

### PRODUCT INFORMATION

Proteinase K. recombinant

Cat. No. 33756

Prod	luct [	Descr	iption:

A recombinant Proteinase K is a subtilisin-related serine protease. It is an endopeptidase with a very high specific activity and a broad spectrum of action. The enzyme is a 28.9 kDa protein expressed in *Pichia pastoris*. It's widely used for digestion of proteins, including DNases and RNases during nucleic acid preparations without compromising the integrity of the isolated DNA or RNA. It is active in a wide range of reaction conditions.

### Application

- Inactivation of RNases and DNases during isolation of DNA or RNA from tissues, cell lines or cultured cells
- Removal of protein impurities
- Improving cloning efficiency of PCR products
- Determination of enzyme localization on membranes
- Removal of nucleases for in situ hybridization

#### **Features**

- Supplied as highly purified lyophilized powder
- Specific activity: approximately 30 U/mg protein
- Free of DNase and RNase activity
- Exhibits broad substrate specificity

## Storage

Recommended temperature for long-term storage: - 20 °C

## Activation

The enzyme is stimulated by addition of denaturing agents 0.2 - 1% (w/v) SDS or 4 M urea. It exhibits prolonged stability due to the presence of  $Ca^{2+}$  (1 - 6 mM), which protects enzyme from autolysis and increases its thermal stability.

## Inhibition

Diisopropylfluorophosphate, phenylmethlysulfonyl fluoride² and mercury ions. Proteinase K is unaffected by metal-chelating agents and sulfhydryl inhibitors.

# Reaction conditions

Proteinase K is typically used at  $50-200 \,\mu g/ml$  in nucleic acid preparations at pH 7.5 - 8.0 and 37 °C - 55 °C.

Incubation times vary from 30 minutes to 18 hours.

Working pH range: 4.0 - 12.0 (optimum activity at pH 7.5 - 8.5)

Working temperature range: 20 °C - 65 °C (optimum at 50 °C - 56 °C)

Note: If necessary, to help the product to be fully soluble before use, heat the solution to 55 °C temperature and maintain the temperature for 15-45 min. After that cool and store at -20 °C. The product will retain its full activity.

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<sup>\*</sup>Unit definition: 1 U is defined as the amount of enzyme that liberates Folin-positive amino acids and peptides, corresponding to 1 μmol tyrosine under assay conditions in 1 minute using haemoglobin as substrate.

<sup>&</sup>lt;sup>1</sup>Betzel, C., Pal, G.P. and Saenger, W. (1988) Eur. J. Biochem. 178, 155-171.

<sup>&</sup>lt;sup>2</sup>Ebeling, W., Hennrich, N., Klockow, M., Metz, H., Orth, H.D and Lang, H. (1974) Eur. J. Biochem. 7, 91-97.