August 2023

Quick-Start Protocol

Proteinase K - Ultrapure

The Proteinase K Ultrapure (cat. nos. RP100N, RP101N, RP102N, RP103N-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 widely used for the digestion of proteins, including DNases and RNases, during nucleic acid preparations without compromising the integrity of isolated DNA or RNA. Upon arrival, Proteinase K Ultrapure Grade should be stored at –20°C. Proteinase K Ultrapure Grade maintains activity ≥ 35 U/mg and specific activity ≥ 45 U/mg for at least 24 months when stored in its original, unopened container, and increased solubility (2.5 fold) and remarkable purity with DNA content ≤0.1 pg/mg. Proteinase K Ultrapure is highly soluble in water (≥50mg/ml) and has little DNA content (≤0.1 pg/mg DNA *Escherichia coli*; ≤0.1 pg/mg DNA *Komagataella phaffii (Pichia pastoris*).

Notes before starting

- One unit of Proteinase K 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 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 Ca^{2+} (calcium chloride, calcium acetate) for immediate use; or 10 mM Tris-HCl, pH = 7.5-8.0, 1-5 mM 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 1–4M 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.
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.
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

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