PROTEINASE K, LYOPHILIZED

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-NUC-001
Product Name	Proteinase K, Lyophilized
Category	Protease / Nuclease Accessory
Pack Size	100g/bottle
Regulatory Status	For Research Use Only (RUO)
Version	1.1
Issue Date	2026-05-28

2. INTENDED USE

Enzovera Proteinase K, Lyophilized is intended for the enzymatic digestion of proteins in nucleic acid purification procedures. This robust serine protease efficiently removes protein contaminants from DNA and RNA samples while remaining active in the presence of detergents (SDS), chelating agents (EDTA), and other denaturing conditions commonly used in molecular biology workflows. Typical applications include genomic DNA extraction from tissues and cells, plasmid preparation, removal of nucleases from nucleic acid solutions, and pre-treatment of samples for PCR or sequencing. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Proteinase K, Lyophilized	100 g	-20°C
Proteinase K Reconstitution Buffer (10×)	50 mL	Room temperature (15-25°C)
Proteinase K Storage Buffer (1×)	100 mL	4°C
Calcium Chloride Solution (100 mM)	10 mL	Room temperature (15-25°C)
Product Insert with Reconstitution Protocol	1 insert	Room temperature (15-25°C)
Certificate of Analysis	1 document	Room temperature (15-25°C)

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Reconstitution buffer (sterile distilled water or 10 mM Tris-HCl, pH 7.5)
- Appropriate reaction buffer (typically 10-50 mM Tris-HCl, pH 7.5-8.0)
- Nucleic acid samples (DNA or RNA)
- Microcentrifuge tubes (1.5 mL or 2.0 mL, nuclease-free)
- Pipettes and sterile pipette tips
- Heat block or water bath (37°C to 65°C)
- Vortex mixer
- Ice bucket for storage during use

5. STORAGE AND STABILITY

Storage Temperature	-20°C, protect from light
Appearance	White or off-white lyophilized powder
Shelf Life	12 months from manufacture date
Shipping Conditions	On dry ice
Freeze-Thaw Cycles	Maximum 3 cycles recommended
Working Solution	Stable on ice for up to 8 hours

6. PRECAUTIONS AND WARNINGS

- For Research Use Only. Not for use in diagnostic procedures.
- Avoid repeated freeze-thaw cycles. Aliquot reagents if needed.
- Handle all reagents on ice. Return to -20°C storage immediately after use.
- Wear appropriate PPE: gloves, lab coat, and eye protection at all times.
- Dispose of waste in accordance with local, state, and federal regulations.
- Do not use reagents past their expiry date.

7. PROTOCOL

PROTEINASE K, LYOPHILIZED – DETAILED PROTOCOL

For Research Use Only

PRODUCT OVERVIEW

Proteinase K is a broad-spectrum serine protease isolated from the fungus Tritirachium album. It exhibits high specific activity (≥30 U/mg) and demonstrates exceptional stability in the presence of detergents (SDS, Triton X-100), chelating agents (EDTA), and denaturing conditions. The enzyme cleaves peptide bonds adjacent to the carboxyl group of aliphatic, aromatic, and hydrophobic amino acids, making it highly effective for digesting proteins that bind or contaminate nucleic acids during DNA and RNA purification procedures.

RECOMMENDED APPLICATIONS

- Genomic DNA extraction from tissues, cultured cells, blood, and buccal swabs

- Plasmid DNA purification from bacterial cultures

- Total RNA isolation from mammalian and plant tissues

- Removal of RNases and DNases from nucleic acid preparations

- Pre-treatment of samples for PCR, RT-PCR, Southern blotting, and Northern blotting

- Chromatin digestion for ChIP assays

- Protein removal from viral nucleic acid preparations

MATERIALS REQUIRED

Provided by User:

- Molecular biology grade water (nuclease-free)

- Lysis buffer appropriate for sample type (see Buffer Recommendations section)

- SDS (sodium dodecyl sulfate), 10% or 20% stock solution

- EDTA (ethylenediaminetetraacetic acid), 0.5 M stock solution, pH 8.0

- Tris-HCl buffer, 1 M stock solution, pH 7.5 to 8.0

- Sodium chloride (NaCl)

- Phenol:chloroform:isoamyl alcohol (25:24:1) or chloroform:isoamyl alcohol (24:1)

- Ethanol, absolute (molecular biology grade) and 70% ethanol

- Microcentrifuge tubes (1.5 mL or 2.0 mL, nuclease-free)

- Water bath or heating block with temperature control

- Microcentrifuge capable of 12,000 to 16,000 x g

- Vortex mixer

- Pipettes and nuclease-free pipette tips

RECONSTITUTION OF LYOPHILIZED PROTEINASE K

1. Remove the vial of lyophilized Proteinase K from storage at -20°C and allow it to reach room temperature (20 to 25°C) for approximately 10 minutes before opening. This prevents condensation from forming inside the vial.

2. Briefly centrifuge the vial at 2,000 x g for 5 seconds to collect lyophilized powder at the bottom of the tube.

3. Prepare reconstitution buffer by combining 10 mM Tris-HCl pH 7.5, 20 mM calcium chloride ($CaCl_2$), and 50% glycerol in molecular biology grade water. Calcium ions enhance enzyme stability, and glycerol prevents freezing during storage at -20°C.

4. Add the appropriate volume of reconstitution buffer to achieve the desired working concentration. For a standard working stock of 20 mg/mL, add 1.0 mL of reconstitution buffer per 20 mg of lyophilized Proteinase K. For example, if the vial contains 100 mg of enzyme, add 5.0 mL of buffer.

5. Gently mix by pipetting up and down 10 to 15 times or by gentle inversion. Do NOT vortex vigorously, as this may denature the enzyme. Avoid creating foam or bubbles.

6. Allow the solution to sit at room temperature for 5 minutes to ensure complete dissolution. Gently swirl or invert the tube 2 to 3 times.

7. Aliquot the reconstituted Proteinase K into smaller volumes (100 to 500 µL) in nuclease-free microcentrifuge tubes to avoid repeated freeze-thaw cycles. Each freeze-thaw cycle may reduce enzyme activity by 10 to 15%.

8. Label each aliquot with the concentration, reconstitution date, and lot number.

9. Store aliquots at -20°C for up to 12 months. Once thawed for use, keep on ice and return to -20°C storage within 2 hours. Do not refreeze more than 3 times.

BUFFER RECOMMENDATIONS

For Genomic DNA Extraction:

- 10 mM Tris-HCl, pH 8.0

- 100 mM NaCl

- 25 mM EDTA

- 0.5% SDS

- Add Proteinase K to final concentration of 100 to 200 µg/mL

For RNA Extraction (with subsequent DNase treatment):

- 10 mM Tris-HCl, pH 7.5

- 10 mM EDTA

- 0.5 to 1.0% SDS

- Add Proteinase K to final concentration of 100 to 400 µg/mL

For PCR Sample Preparation:

- 10 mM Tris-HCl, pH 8.0

- 50 mM KCl

- 2.5 mM $MgCl_2$

- 0.1% Triton X-100

- Add Proteinase K to final concentration of 50 to 100 µg/mL

PROTOCOL 1: GENOMIC DNA EXTRACTION FROM MAMMALIAN TISSUE

1. Weigh 10 to 25 mg of fresh or frozen tissue and transfer to a 1.5 mL microcentrifuge tube. If using frozen tissue, do not allow it to thaw before adding lysis buffer.

2. Add 500 µL of tissue lysis

8. EXPECTED RESULTS

When used at the recommended concentration of 100–200 µg/mL in nucleic acid isolation protocols, Proteinase K should exhibit complete digestion of protein contaminants within 30–60 minutes at 55–65°C, as evidenced by clear lysates and high-purity nucleic acid yields (A260/A280 ratio ≥1.8 for DNA, ≥2.0 for RNA). The enzyme remains fully active in the presence of 0.5–2% SDS, 10–20 mM EDTA, and chaotropic salts, enabling one-step lysis and deproteinization without buffer exchange. Downstream applications such as PCR, RT-PCR, restriction digestion, and sequencing should proceed without inhibition, confirming effective removal of proteinaceous RNase and DNase activity.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

Document Number	IFU-EV-NUC-001
Version	1.1
Status	DRAFT — Pending Authorisation
Issue Date	2026-05-28
Prepared By	Enzovera Life Sciences Technical Documentation
Approved By	Pending
Next Review	12 months from issue date