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

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

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.

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).

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.

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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: