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PRODUCT INFORMATION

Proteinase K, recombinant, NGS grade — Cat. No. 33757

Product Description:

General

Proteinase K is a subtilisin-related serine protease 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 is widely used for digestion of proteins, including DNases and RNases during nucleic acid preparations without compromising the integrity of the isolated DNA or RNA.
An additional purification step results in a 2.5-fold increase in solubility, a higher specific activity and an extremely low DNA content, making this preparation particularly suitable for methods that require the highest quality such as Next Generation Sequencing (NGS).

Application

- Isolation of nucleic acids for PCR, RT-PCR, NGS
- Removal of protein impurities
- Improving cloning efficiency of PCR products
- Determination of enzyme localization on membranes
- Antigen retrieval in in situ hybridization

Features

- Supplied as highly purified lyophilized powder
- Specific activity: min. 45 U/mg protein, min. 35 U/mg lyophilizate
- Free of DNase and RNase activity
- DNA: ≤ 0.1 pg/mg enzyme
- Solubility in water ≥ 50 mg/ml

Storage

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

Activation

- By addition of 0.2 - 1 % (w/v) SDS or 4 M urea
- Extended stability due to protection against autolysis and increased thermal stability in the presence of Ca^{2+} (1 - 6 mM)

Inhibition

- By DFP, PMSF[2] and mercury ions
- Unaffected by metal-chelating agents and sulfhydryl inhibitors

Reaction conditions

Usually used in nucleic acid preparation in a concentration of 50 - 200 µg/ml 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.

***Unit definition:** 1 U is defined as the amount of enzyme that hydrolyzes urea-denaturated hemoglobin producing color equivalent of 1 µmol tyrosine per 1 min at 37 °C and pH 7.5 (Folin & Ciocalteu's method). 1 U = 1 mAnsonU.

[1]Betzel, C., Pal, G.P. and Saenger, W. (1988) Eur. J. Biochem. 178, 155-171.
[2]Ebeling, W., Hennrich, N., Klockow, M., Metz, H., Orth, H.D and Lang, H. (1974) Eur. J. Biochem. 7, 91-97.

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