Factor IX. Alternative splicing generates an additional isoform that lacks the first EGF-like domain. The activity of recombinant rabbit Coagulation Factor IX is measured by its ability to cleave a fluorogenic peptide substrate Mca-RPKPVE-Nval-WRK(Dnp)-NH₂ in the assay buffer 100 mM Tris, 150 mM NaCl, 10 mM CaCl₂, 0.05% (w/v) Brij-35, pH 8.0. The Coagulation Factor IX is diluted to 100 ug/ml in assay buffer, then activated with a final concentration of 10 ug/ml Thermolysin at 37 °C for 90min. Adding a final concentration of 10 mM 1,10 Phenanthroline to stop the activation. The activated Coagulation Factor IX is diluted to 6 ug/mL in assay buffer. Loading

into a black well plate 50 μ L of 6 μ g/mL Coagulation Factor IX and start the reaction by adding 50 μ L of 20 μ M substrate, with a substrate blank containing 50 μ L assay buffer, 50 μ L substrate, and no Coagulation Factor IX. Then read at 320/405 nm in kinetic mode for 5 minutes. The specific activity of recombinant rabbit Coagulation Factor IX is > 20 pmol/min/ μ g.



RFU (320/405 nm)	MCA-Pro-Leu-OH(μ M)
340.325	14.0625
169.525	7.03125
83.895	3.515625
47.155	1.7578125
22.925	0.87890625
13.285	0.439453125
7.327	0.219726563
3.885	0.109863281
1.916	0.054931641

Figure 1. The standard curve of MCA-Pro-Leu-OH

Specific Activity (pmol/min/ μ g) =

$$\frac{\text{Adjusted Vmax} * (\text{RFU/min}) \times \text{Conversion Factor} ** (\text{pmol/RFU})}{\text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH

[IDENTIFICATION]

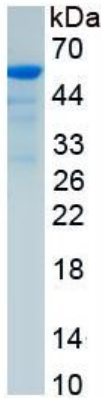


Figure 2. SDS-PAGE

Sample: Active recombinant F9, Rabbit

[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.