PRODUKT INFORMATION

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

Product Description:

General

Proteinase K[1] 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).

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

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

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, PMSF2 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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