

Certificate of Analysis

CORNING® COLLAGEN I High Concentration (HC), RAT TAIL

Collagen I is found in most tissues and organs, but is most plentiful in dermis, tendon and bones. Type I molecule is a heterotrimer [$\alpha_1(I)_2 \alpha_2(I)$] of 300 nm length being composed of two $\alpha_1(I)$ chains and one $\alpha_2(I)$ chain.¹ Collagen binding integrin receptors are $\alpha_1 \text{Beta}_1$, $\alpha_2 \text{Beta}_1$, and $\alpha_3 \text{Beta}_1$.² When used as a gel, collagen facilitates successful adaptation *in vitro* culture and enhances expression of cell-specific morphology and function. The Collagen HC is used in applications where a sturdy gel provides maximal support to maintain the three dimensional structure.³⁻⁵

CATALOG NUMBER: 354249 LOT NUMBER: _____
 SOURCE: Rat tail tendon
 QUANTITY: 100 milligrams protein (measured by Pyrochemiluminescence)
 CONCENTRATION: _____ mg/mL
 FORMULATION: 0.02 N Acetic Acid
 USE: Corning Collagen I HC, rat tail, is used as a three dimensional gel. Please see reverse for gelling procedures. Use these as guidelines only - we recommend that each investigator empirically determine the optimal conditions for their unique applications.
 QUALITY CONTROL: $\geq 90\%$ by SDS PAGE.

This product has been tested for its ability to promote the attachment and spreading of HT-1080 Human Fibrosarcoma cells.

Corning Collagen I HC, rat tail, is a membrane-filtered (0.2 micron) preparation. Tested and found negative for the presence of bacteria, fungi and mycoplasma.

STORAGE: Stable when stored at 2-8°C. **DO NOT FREEZE.**

On release this product has been successfully gelled over a wide range of dilutions and will form a gel up to a concentration of 0.3 mg/mL. Further dilution may decrease the rigidity of the gel as will the time from manufacture.

EXPIRATION DATE: _____

REFERENCES: 1. Linsenmayer, T.F., Collagen, in Cell Biology of Extracellular Matrix (ed., E.D. Hay) pp 5-37, Plenum Press, NY (1991).
 2. Chan, B.M., and Hemler, M.E., *J. Cell Biol.*, **120**:537 (1993).
 3. Gautreau, A., et.al., *PNAS*, **96**:7300 (1999).
 4. Abir, R., et.al., *Hum. Reprod.*, **14**:1299 (1999).
 5. Abir, R., et.al., *Fertil. Steril.*, **75**:141 (2001).

Suggested Coating Procedures

Gelling Procedure- Corning Collagen I HC, rat tail, will gel when its pH is brought to alkalinity using the procedure below. Please use this as a guideline for determining the optimum concentration for your application.

- 1) Prepare ammonia vapor chamber by taping a sterile 2 inch gauze sponge to the inside lid of a 150 mm petri dish. Saturate the gauze with ammonium hydroxide. Place lid on 150 mm dish and set aside.

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- 2) Place an even coating of Corning® Collagen I HC, rat tail, on surface to be coated. Thickness may be varied as desired. 50-100 µl of Corning Collagen I HC, rat tail, is sufficient to coat a 22 mm coverslip. For dishes of 100 mm diameter, add approximately 6.0 mL per dish; for 60 mm dishes add approximately 2.3 mL and for 35 mm dishes add approximately 1.0 mL.
- 3) Transfer coated coverslips or dishes with lids off to ammonia vapor chamber and expose for three minutes.
- 4) Soak coated coverslip or dishes in sterile dH₂O for 30 minutes (5 mL for 35 mm dishes, 10 mL for 60 mm dishes, etc.). Aspirate and replace with 0.5-1.0 mL of sterile dH₂O and let sit overnight lidded in a laminar flow hood.
- 5) Aspirate the dH₂O and replace with serum supplemented balanced salt solution and store at 2-8°C.

Alternate Gelation Procedure for Corning Collagen I HC, Rat tail

- 1.0 Place on ice the following:
 - 1.1 Corning Collagen I HC, rat tail
 - 1.2 Sterile 10X phosphate buffered saline (10X PBS)
 - 1.3 Sterile dH₂O
 - 1.4 Sterile 1 N NaOH
- 2.0 Determine the final volume of Corning Collagen I HC, rat tail, solution to be used and the desired final collagen concentration.
- 3.0 Place on ice a sterile tube of sufficient capacity to contain the final volume of Corning Collagen I HC, rat tail.
- 4.0 Perform the following steps using aseptic technique in a Class 100 Hood.
 - 4.1 Add to the tube the following volume of 10X PBS:

$$\frac{\text{Final Volume}}{10} = \text{mL 10X PBS}$$
 - 4.2 Calculate the volume of Corning Collagen I HC, rat tail, to be used (do not add to the tube until step 4.6):

$$\frac{\text{Final volume} \times \text{Final collagen concentration in mg/mL}}{\text{Concentration in bottle (see lot specific spec. sheet)}} = \text{volume collagen to be added}$$
 - 4.3 Add to the 10X PBS the following volume of sterile ice cold 1 N NaOH:

$$(\text{volume collagen to be added}) \times 0.023 \text{ mL} = \text{volume 1 N NaOH}$$
 - 4.4 Add to the 10X PBS/1 N NaOH the following volume of sterile ice-cold dH₂O:

$$(\text{Final volume}) - (\text{Volume collagen}) - (\text{Volume 10X PBS}) - (\text{Volume 1 N NaOH}) = \text{Volume dH}_2\text{O to add}$$

- 4.5 Mix the contents of tube and hold in ice.
- 4.6 Add the calculated volume of Corning® Collagen I HC, rat tail, and mix. Leave on ice until ready for use.

- 5.0 The Corning Collagen I HC, rat tail, solution can be used immediately or held on ice for 2-3 hours.
- 6.0 When ready to use, aseptically deliver the solution into the cell culture device and allow to gel at 37°C for 30 minutes.

NOTE: For more details on Corning Collagen products and technical resources please visit support page at www.corning.com/lifesciences

Quality Assurance

Date