

Quick-Start Protocol

August 2023

Proteinase K - Powder

The Proteinase K provided as lyophilized powder (cat. nos. RP100B, RP101B, RP102B, and RP103B-20MG) is a subtilisin-related serine protease. A recombinant Proteinase K from *Parengyodontium album (Tritirachium album)* is a 28.9 kDa protein expressed in *Komagataella phaffii (Pichia pastoris)*. It is a broad-spectrum endopeptidase with an extraordinary specificity allowing highly effective digestion of proteins in many laboratory applications. Upon arrival, Proteinase K Lyophilized Powder should be stored at –20°C. Proteinase K Lyophilized powder maintains activity \geq 30 U/mg and specific activity \geq 40 U/mg for at least 24 months when stored in its original, unopened container.

Further information

- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

 One unit of Proteinase K Lyophilized powder hydrolyzes urea-denatured hemoglobin producing color equivalent of 1 µ mol tyrosine per 1 min at 37°C and pH 7.5 (Folin & Ciocalteu's method), 1 U = 1 mAnsonU.

Things to do before starting

Proteinase K stock solution preparation:

- 20 mg/mL solutions: use purified water for immediate use.
- 20 50 mg/mL solutions: use 50 mM Tris-HCl, pH = 7.5-8.0, 1-5 mM Ca²⁺ (calcium chloride, calcium acetate) for immediate use; or 10 mM Tris-HCl, pH = 7.5-8.0, 1-5 mM Ca²⁺ (calcium chloride, calcium acetate), 50% glycerol for long-term storage.

Sample to Insight

Considerations for use

- The enzyme is typically used at 50–200 µg/mL nucleic acid preparations at pH 7.5–8.5 and 37–55°C. Incubation times vary from 30 minutes to 18 hours.
- 2. The Proteinase K cleaves proteins preferably behind hydrophobic amino acids. The smallest peptide to be hydrolyzed is a tetrapeptide.
- 3. Working pH range is 4.0–12.0 with optimum activity at pH 7.5–8.5. Full activity is maintained over several hours over a pH range of 6.5–10.0.
- 4. Working temperature range is 20–65°C with optimum activity at 50–56°C.
- 5. The enzyme is stimulated by addition of denaturing agents 0.2–1% SDS or 1–4 M urea. It exhibits prolonged stability due to the presence of Ca²⁺ (1–6 mM), which protects enzyme from autolysis and increases its thermal stability.
- 6. The enzyme is not inactivated by chelating agents (e.g. EDTA), chaotropic salts, detergents (e.g. 1% SDS, 1–4M urea), metal ions, thiol reagents or trypsin-specific inhibitors.
- Proteinase K is usually denatured by subsequent phenol extractions. It can be also inactivated by heating above 65°C or using inhibitors such as PMSF or DIFP.

Document Revision History

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Changes

August 2023

Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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