

APB267Mu01 100µg

Active Kininogen 1 (KNG1)

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: Prokaryotic expression.

Host: *E. coli*

Residues: Glu22~Arg379

Tags: N-terminal His-tag

Purity: >90%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 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: 5.6

Predicted Molecular Mass: 41.0kDa

Accurate Molecular Mass: 47kDa 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.

[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]

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EAQEIDCND EAVFQAVDFS LKQFNPGVKS
GNQYMLHRVI EGTKTDGSPT FYSFKYLIKE GNCSAQSGLA WQDCDFKDAE
EAATGECTAT VGKRENEFFI VTQTCKIAPS KAPILKAYFP CIGCVHAIST
DSPDLEPVLK HSIEHFNNNT DHSHLFTLRK VKSAHRQVVA GLNFDITYTI
VQTNCSKERF PSLHGDCVAL PNGDDGECRG NLFMDINNKI ANFSQSCTLY
SGDDLVEALP KPCPGCPRDI PVDSPELKEV LGHSIAQLNA ENDHPFYKYI
DTVKKATSQV VAGTKYVIEF IARETKCSKE SNTELAEDCE IKHLGQSLDC
NANVYMRPWE NKVVPTVKCQ ALDMTEMAR
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[ACTIVITY]

Kininogen 1 (KNG1), specifically its high-molecular-weight form (HMWK), is a multifunctional plasma glycoprotein that plays a critical role in the contact activation system of intrinsic blood coagulation. It acts as a non-enzymatic cofactor, circulating in a complex with prekallikrein and factor XI. Upon binding to negatively charged surfaces, HMWK facilitates the activation of these factors, leading to the initiation of coagulation and the inflammatory kallikrein-kinin system. The activity of Kininogen 1 (KNG1) was measured by its ability to inhibit papain cleavage of the fluorogenic peptide substrate Z-Phe-Arg-AMC in assay

buffer (50 mM Tris, pH 7.0). Papain was diluted to 100 μ g/mL in activation buffer (50 mM Tris, 5 mM DTT, pH 7.0) and allowed to stand at room temperature for 10 minutes, then further diluted to 2 μ g/mL with assay buffer. KNG1 was serially diluted 2-fold with assay buffer. Subsequently, 50 μ L of diluted papain (2 μ g/mL) was incubated with 50 μ L of KNG1 at room temperature for 10 minutes. The incubated mixture was diluted 1:5 with assay buffer, and 50 μ L of this diluted mixture was mixed with 50 μ L of Z-Phe-Arg-AMC substrate (200 μ M, prepared by dissolving in DMSO to 10 mM and then diluting with assay buffer). A substrate blank containing 50 μ L of assay buffer and 50 μ L of substrate was also included. Reading was performed in kinetic mode at excitation and emission wavelengths of 380 nm and 460 nm, respectively, for 5 minutes. The results showed that KNG1 significantly inhibited papain activity. The result was shown in Figure 1 and it was obvious that recombinant mouse KNG1 significantly decreased trypsin activity. The inhibition IC₅₀ was <39 nM.

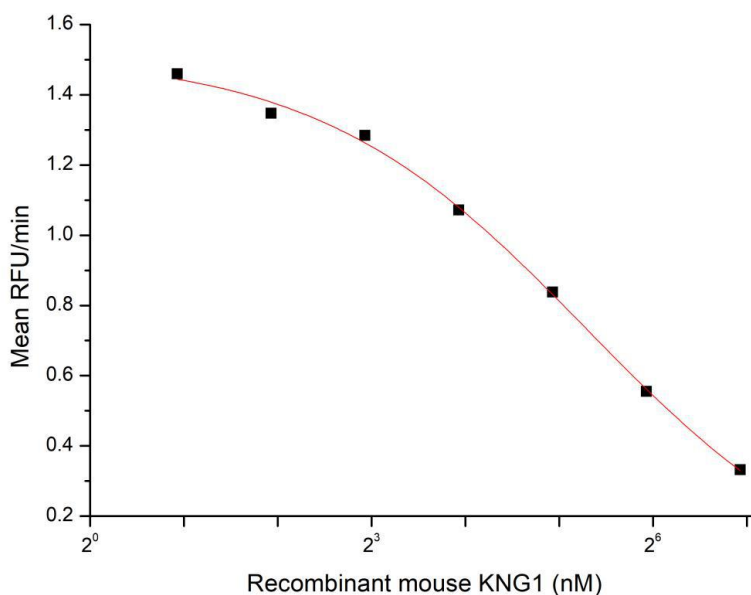


Figure 1. Inhibition of trypsin activity by recombinant mouse KNG1

[IDENTIFICATION]

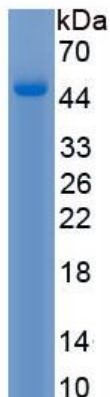


Figure 2. SDS-PAGE

Sample: Active recombinant KNG1, Mouse

[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.