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Product Information

Version 1.2.04

A3650 – Proteinase K

Description

Proteinase K is a non-specific serine protease having a very high specific activity. It is used to purify target material from contaminating proteins, for the isolation of mRNA or high molecular weight DNA and to inactivate other enzymatic activities. Proteinase K exhibits high activity in the presence of SDS, EDTA and Urea as well as over a wide pH range. Proteinase K is isolated from *Tritirachium album* and it is very stable in the storage buffer at +4 °C and at –20 °C.

Proteinase K powder is highly soluble (>50mg/ml), and commonly made as a 20mg/ml liquid stock solution. The proteinase K is completely nuclease-free with a specific activity >30 units/mg at 37°C. Proteinase K has two binding sites for Ca^{2+}. Calcium acts as a stabilizing factor of the enzyme. When calcium is removed from the solution, the activity of proteinase K decreases slowly.

Proteinase K activity is greatly increased by addition of denaturing agents like SDS or urea (Hilz et al., 2008), indicating that the denaturation of the substrates helps Proteinase K to degrade them. Increasing the temperature to 50°C will also unfold some proteins already, making it easier for the Proteinase K to degrade them. The proteinase K seems to be a pretty stable enzyme, and can still work at this temperature. Proteinase K can be inactivated by heat, eg. Incubating at >55 °C. It can also be inactivated by changing the pH significantly.

Proteinase K Activity in Commonly Used Buffers

Buffer	Application Example	Activity (%)
20 mM Tris-HCl, pH 8.0	Reference	100
10 mM Tris-HCl, 1 mM EDTA, 0.5 % SDS, pH 8.0	Bacterial genomic DNA isolation	108
10 mM Tris-HCl, 100 mM NaCl, 25 mM EDTA, 1 % SDS, pH 8.0	Genomic DNA isolation from mammalian tissues	171
100 mM Tris-HCl, 100 mM EDTA, 250 mM NaCl, 1% Sarkosyl, pH 8.0	Plant tissue genomic DNA isolation	118
10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 5 mM EDTA, 0.5 % SDS, pH 7.9	Inactivation of Calf Intestinal Alkaline Phosphatase	104
50 mM Tris-HCl, 1 mM $CaCl_2$, 3 mM DTT, 2 M Urea, pH 8.0	Denaturation of proteins	66
10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 % Triton X-100, pH 8.8	PCR buffer	158

Application Guidelines:

1. Isolation of high molecular weight DNA
Chromosomal DNA that has been embedded in agarose plugs can be treated with Proteinase K to inactivate rare-cutting restriction enzymes used to digest the DNA. Proteinase K is used for this method at a concentration of 1mg/ml in a buffer containing 0.5M EDTA and 1% N-lauroylsarcosine (v/v).
Incubate 24-48 hours at 37°C.

2. Isolation of plasmid and genomic DNA
Genomic or plasmid DNA can be isolated from liquid nitrogen frozen cells or cultured cells using Proteinase K. Incubate 50-100 mg of tissue or 1x108 cells in 1 ml of buffer containing 0.5% SDS (w/v) with Proteinase K at a concentration of 1mg/ml, for 12-18 hours at 50°C.

3. Isolation of RNA
For cytoplasmic RNA isolation, centrifuge the cell lysate, remove the supernate and add 200ug/ml Proteinase K and SDS to 2%(w/v). Incubate for 30 minutes at 37°C. Total RNA can be isolated by passing the lysate through a needle fitted to a syringe prior Proteinase K treatment.

4. Inactivation of RNases , DNases and enzymes in reactions
Proteinase K is active in a wide variety of buffers (see FAQ "What is the Proteinase K activity in commonly used buffers?"). The enzyme should be used at a ratio of approximately 1:50 (w/w, proteinase K:enzyme). Incubation is at 37°C for 30 minutes.

Storage Condition:

In storage buffer at –20 °C Proteinase K is stable for at least 1 year.

**** The product is designed for laboratory research purpose only. Not for human or animal diagnostic and therapeutic use.***