APX263Ge51 10ug
Active Proteinase K (PROK)
Organism Species: Pan-species (General)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.
Host: *Yeast*
Residues: Ala16~Ala384
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.
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 7.1
Predicted Molecular Mass: 40.6kDa
Accurate Molecular Mass: 30kDa 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 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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]

APAVE QRSEAAPLIE ARGEMVANKY IVKFKEGSAL
SALDAAMEKI SGKPDHVYKN VFSGFAATLD ENMVRVLRAH PDVEYIEQDA
VVTINAAQTN APWGLARISS TSPGTSTYYY DESAGQGSCV YVIDTGIEAS
HPEFEGRAQM VKTYYYSSRD GNGHGTHCAG TVGSRTYGVA KKTQLFGVKV
LDDNGSGQYS TIIAGMDFVA SDKNNRNCPK GVVASLSLGG GYSSSVNSAA
ARLQSSGVMV AVAAGNNNAD ARNYSPASEP SVCTVGASDR YDRRSSFSNY
GSVLDIFGPG TSILSTWIGG STRSISGTSM ATPHVAGLAA YLMTLGKTTA
ASACRYIADT ANKGDLSNIP FGTVNLLAYN NYQA

[ACTIVITY]

Proteinase K is a typical member of the subtilisin family of proteinases .It exhibits broad substrate specificity and degrades many proteins in the native state, even in the presence of detergents.The predominant site of cleavage is the peptide bond adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked alpha amino groups. Thus,the activity of recombinant Proteinase K measued by hydrolyzing casein.The reaction was performed in adding 200μl recombinant Proteinase K(dilued by 0.01mol/L Tris-HCL,pH 8.0) to 200μl 10g/L casein ,rapidly mixing,incubated for 5min at 55 ℃ ,stop the reaction with 400μl 0.4mol/L TCA.Clarify by filtration through a 0.45 m filter or by centrifugation. Read the supernatant an 275nm. The blank use 200μl 0.01mol/L Tris-HCL,pH 8.0 replace the Proteinase K. One unit is described as that amount of enzyme that liberates 1 μg of

L- tyrosine within one minute at 55°C using casein as a substrate.

Calculation

$$\text{PROK activity（U/mg）} = \frac{N \times 0.8}{5 \times 0.2d}$$

N= released L- tyrosine μg/ml

0.8= Total volume (in milliliters) of stopped reaction

5= Time of assay (inminutes)

0.2= Volume of enzyme (in milliliter) of enzyme used

d= Original enzyme concentration (mg/ml)

The specific activity of recombinant Proteinase K is 40000U/mg

[IDENTIFICATION]

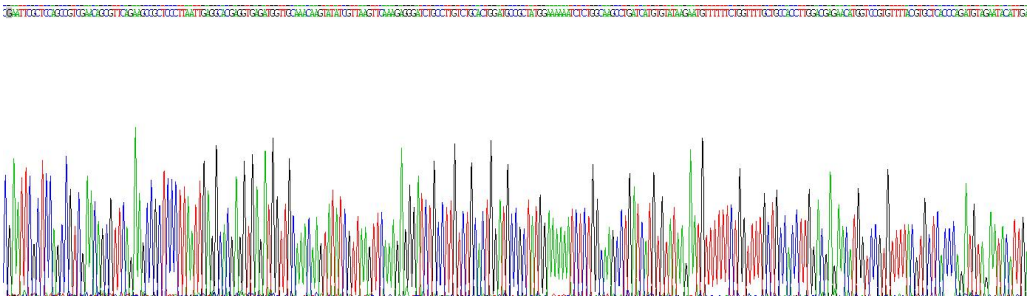

Figure 1. Gene Sequencing (extract)

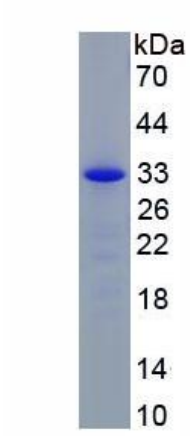

Figure 2. SDS-PAGE

Sample: Active recombinant PROK, General

[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.