RECOMBINANT PORCINE TRYPSIN, LYOPHILIZED

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-PRO-001
Product Name	Recombinant Porcine Trypsin, Lyophilized
Category	Serine Protease
Pack Size	500mg/tube, 5g/bottle
Regulatory Status	For Research Use Only (RUO)
OEM Reference	GPE010001
Version	1.0
Issue Date	2026-05-14

2. INTENDED USE

Recombinant Porcine Trypsin is a serine protease with amino acid sequence identical to porcine pancreatic trypsin, specifically cleaving peptide bonds at the C-terminus of lysine and arginine residues. This animal-origin-free enzyme, produced through yeast gene recombinant expression, is intended for recombinant insulin production, cell culture dissociation and fermentation, protein digestion in proteomics workflows, and cell separation in diverse tissue types. With specific activity exceeding 10,000 U/mg lyophilized powder, this molecular biology grade trypsin provides a compliant alternative to animal-sourced enzymes for biopharmaceutical manufacturing and cell culture applications. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Recombinant Porcine Trypsin, Lyophilized	500 mg (1 tube) or 5 g (1 bottle)	-20°C

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Materials Required but Not Provided:
- Reconstitution buffer: 1 mM hydrochloric acid (pH 3.0) or 50 mM acetic acid for enzyme solubilization
- Substrate for enzymatic activity assay (e.g., N-α-benzoyl-DL-arginine-p-nitroanilide, BAPNA)
- Appropriate reaction buffer compatible with target protein or cell culture application
- Calcium chloride ($CaCl_2$) solution for enzyme stabilization during proteolysis reactions
- Serine protease inhibitors (e.g., PMSF, aprotinin) for terminating enzymatic reactions
- Protein concentration determination reagents (e.g., Bradford or BCA assay kit)
- pH meter or pH indicator strips for buffer verification
- Sterile tubes or containers for aliquoting reconstituted enzyme solutions

5. STORAGE AND STABILITY

Storage Temperature	-20°C recommended, sealed for optimal preservation
Appearance	White or off-white lyophilized powder
Shelf Life	One year from the date of manufacture
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

RECOMBINANT PORCINE TRYPSIN PROTOCOL

ENZOVERA LIFE SCIENCES

Product: Recombinant Porcine Trypsin, Lyophilized

Catalog Number: Contact Enzovera Technical Support

Specific Activity: >10,000 U/mg dry weight

Molecular Weight: ≥24 kDa

Expression System: Yeast (Pichia pastoris or Saccharomyces cerevisiae)

Storage: -20°C, sealed container, desiccated

Stability: One year from date of manufacture when stored properly

INTRODUCTION

Enzovera Recombinant Porcine Trypsin is an animal-origin-free (AOF) serine protease with amino acid sequence identical to native porcine pancreatic trypsin. This molecular biology grade enzyme specifically cleaves peptide bonds at the C-terminal side of lysine and arginine residues, making it suitable for cell dissociation, recombinant protein production, proteomics sample preparation, and biopharmaceutical manufacturing applications. The recombinant production in yeast eliminates risks associated with animal-derived materials including adventitious agents, lot-to-lot variability, and regulatory compliance challenges.

MATERIALS REQUIRED

Recombinant Porcine Trypsin, lyophilized (Enzovera)

Storage/Stock Solution Buffer: 1 mM hydrochloric acid (HCl), pH 3.0, or 50 mM acetic acid

Working Dilution Buffer: 1 mM HCl, pH 3.0

Calcium chloride (CaCl2), optional for stabilization

Phosphate-buffered saline (PBS), pH 7.4, sterile filtered

Neutralization buffer: growth medium with serum or trypsin inhibitor (for cell culture applications)

Microcentrifuge tubes, sterile

Pipettes and sterile filter tips

Ice bucket

Vortex mixer

pH meter

SAFETY PRECAUTIONS

Wear appropriate personal protective equipment including lab coat, gloves, and safety glasses.

Handle trypsin in a biological safety cabinet when used for cell culture applications.

Trypsin is a potent protease and may cause irritation to skin, eyes, and respiratory tract.

Avoid inhalation of lyophilized powder.

Activity is inhibited by serine protease inhibitors such as phenylmethylsulfonyl fluoride (PMSF).

Metal ion chelators such as EDTA can modulate enzymatic activity.

PROTOCOL

SECTION A: RECONSTITUTION AND STOCK SOLUTION PREPARATION

1. Remove the vial of lyophilized Recombinant Porcine Trypsin from -20°C storage and equilibrate to room temperature (18-25°C) for approximately 5 minutes while keeping the vial sealed to prevent moisture condensation.

2. Briefly centrifuge the vial at 1,000 x g for 10 seconds to ensure all lyophilized powder is collected at the bottom of the tube.

3. Prepare Storage Buffer by dissolving reagent-grade hydrochloric acid in ultrapure water to a final concentration of 1 mM HCl (pH 3.0). Alternatively, prepare 50 mM acetic acid in ultrapure water. Verify pH using a calibrated pH meter. Store at 4°C for up to one month or prepare fresh.

4. Calculate the volume of Storage Buffer needed to achieve the desired stock concentration. For most applications, a stock concentration of 1-10 mg/mL is recommended. Example: For 5 mg lyophilized trypsin, add 500 µL of 1 mM HCl to obtain a 10 mg/mL stock solution (approximately 100,000 U/mL based on specific activity >10,000 U/mg).

5. Using a sterile pipette tip, slowly add the calculated volume of ice-cold Storage Buffer directly to the lyophilized powder. Avoid creating foam.

6. Gently swirl or tap the vial to initiate dissolution. Do not vortex vigorously as this may denature the enzyme.

7. Incubate the vial on ice for 5-10 minutes with occasional gentle mixing by inversion or tapping.

8. Once fully dissolved, gently mix by pipetting up and down 3-5 times or invert the tube 10 times. Avoid introducing air bubbles.

9. Inspect the solution for complete dissolution. The solution should be clear to slightly opalescent with no visible particulates.

10. If sterility is required for cell culture applications, filter the reconstituted trypsin solution through a sterile 0.22 µm low-protein-binding syringe filter into a sterile microcentrifuge tube. Perform filtration on ice.

SECTION B: WORKING SOLUTION PREPARATION

11. Prepare Working Dilution Buffer consisting of 1 mM HCl, pH 3.0, as described in Step 3. Keep buffer on ice.

12. Calculate the required activity or concentration for your specific application. For cell dissociation, typical working concentrations range from 0.05% to 0.25% (w/v) (approximately 500-2,500 U/mL). For protein digestion, concentrations of 10-100 µg/mL are commonly used.

13. Dilute the stock solution in ice-cold Working Dilution Buffer to achieve the desired working concentration. Perform serial dilutions if necessary to maintain accuracy.

14. For cell culture applications, transfer the working trypsin solution to a sterile container and warm to 37°C in a water bath or incubator for 5-10 minutes immediately before use.

15. If calcium stabilization is desired, supplement the working solution with 1-5 mM CaCl2. Calcium ions enhance trypsin stability but are not required for enzymatic activity.

SECTION C: CELL DISSOCIATION PROTOCOL

16. Remove culture

8. EXPECTED RESULTS

When reconstituted and assayed under standard conditions (pH 8.0, 37°C, BAEE substrate), Recombinant Porcine Trypsin demonstrates specific activity >10,000 U/mg dry weight with selective cleavage at the C-terminal side of lysine and arginine residues. SDS-PAGE analysis confirms molecular mass ≥24 kDa with isoelectric point of 8.9, consistent with native porcine pancreatic trypsin structure. The enzyme exhibits typical serine protease inhibition profile with PMSF and activity modulation by EDTA, suitable for cell dissociation (≥95% viability in adherent mammalian cultures), proteomics-grade protein digestion, and biomanufacturing applications including recombinant insulin production.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

Document Number	IFU-EV-PRO-001
Version	1.0
Status	DRAFT — Pending Authorisation
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