RECOMBINANT HUMAN TYPE III COLLAGEN (55.9 KDA)

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-COL-003
Product Name	Recombinant Human Type III Collagen (55.9 kDa)
Category	Structural Protein / Collagen
Pack Size	50g/bag, 100g/bag
Regulatory Status	For Research Use Only (RUO)
OEM Reference	GPE012003
Version	1.0
Issue Date	2026-05-14

2. INTENDED USE

Recombinant Humanized Type III Collagen is a high-purity (≥95%) structural protein derived from a yeast expression system containing the cloned human collagen type III gene, designed for research and development applications in tissue engineering, medical device development, wound healing studies, and regenerative medicine. This 55.9 kDa water-soluble collagen exhibits excellent hydrophilicity, biocompatibility, and permeability, making it suitable for 3D bioprinting matrices, scaffold fabrication, skin hydration research, and tissue repair investigations. The product is manufactured using a 28,000-liter yeast expression platform with advanced chromatographic purification, providing a virus-free, animal-free collagen source with no risk of immunogenic reactions and optimal performance in pH range 5–8. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Recombinant Human Type III Collagen, lyophilized	50 g or 100 g (depending on pack size)	-20°C, dry and sealed
Reconstitution Buffer (10x PBS, pH 7.4)	100 mL	2-8°C
Sterile Water for Injection, WFI grade	50 mL	Room temperature (15-25°C)
Desiccant Packs (silica gel)	2 packs	Room temperature (15-25°C)
Certificate of Analysis (CoA)	1 document (lot-specific)	N/A
Instructions for Use (IFU)	1 document	N/A

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Materials Required But Not Provided:
- Sterile phosphate buffered saline (PBS) or ultrapure water for reconstitution
- Sterile microcentrifuge tubes or vials for aliquoting reconstituted collagen
- Low-protein binding pipette tips for accurate dispensing
- pH-adjusted buffer system (pH 5-8) appropriate for downstream application
- Desiccant storage containers for protecting lyophilized powder from moisture absorption
- Cell culture media or formulation base for experimental applications

- Analytical balance accurate to 0.1 mg for weighing lyophilized material
- -20°C freezer with temperature monitoring for long-term storage

5. STORAGE AND STABILITY

Storage Temperature	Store at -20°C in dry, sealed condition; refrigeration temperature recommended
Appearance	White or off-white lyophilized collagen sponge
Shelf Life	1 year under proper storage conditions
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

PROTOCOL: RECOMBINANT HUMAN TYPE III COLLAGEN (55.9 kDa)

PREPARATION AND APPLICATION PROCEDURE

Product: Recombinant Humanized Type III Collagen

Catalog Number: GPE012003

Molecular Weight: 55.9 kDa

Purity: ≥95% (SDS-PAGE), Protein content ≥90%

For Research Use Only

OVERVIEW:

This protocol describes the reconstitution, handling, and application of recombinant humanized Type III collagen derived from yeast expression system. The product is suitable for 3D bioprinting matrices, large-area wound healing scaffolds, tissue engineering constructs, skin hydration studies, and tissue repair research applications.

MATERIALS REQUIRED:

- Recombinant Human Type III Collagen, lyophilized powder

- Sterile ultrapure water (18.2 MΩ·cm, nuclease-free)

- Sterile phosphate buffered saline (PBS), pH 7.4, or appropriate buffer (pH 5-8 range)

- Sterile microcentrifuge tubes (1.5-2.0 mL)

- Sterile pipettes and filter tips

- Refrigerated microcentrifuge

- Vortex mixer

- pH meter (calibrated)

- Sterile culture vessels or scaffolds (application-dependent)

- Personal protective equipment (lab coat, gloves, safety glasses)

SAFETY PRECAUTIONS:

1. Handle lyophilized protein powder in a laminar flow hood or biosafety cabinet to maintain sterility and prevent moisture absorption.

2. Wear appropriate personal protective equipment throughout the procedure.

3. This product is virus-free with no risk of immunogenic reactions; however, follow standard biosafety practices for recombinant protein handling.

4. Avoid repeated freeze-thaw cycles which may compromise protein structure and bioactivity.

RECONSTITUTION PROCEDURE:

1. Remove the vial of lyophilized Recombinant Human Type III Collagen from -20°C storage and allow it to equilibrate to room temperature (20-25°C) for 10-15 minutes while keeping the vial sealed to prevent moisture absorption.

2. Briefly centrifuge the sealed vial at 1000 × g for 30 seconds to collect all lyophilized material at the bottom of the tube.

3. Calculate the required volume of sterile ultrapure water or buffer to achieve the desired final concentration. Recommended starting concentration: 0.5-2.0 mg/mL for most applications. Higher concentrations (2-5 mg/mL) may be used for bioprinting or high-density scaffold fabrication.

4. Aseptically open the vial in a laminar flow hood and add the calculated volume of sterile ultrapure water or buffer (pH 5-8) directly onto the lyophilized powder. For a 1 mg vial, add 500 µL to 1000 µL for 1-2 mg/mL concentration.

5. Close the vial and gently swirl or rotate the tube for 2-3 minutes to initiate dissolution. Avoid vigorous vortexing which may denature the collagen or create excessive foam.

6. Allow the solution to stand at room temperature (20-25°C) for 10-15 minutes to ensure complete hydration of the water-soluble collagen protein.

7. Gently pipette the solution up and down 5-10 times using a sterile pipette to ensure homogeneous mixing. The solution should appear clear to slightly opalescent.

8. If any undissolved particles are visible, centrifuge the solution at 10,000 × g for 5 minutes at 4°C and transfer the clear supernatant to a fresh sterile tube. Discard any insoluble pellet.

9. Measure the pH of the reconstituted solution using a calibrated pH meter. Verify that pH is within the range of 5-8 (optimal range for Type III collagen stability). Adjust if necessary using sterile 0.1 M HCl or 0.1 M NaOH, dropwise with continuous monitoring.

QUALITY VERIFICATION:

10. Prepare a small aliquot (10-20 µL) for protein concentration determination using UV spectrophotometry at 280 nm or Bradford/BCA protein assay according to manufacturer instructions. Verify that protein content is ≥90% of expected value.

11. If required, verify purity by SDS-PAGE analysis. Prepare sample in reducing conditions (with β-mercaptoethanol or DTT) and run on 10-12% polyacrylamide gel. Collagen should migrate as a single predominant band at approximately 55.9 kDa with purity ≥95%.

PREPARATION FOR SPECIFIC APPLICATIONS:

FOR 3D BIOPRINTING MATRICES:

12. Prepare bioink formulation by combining reconstituted Type III collagen (2-5 mg/mL final concentration) with appropriate crosslinking agents (e.g., genipin 0.1-0.5%, transglutaminase 0.5-2

U/mL) or blending with other biopolymers (alginate, hyaluronic acid) according to your specific bioprinting protocol.

13. Maintain the bioink at 4°C until loading into bioprinter cartridge to prevent premature gelation or aggregation.

14. Load bioink into sterilized bioprinter cartridge and proceed with printing according to equipment specifications, typically at controlled temperature (10-25°C) and controlled extrusion pressure.

15. After printing, allow printed constructs to stabilize for 30-60 minutes at room temperature or initiate crosslinking procedure as required by your specific application.

FOR WOUND HEALING SCAFFOLDS:

16. Prepare scaffold coating solution by diluting reconstituted Type III collagen to working concentration of 0.5-1.0 mg/mL in sterile PBS

8. EXPECTED RESULTS

Expected Results

When analyzed by SDS-PAGE under reducing conditions, Recombinant Human Type III Collagen migrates as a single dominant band at approximately 55.9 kDa with purity ≥95%, and total protein content is ≥90% as determined by Bradford or BCA assay. The lyophilized product exhibits complete water solubility at pH 5–8 (pI 6.59), forming clear, colorless solutions suitable for 3D bioprinting, scaffold fabrication, and tissue engineering applications. Quality control testing confirms endotoxin levels ≤10 EU/mg and bioburden ≤5 cfu/g, consistent with recombinant yeast expression from a 28,000-liter platform using cloned human COL3A1 gene sequences.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

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