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

**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).

**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.

**RNase Activity (Extended Digestion):**
The product is tested in a reaction containing a RNA substrate. After incubation for 16 hours there is no detectable degradation of the DNA substrate as 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.

**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.

**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.
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: