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CAUTION! Professional use only.
Follow instruction manual before use.
Reading and carefully following these warnings will minimize usage errors, other possible risks and hazards.
Wear safety glasses, clothes, and gloves.

PROTEINASE K (LYOPHILIZED)

User Guide

Catalog Number	Pack Size
ZT-PK-20	20 MG
ZT-PK-100	100 MG
ZT-PK-1000	1 G

Molecular Biology Grade,
For Research Use Only

Content and Storage Condition

Content	Shipping Condition	Storage Condition
PROTEINASE K (LYOPHILIZED)	RT for short periods of time	-20°C after dissolving
PROTEINASE K STOCK SOLUTION	RT	4°C

Features

- Kit content can be stored at 4°C before dissolving. It must be stored at -20°C after dissolving until expiry date.
- To avoid repeated freezing and thawing as well as to minimize the contamination risk of stock solutions, it is highly recommended to divide large-volume stocks into several smaller aliquots and store them at -20°C.
- The product is compatible with all nucleic acid extraction protocols and kits.

Product Description

Proteinase K is a non-specific serine protease having a very high specific activity. It has been used for isolation of mRNA, high molecular weight DNA and to inactivate other enzymatic activities. Proteinase K is active with or without the presence of SDS and EDTA.
Source: *Tritirachium album*
Appearance: White Powder
Molecular Weight: 29.3 kDa monomer.

Definition of Activity Unit

One unit of the enzyme liberates Folin-positive amino acids and peptides corresponding to 1 µmol tyrosine in 1 min at 37°C , pH 7.5 using denatured hemoglobin as substrate.
Enzyme activity is assayed in the following mixture: 0.08 M potassium phosphate (pH 7.5), 5 M urea, 4 mM NaCl, 3 mM CaCl2 and 16,7 mg/ml hemoglobin.

Inhibition and Inactivation

Inhibitors: Proteinase K is not inactivated by metal chelators, by thiol-reactive reagents or by specific trypsin and chymotrypsin inhibitors. Phenylmethylsulfonyl fluoride and diisopropyl phosphorofluoridate completely inhibit the enzyme. Inactivated by heating at 95°C for 10 minutes.

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Note

Optimum activity at 50-55°C.
Rapid denaturation of enzyme occurs at temperatures above 65°C.
The activity of the enzyme is stimulated by 0.2-1% SDS or by 1-4 M urea.
Ca2+ protects Proteinase K against autolysis, increases the thermal stability and has a regulatory function for the substrate binding site of Proteinase K.
Stable over a wide pH range: 4.0-12.5 (optimum pH 7.5-8.0).

Applications

Isolation of genomic DNA from cultured cells and tissues
Removal of DNases and RNases when isolating DNA and RNA from tissues or cell lines
Determination of enzyme localization
Improving cloning efficiency of PCR products

Preparation of Proteinase K Solution

The standard concentration of a Proteinase K solution is 20mg/mL. For preparing this solution, 20mg of Proteinase K is dissolved in Proteinase K Stock Solution. The solution is stable at -20°C for 2 years but can also be stored at +2°C to +8°C°C for several months.

QUALITY CONTROL

16-hour incubation:

50 µL reaction solution containing 1µg of lambda-DNA and 1.8 units of enzyme incubated for 16 hours at 37°C resulted in the same DNA band pattern after gel electrophoresis as compared to the pattern produced without enzyme.

Exonuclease activity:

Incubation of 6 units of the enzyme for 4 hours at 37°C in 50µL assay buffer with 1g sonicated 3H DNA (3 x 10(5) cpm/µg) released less than 0.2% of radioactivity.

Endonuclease activity:

Incubation of 1.8 units of enzyme with 1µg PhiX174 RFI DNA in 50µL assay buffer for 4 hours at 37°C gave less than 1.5% conversion of RFI.

RNase contamination:

Incubation of 6 units of enzyme with 1 µg MS2 RNA in 50µL assay buffer for 4 hours at 37°C resulted in the same RNA band pattern after gel electrophoresis as compared to the pattern produced without the enzyme.

Usage

Proteinase K is used for the destruction of proteins in cell lysates (tissue, cell culture cells) and for the release of nucleic acids since it inactivates DNases and RNases very effectively.

Warning

Do not preserve the product when the package is damaged.