

**Proteinase K,  
Molecular  
Biology Grade**



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



P8107S 009140916091

**P8107S**

**2 x 1 ml**      **Lot: 0091409**      **Exp: 9/16**  
**800 U/ml**      **Store at -20°C**

**Description:** Proteinase K is a subtilisin-related serine protease that will hydrolyze a variety of peptide bonds. Proteinase K is active in a wide range of temperatures and buffers with optimal activity between 20 and 60°C and a pH between 7.5 and 12.0 (1, 2). Activity is stimulated when up to 2% SDS or up to 4 M urea are included in the reaction (3). 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<sub>2</sub> 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.

**Molecular Weight:** 28.9 kDa

**Protein Concentration (A<sub>280</sub>):** 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 (Nickin):**

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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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.

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**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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SYBR® is a registered trademark of Life Technologies, Inc.

**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.
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