Proteinase K, Molecular Biology Grade

1-800-632-7799
info@neb.com
www.neb.com

P8107S 015161018103

P8107S

2 x 1 ml **Lot: 0151610** **Exp: 10/18**

800 U/ml **Store at –20°C**

Description: Proteinase K is a subtilisin-related serine protease that will hydrolyze a variety of peptide bonds. Calcium is important for thermostability of Proteinase K but it is not required for catalysis, therefore Proteinase K is also active in buffers containing chelating agents such as EDTA (4).

Source: *Engyodontium album (Tritirachium album)*

Applications:

- Isolation of plasmid and genomic DNA
- Isolation of RNA
- Inactivation of RNases, DNases and enzymes in reactions
- Removal of enzymes from DNA to improve cloning efficiency (5)
- PCR purification

Supplied in: 20 mM Tris-HCl (pH 7.4), 1 mM $CaCl_2$ and 50% glycerol.

Reaction Conditions: Proteinase K is active in a wide range of buffers including all NEB specific restriction endonuclease buffers. It is highly active between pH 7.5 and 12.0 and temperatures between 20 and 60°C (1,2). Proteinase K is also active in chelating agents such as EDTA (4) and activity is stimulated in up to 2% SDS or 4 M urea (3).

Unit Definition: One unit will digest urea-denatured hemoglobin at 37°C (pH 7.5) per minute to produce equal absorbance as 1.0 µmol of L-tyrosine using Folin & Ciocalteu's phenol reagent (6).

Unit Assay Conditions: 0.5–2 µg of Proteinase K is incubated with 2% denatured hemoglobin solution for 10 minutes at 37°C (pH 7.5). After precipitation, neutralization and addition of Folin & Ciocalteu's phenol reagent, absorbance of soluble cleavage products are measured at 750 nm. Absorbance is compared to a standard curve of L-tyrosine absorbance prepared similarly.

Heat Inactivation: 95°C for 10 minutes.

Molecular Weight: 28.9 kDa

Protein Concentration (A_{280}): The concentration of Proteinase K is approximately 20 mg/ml as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using an extinction coefficient of 36,580 and a molecular weight of 28,907 daltons for Proteinase K (7).

Quality Control Assays

Endonuclease Activity (Nicking):
The product is tested in a reaction containing a supercoiled DNA substrate. After incubation for 4 hours the percent converted to the nicked form is determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release):
The product is tested in a reaction containing a radiolabeled mixture of single and double-stranded DNA. After incubation for 4 hours the exonuclease activity is determined by the % release of radioactive nucleotides.

Non-Specific DNase Activity (16 hour):
The product is tested in a reaction containing a DNA substrate. After incubation for 16 hours there is no detectable degradation of the DNA substrate as determined by agarose gel electrophoresis.

Single Stranded DNase Activity (FAM Labeled Oligo): The product is tested in a reaction containing a fluorescent internal labeled single stranded oligonucleotide. The percent degradation is determined by capillary electrophoresis.

RNase Activity (Extended Digestion):
The product is tested in a reaction containing a RNA substrate. After incubation for 16 hours > 90% of the substrate RNA remains intact as determined by gel electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

qPCR DNA Contamination (eukaryotic genomic):
The product is screened for the presence of eukaryotic genomic DNA using SYBR® Green qPCR with primers specific to the eukaryotic 18S rRNA locus. Results are quantified using a standard curve generated from purified *Engyodontium album* genomic DNA.

Note:
Proteinase K is stable for at least 2 years at –20°C. No loss of activity is observed after 10 freeze-thaw cycles.

References:

1. Bajorath, J. et al. (1988) *Biochimica et Biophysica Acta* 954, 176–182.
2. Ebeling, W. et al. (1974) *Eur. J. Biochem.* 47, 91–97.
3. Hilz, H. et al. (1975) *Eur. J. Biochem.* 56, 103–108.
4. Bajorath, J. et al. (1988) *Eur. J. Biochem.* 176, 441–447.
5. Crowe, J.S. et al, (1991) *Nucleic Acids Research* 19, 184.
6. Anson, M.L. (1939) *J. Gen. Physiol.* 22,79–89.
7. Pace, C.N. et al. (1995) *Protein Sci.*, 4, 2411–2423.

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