

## Proteinase K - Powder

The Proteinase K provided as lyophilized powder (cat. nos. RP100B, RP101B, RP102B, RP103B-20MG, RP103B-10, RP103B-25, RP103B-50, and RP103B-100) 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  $-20^{\circ}\text{C}$ . 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](http://www.qiagen.com/safety)
- Technical assistance: [support.qiagen.com](mailto:support.qiagen.com)

### Notes before starting

- One unit of Proteinase K Lyophilized powder hydrolyzes urea-denatured hemoglobin producing color equivalent of  $1\ \mu\text{mol}$  tyrosine per 1 min at  $37^{\circ}\text{C}$  and pH 7.5 (Folin & Ciocalteu's method),  $1\ \text{U} = 1\ \text{mAnsonU}$ .

### 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  $\text{Ca}^{2+}$  (calcium chloride, calcium acetate) for immediate use; or 10 mM Tris-HCl, pH = 7.5–8.0, 1–5 mM  $\text{Ca}^{2+}$  (calcium chloride, calcium acetate), 50% glycerol for long-term storage.

## Considerations for use

1. 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 Ca<sup>2+</sup> (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, 1–4 M urea), metal ions, thiol reagents, or trypsin-specific inhibitors.
7. 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

Date	Changes
August 2023	Initial release.
July 2024	Added cat. nos. RP103B-10, RP103B-25, RP103B-50, and RP103B-100.

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