Proteinase K, recombinant, PCR Grade

From Pichia pastoris in solution

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>03 115 887 001</td>
<td>1.25 ml</td>
</tr>
<tr>
<td>03 115 828 001</td>
<td>5 ml</td>
</tr>
<tr>
<td>03 115 844 001</td>
<td>25 ml</td>
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</tbody>
</table>

1. What this Product Does

Contents and Concentration

Solution

The concentration of the enzyme solution is 14 – 22 mg/ml in 10 mM Tris-HCl, pH 7.5. Refer to the Certificate of Analysis for specific values of the concentration for the present lot. The solution contains calcium acetate as a stabilizer.

Storage and Stability

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

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 below. 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.

Typical Experiments

Isolation of nucleic acids: Use Proteinase K, recombinant, PCR grade, with the High Pure PCR Template Preparation Kit to isolate nucleic acids from:

- 200 μl mammalian blood
- 200 μluffy coat
- 10^4 – 10^5 cultured mammalian cells
- 25 – 50 mg mammalian tissue
- 0.2 – 0.5 cm (25 – 50 mg) mouse tail
- 10^3 bacteria or 10^3 yeast cells
- 25 – 50 mg formalin-fixed, paraffin-embedded tissue sections

Add 40 μl of the Proteinase K solution to each sample. Then follow the procedure described in the package insert of the High Pure PCR Template Preparation Kit.

Isolation of cytoplasmic RNA from cultured cells (5): Lyse cells in a buffer containing 0.5% (v/v) Nonidet P40* (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 × 10^6 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, Trichirachium 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 Proteinase K, recombinant, PCR grade, is DNA-free according to the current Quality Control procedures, it is especially suitable for isolating PCR and RT-PCR templates.

Enzyme Characteristics

<table>
<thead>
<tr>
<th>Volume Activity</th>
<th>≥50 U/ml. One unit is the enzyme activity which cleaves at +25°C in 1 min 18 mmol Chromozym TRY (equivalent to ≥600 U/ml with the hemoglobin assay). Refer to the Certificate of Analysis for specific values for the present lot.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Activity</td>
<td>≥2.5 U/mg, when assayed with the Chromozym assay (equivalent to ≥30 U/mg with the hemoglobin assay). Refer to the Certificate of Analysis for specific values for the present lot.</td>
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</table>

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.

For life science research only. Not for use in diagnostic procedures.
Recombinant proteinase K is stable from pH 4.0 to pH 12.5. It retains full activity for several hours when incubated at pH 8.5 – 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).

**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 seven-fold (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**

A Eco Rf/Hind 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 Ribonuclease Activity**

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 the Threshold DNA Detection Assay according to the supplier's instructions. The amount of total DNA in Proteinase K, recombinant, PCR Grade is less than 10 pg/mg enzyme.

**Bioburden**

The number of viable microorganisms is determined with a stringent bioburden test described in the European Pharmacopoeia. The total number of viable microorganisms in Proteinase K, recombinant, PCR Grade is less than 5 cfu/ml. This extremely low bioburden ensures that the product will be very stable and safe.

### References

5. Ausubel F M et al. (Eds.) Current Protocols in Molecular Biology, Wiley & Sons; (2004); Four volumes 0-471-50386-X (Looseleaf or 0-471-30661-4 CD-ROM).

### Contact and Support

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

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