For life science research only. Not for use in diagnostic procedures.

Proteinase K, recombinant, PCR Grade

From *Pichia pastoris*
Lyophilizate

Cat. No. 03 115 836 001	25 mg
Cat. No. 03 115 879 001	100 mg
Cat. No. 03 115 801 001	2× 250 mg
Cat. No. 03 115 852 001	4× 250 mg

Version 07
Content version: May 2019
Store at +2 to +8°C

1. What this Product Does

Stability and Storage

The lyophilized enzyme is stable at +2 to +8°C until the expiration date printed on the label.

⚠ Reconstitute the lyophilizate in double-distilled water or Tris buffer and store in aliquots at −15 to −25°C. If stored properly, aliquots are stable up to 12 months. Avoid repeated freezing and thawing since this may lead to precipitation of the protein.

For your convenience we offer a ready-to-use solution* of recombinant, PCR grade proteinase K.

Applications

Proteinase K, recombinant, PCR grade, digests native proteins very effectively. It can therefore be used to rapidly inactivate endogenous RNases and DNases (1, 2) during nucleic acid isolation (4, 5). This property makes proteinase K particularly suitable for the isolation of native RNA and DNA from tissues or cell lines. The enzyme also promotes cell lysis by activating a bacterial autolytic factor. Proteinase K is also used for the analysis of membrane structures by modifying proteins and glycoproteins on cell surfaces. Because the solution is tested for the absence of RNases and DNases, and is virtually free of DNA, it is especially suitable for isolating PCR and RT-PCR templates. Proteinase K can also be used to remove cellular debris during the preparation of colony lifts (15), and to treat tissue sections to ensure efficient probe infiltration during *in situ* hybridization (6).

2. How to Use this Product

Suggested Buffers

The best buffer for proteinase K will vary from application to application. Always follow the pH and temperature guidelines above. As a general rule, proteinase K is stable and very active in buffers that contain denaturing reagents such as urea, sodium dodecyl sulfate (SDS), and guanidinium salts.

Reconstitution in Double-Distilled Water

Proteinase K is soluble at least up to 20 mg/ml in double-distilled water.

Typical Experiments

Isolation of nucleic acids: Dissolve the lyophilized Proteinase K, recombinant, PCR Grade, in double-distilled water (90 mg lyophilizate in 4.5 ml double-distilled water) and use it with the High Pure PCR Template Preparation Kit* to isolate nucleic acids from:

- 200 µl mammalian blood
- 200 µl buffy coat
- 10^4–10^5 cultured mammalian cells
- 25–50 mg mammalian tissue
- 0.2–0.5 cm (25–50 mg) mouse tail
- 10^9 bacteria or 10^8 yeast cells
- 25–50 mg formalin-fixed paraffin-embedded tissue section

Add 40 µl of the reconstituted proteinase K solution to each sample. Then follow the procedure described in the document of the High Pure PCR Template Preparation Kit, which is available online at lifescience.roche.com/Instructions for Use/11796828001.pdf.

Isolation of cytoplasmic RNA from cultured cells (5): Lyse cells in a buffer containing 0.5% (v/v) Nonidet P-40* (non-ionic detergent). Centrifuge the lysate then transfer the supernatant to a clean tube containing 4 µl of 20% SDS. Immediately vortex the tube to mix the contents. Add 2.5 µl of 20 mg/ml proteinase K to the tube and incubate for 15 min at +37°C.

Isolation of genomic DNA from mammalian tissue (5): The starting material can be 80 mg minced mammalian tissue, 80 mg of tissue that has been frozen in liquid nitrogen, or 1×10^8 cultured mammalian cells (5). Incubate the starting material for 12–18 h at +50°C in 1 ml digestion buffer that contains 100 µg/ml proteinase K and 0.5% SDS (w/v).

Preparation of tissue sections for *in situ* hybridization: For some tissues, treatment of cytological sections with proteinase K will improve the likelihood that probes will reach cellular nucleic acids. The effectiveness of proteinase K treatment and the optimal concentration of proteinase K depend greatly on the kind of tissue and how it was fixed. For example, to treat blood vessel or myocardial tissue, Plenz et al (7) used the following concentrations of proteinase K:

- Cryosections: up to 2 µg/ml
- Paraffin-embedded sections: up to 20 µg/ml
- Methacrylate-embedded sections: up to 50 µg/ml

3. Additional Information on this Product

Background Information

Proteinase K is a subtilisin-related serine protease. The recombinant enzyme is identical to the native protease originally isolated from the mold, *Tritirachium album*. The specifications of the recombinant enzyme are the same as those of the native protease. The amino acid sequence (molecular weight) and the molecule structure (enthalpy for denaturation) are identical.

However, the recombinant preparation is much purer than the native enzyme. In particular, since recombinant, PCR grade proteinase K is DNA-free, it is especially suitable for isolating PCR and RT-PCR templates.

Enzyme Characteristics

Cleavage Specificity	Proteinase K is one of the most active endopeptidases known and does not show any pronounced cleavage specificity. Proteinase K cleaves proteins as follows: X-_«-Y-, where X = aliphatic, aromatic or hydrophobic amino acid and Y = any amino acid. If excess proteinase K is incubated with proteins for a long time, the enzyme will degrade the proteins to free amino acids.

Specific Activity	Approx. 2.5 U/mg protein, when assayed with the Chromozymassay (equivalent to 30 U/mg protein with the haemoglobin assay). Approx. 2.0 U/mg lyo, when assayed with the Chromozymassay (equivalent to 24 U/mg lyo with the haemoglobin assay).
pH and Temperature	Recombinant proteinase K is stable from pH 4.0 to pH 12.5. It retains full activity for several hours when incubated at pH 6.5–9.5. The enzyme is 12 times more active at +65°C than at +25°C. However, it is rapidly denatured at temperatures above +65°C. For more information, see the article on pp. 16-17 of Biochemica 2003 (3). This article is available online at: diagnostics.roche.com
Activators	To stimulate proteinase K activity, add denaturing agents (SDS and urea). For example, SDS can increase the activity of proteinase K as much as sevenfold (3).
Inhibitors	Proteinase K is inhibited by diisopropyl fluorophosphate and phenylmethylsulfonyl fluoride (PMSF). It is also totally inactivated by mercury ions. Pefabloc SC* and Pefabloc PLUS* are specific, irreversible, non-toxic inhibitors of proteinase K. Proteinase K is not inactivated by metal ions, chelating agents (*e.g.*, EDTA), sulfhydryl reagents or trypsin/chymotrypsin inhibitors.
Autolysis	Autolysis of the enzyme occurs more rapidly at alkaline pH. However, proteinase K is not completely inactivated by autolysis. Some enzyme fragments retain complete proteolytic activity.

Quality Control

Absence of endonucleases	*Eco* RI/*Hin*d III fragments (4.0 µg) are incubated with proteinase K for 16 h at +37°C in 36 µl 10 mM Tris-HCl, pH 7.5. The DNA shows no degradation after incubation with up to 200 µg proteinase K.
Absence of Nicking Activity	pBR322 DNA (4.0 µg) is incubated with proteinase K for 16 h at +37°C in 36 µl 10 mM Tris-HCl, pH 7.5. The DNA does not lose its supercoiled structure after incubation with up to 200 µg Proteinase K.
Absence of Ribonucleases	MS2 RNA (4.0 µg) is incubated with proteinase K in 36 µl 10 mM Tris-HCl, pH 7.5. The RNA shows no degradation after incubation with up to 40 µg proteinase K.
DNA Content	Total DNA content (in pg/mg) is determined with Threshold according to the supplier's instructions. The amount of total DNA in recombinant, PCR grade proteinase K is less than 10 pg/mg enzyme.
Bioburden	The number of viable microorganisms is determined with the most stringent bioburden test in the European Pharmacopoeia. The total number of viable microorganisms in Proteinase K, recombinant, PCR grade, is less than 125 cfu/g. This extremely low bioburden ensures that the product will be very stable and safe.

References

1 Wieger U & Hilz H. *FEBS Lett.* (1972);**23**:77.
2 Wieger U & Hilz H. *Biochem. Biophys. Res. Commun.* (1971);**44**:513.
3 Hilz, H. et al. *Eur. J. Biochem.* (1975);**56**:103–108.
4 Sambrook J et al. Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989).
5 Ausubel F M et al (Eds). Current Protocols in Molecular Biology, Wiley & Sons; (2004); Four Volumes 0-471-50338-X-Looseleaf or 0-471-30661-4-CD-Rom.
6 Roche Applied Science, *DIG Application Manual for Nonradioactive In Situ Hybridization* (2002), 3rd edition.
7 Proteinase K, Recombinant, PCR Grade: The Ideal Tool for Template Preparations *Biochemica* (2003);2:12.
8 Jackson P J et al. PCR analysis of tissue samples from 1979 Sverdlovsk anthrax victims: The presence of multiple Bacillus anthracis strains in different victims. *Proc. Natl. Acad. Sci.* (1998); **95**(3):1224–1229.
9 González-Huici V et al. Binding of phage F29 architectural protein p6 to the viral genome: evidence for topological restriction of the phage linear DNA. *Nucleic Acids Res.* (2004);**32**:3493–3502.
10 Ursic D et al. Multiple protein/protein and protein/RNA interactions suggest roles for yeast DNA/RNA helicase Sen1p in transcription, transcription-coupled DNA repair and RNA processing. *Nucleic Acids Res.* (2004);**32**:2441–245.
11 Han S *et al.* Rectification of age-related impairment in Ig gene hypermutation during a memory response. *Int. Immunol.* (2004);**16**:525–532.
12 Clark LJ *et al.* Theca Interna: The Other Side of Bovine Follicular Atresia. *Biol. Reprod.* (2004);**71**:1071–1078.
13 Frick C et al. Appropriate Function of 11b-Hydroxysteroid Dehydrogenase Type 1 in the Endoplasmic Reticulum Lumen Is Dependent on Its N-terminal Region Sharing Similar Topological Determinants with 50-kDa Esterase. *J. Biol. Chem.* (2004);**279**:31131–31138.
14 Shigemori Y & Oishi M. Specific cleavage of DNA molecules at RecA-mediated triple-strand structure. *Nucleic Acids Res.* (2004);**32**:4.

4. Supplementary Information

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Use
Asterisk *	Denotes a product available from Roche Diagnostics

Symbols

In this document the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

Ordering Informationr

Product	Pack Size	Cat. No.
Proteinase K, recombinant, PCR Grade (solution)	1.25 ml 5 ml 25 ml	03 115 887 001 03 115 828 001 03 115 844 001
High Pure PCR Temp. Prep. Kit	1 Kit	11 796 828 001
Nonidet P-40	100 ml	11 754 599 001
Pefabloc SC	100 mg	11 429 868 001
Pefabloc SC	Set I Set II	11 873 601 001 11 873 628 001

Changes to previous version

- Editorial changes.

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Disclaimer of License

For patent license limitations for individual products please refer to:
List of biochemical reagent products

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany