

Proteinase K

-----PCR Grade

Cat.NO.:ZB117

Price:EUR500.00/g

Description:

Proteinase K is a subtilisin-like endolytic protease that is isolated from the saprophytic fungus *Tritirachium album*. It has a high activity that is stable across a wide range of pH and temperature conditions and is suited to short digestion times. The activity of proteinase K is increased at elevated temperatures up to 65°C. Calcium is not essential to the function of proteinase K. Therefore, EDTA and other chelating agents do not interfere with the activity and may be used alongside proteinase K to inactivate calcium-dependent nucleases in DNA and RNA preparation.

Properties of Proteinase K

Alternate names	Peptidase K, <i>Tritirachium</i> alkaline proteinase
Specificity	Cleaves at the carboxyl side of aliphatic, aromatic or hydrophobic residues
Proteinase K Source	<i>Tritirachium album</i>
Appearance	White Lyophilized Powder
Molecular weight	28,900
Form	Lyophilized form
Concentration/activity	>30 units/mg at 35°C
RNase/DNase	RNase-free and DNase-free
Protease type	Serine protease
Uses/applications	Inactivation of RNase and DNase during nucleic acid purification
Reaction conditions	0.05-1 mg/ml proteinase K, pH 7.5-8, often containing 0.5-1% SDS
Storage conditions	Store at -20°C, shipped in dry ice.
Inhibitors	PMSF or DFP

Applications

- Isolation of high molecular weight DNA
- Isolation of plasmid and genomic DNA

- Isolation of RNA
- Inactivation of RNase and DNase activities

Storage buffer

20 mM Tris-HCl (pH 7.4), 1 mM CaCl₂, 50 % Glycerol.

Quality control

Unit definition

One unit is defined as the amount of enzyme that liberates folin-positive amino acids and peptides corresponding to 1 μmol tyrosine under the assay conditions in 1 minute using hemoglobin as substrate.

16-hour incubation

A 50 μl reaction containing 1 μg of λ DNA and 1.8 U of enzyme incubated for 16 hours at 37°C resulted in the same DNA band as that produced without the enzyme.

Exonuclease activity

Incubation of 6 U for 4 hours at 37°C in 50 μl assay buffer with 1 μg sonicated [³H]-DNA (2 x 10⁵ cpm/μg) released <0.2 % of radioactivity.

Endonuclease activity

Incubation of 1.8 U with 1 μg φX174 RFI DNA (4 hours, 37°C, 50 μl) gave <5 % conversion to RFII.

RNase activity

Incubation of 6.0 U with 1 μg MS2 RNA (4 hours, 37°C, 50 μl) resulted in the same RNA band as that produced without the enzyme.

Common features

Proteinase K has two binding sites for Ca²⁺. Calcium acts as a stabilizing factor of the enzyme. When calcium is removed from the solution, the activity of proteinase K decreases slowly.



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