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

Proteinase K, recombinant, PCR Grade

from *Pichia pastoris*

Version: 06
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Solution

Cat. No. 03 115 887 001	1.25 ml > 50 U/ml
Cat. No. 03 115 828 001	5 ml > 50 U/ml
Cat. No. 03 115 844 001	25 ml > 50 U/ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Proteinase K, recombinant, PCR Grade	▪ Subtilisin-related serine protease. ▪ Concentration of the enzyme solution is 14 to 22 mg/ml in 10 mM Tris-HCl, pH 7.5. *See the Certificate of Analysis for the concentration of the present lot.* ▪ The solution contains calcium acetate as stabilizer.	03 115 887 001	1 vial, 1.25 ml
			03 115 828 001	1 vial, 5 ml
			03 115 844 001	1 vial, 25 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Proteinase K, recombinant, PCR Grade	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

For isolation of nucleic acids
- High Pure PCR Template Preparation Kit*

For isolation of cytoplasmic RNA from cultured cells
- Nonidet P-40*
- Digestion buffer
- 0.5% SDS*

For isolation of genomic DNA from mammalian tissue
- Digestion buffer
- 0.5% SDS*

1.4. Application

Proteinase K, recombinant, PCR Grade digests native proteins very effectively and can be used in a variety of applications:
- Rapidly inactivates endogenous RNases and DNases during nucleic acid isolation; it is particularly suitable for the isolation of native RNA and DNA from tissues or cell lines.
- Promotes cell lysis by activating a bacterial autolytic factor.
- For the analysis of membrane structures by modifying proteins and glycoproteins on cell surfaces.
- Since 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.
- Removes cellular debris during the preparation of colony lifts, and to treat tissue sections to ensure efficient probe infiltration during *in situ* hybridization.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Buffer selection

The best buffer for Proteinase K varies with the application. For best results:
- Always follow the pH and temperature guidelines.
- 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.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Isolation of nucleic acids

1. Use Proteinase K with the High Pure PCR Template Preparation Kit* to isolate nucleic acids from:

Sample Material	Amount
Mammalian blood	200 µl
Buffy coat	200 µl
Cultured mammalian cells	10^4 – 10^5
Mammalian tissue	25 – 50 mg
Mouse tail	0.2 – 0.5 cm (25 – 50 mg)
Bacteria	1×10^9
Yeast cells	1×10^8
Formalin-fixed, paraffin-embedded tissue sections	25 – 50 mg

2. Add 40 µl of the Proteinase K solution from Step 1 to each sample.

3. Follow the procedure described in the Instructions for Use of the High Pure PCR Template Preparation Kit*.

Isolation of cytoplasmic RNA from cultured cells

1. Lyse cells in a buffer containing 0.5% (v/v) Nonidet P-40* (non-ionic detergent).
2. Centrifuge the lysate and transfer the supernatant to a clean tube containing 4 µl of 20% SDS*.
3. Immediately vortex the tube to mix the contents.
4. Add 2.5 µl of 20 mg/ml Proteinase K, recombinant to the tube and incubate for 15 minutes at +37°C.

Isolation of genomic DNA from mammalian tissue

1. Use any of the starting materials shown in the table:

Sample Material	Amount
Mammalian tissue	80 mg, minced
Tissue that has been frozen in liquid nitrogen	80 mg
Cultured mammalian cells	1×10^8

2. Incubate the starting material for 12 to 18 hours 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 vessels or myocardial tissue, use the following concentrations of Proteinase K, recombinant:

Sample Material	Amount [µg/ml]
Cryosections	≤2
Paraffin-embedded sections	≤20
Methacrylate-embedded sections	≤50

2.3. Parameters

Activator

To stimulate Proteinase K activity, add denaturing agents, such as SDS* and urea.

SDS can increase the activity of Proteinase K as much as sevenfold compared with the activity exhibited in the absence of SDS.

Inactivation

Autolysis

Autolysis of the enzyme occurs more rapidly at alkaline pH. However, Proteinase K is not completely inactivated by autolysis. Some enzyme fragments retain full proteolytic activity.

Inhibition

Proteinase K is inhibited by diisopropyl fluorophosphate and phenylmethylsulfonyl fluoride (PMSF). It is also totally inactivated by mercury ions. Pefabloc SC* and Pefabloc SC PLUS* are specific, irreversible, nontoxic inhibitors of Proteinase K.

Proteinase K is not inactivated by metal ions, chelating agents, such as EDTA, sulfhydryl reagents, or trypsin/chymotrypsin inhibitors.

pH Stability

The enzyme is stable from pH 4.0 to pH 12.5. It retains full activity for several hours when incubated at pH 6.5 to 9.5.

Specific Activity

≥2.5 U/mg protein when assayed with the Chromozym assay (equivalent to ≥30 U/mg with the hemoglobin assay).

See the Certificate of Analysis for specific values of the present lot.

Specificity

Proteinase K is one of the most active endopeptidases known and does not exhibit any pronounced cleavage specificity.
The enzyme cleaves proteins as shown:
X-↓-Y-, where X = aliphatic, aromatic, or hydrophobic amino acid, and
Y = any amino acid.

The enzyme can break down protein material into free amino acids when used in large excess and over long incubation periods.

Temperature Stability

The enzyme is 12 times more active at +65°C than at +25°C. However, it is rapidly denatured at temperatures above +65°C.

Unit Definition

One U is the enzyme activity which cleaves at +25°C in 1 minute 18 mmol Chromozym TRY (equivalent to 600 U/ml with the hemoglobin assay).

See the Certificate of Analysis for specific values of the present lot.

Volume Activity

≥50 U/ml

3. Additional Information on this Product

3.1. Test Principle

Characteristics

The recombinant enzyme is identical to the native protease originally isolated from the mold, *Tritirachium album*.

- Specifications of the recombinant enzyme are the same as those of the native protease.
- 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.

3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
High Pure PCR Template Preparation Kit	1 kit, up to 100 purifications	11 796 828 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Nonidet P-40 Substitute	50 ml, 5 x 10 ml	11 332 473 001
	100 ml	11 754 599 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

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

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or 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.

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