

**Certificate of Analysis****CORNING® COLLAGEN I, RAT TAIL**

Collagen I is found in most tissues and organs, but is most plentiful in dermis, tendon and bones. The 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.<sup>1,2</sup> Collagen binding integrin receptors are  $\alpha_1 \text{Beta}_1$ ,  $\alpha_2 \text{Beta}_1$ , and  $\alpha_3 \text{Beta}_1$ .<sup>3</sup> When used as a gel, collagen facilitates successful adaptation *in vitro* culture and enhances expression of cell-specific morphology and function. Collagen may also be used in a thin layer to promote attachment. Applications include the study of tumor cell invasion and migration,<sup>4,5</sup> fibrillogenesis studies,<sup>6</sup> culture and/or differentiation of monocytes and/or macrophages,<sup>7</sup> and autoradiographic studies of granulocytes and macrophages.<sup>8</sup> Collagen I is also used in the maintenance of hepatocyte function, state of differentiation and elevated levels of liver cell gene transcription.<sup>9,10</sup> Collagen gels will maintain the differentiated state of cultured avian skeletal myotubes,<sup>11</sup> and can be used to study secretory epithelium<sup>12</sup> and growth patterns of normal and neoplastic mammary cells.<sup>13,14</sup>

CATALOG NUMBER: 354236 LOT NUMBER: 8169008

SOURCE: Rat tail tendon

QUANTITY: 100 milligrams protein (measured by Pyrochemiluminescence)

CONCENTRATION: 3.50 mg/mL

FORMULATION: 0.02 N Acetic acid

USE: Corning Collagen I, rat tail, may be used as a gel or as a thin coating. Please see reverse for coating 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, 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 firm gel up to a dilution of 1:10. Further dilution may decrease the rigidity of the gel as will the time from manufacture.

EXPIRATION DATE: September 2, 2020

## REFERENCES:

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2. Linsenmayer, TF. Collagen, in Cell Biology of Extracellular Matrix (ed., E.D. Hay) pp 5-37, Plenum Press, NY (1991).
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4. De Wever, O., et.al., *Int. J. Dev. Biol.*, **54**:887 (2010).
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**SAFETY RECOMMENDATION:** Handle in accordance with good industrial hygiene and laboratory safety practices.

### Suggested Coating Procedures

Corning® Collagen I, rat tail, may be gelled onto coverslips or tissue culture dishes, or used as a thin coating for cell attachment. Cells may be cultured on top of the gel, within the gel, or between gel layers.

Thin Coating - We recommend using Corning Collagen I, rat tail, as a thin coating at 5 µg/cm<sup>2</sup>. Please use this as a guideline for determining the optimum concentration for your application.

- 1) Dilute material to 50 µg/mL using 0.02 N acetic acid. Corning Collagen I, rat tail, is insoluble at neutral pH.
- 2) Add enough diluted material to coat dishes with 5 µg/cm<sup>2</sup>.

For example: A 35 mm dish has a surface area of approximately 10 cm<sup>2</sup>. One to two ml of the above solution would be sufficient to cover the dish.

- 3) Incubate at room temperature for one hour.
- 4) Carefully aspirate remaining solution.
- 5) Rinse well to remove acid, using PBS or serum free medium.
- 6) Plates may be used immediately or may be air dried. They may be stored at 2-8°C for up to one week under sterile conditions.

Gelling Procedure - Corning Collagen I, rat tail, will gel when its pH is brought to alkalinity using the procedure below;

- 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.

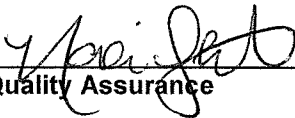
- 2) Place an even coating of Corning Collagen I, rat tail, on surface to be coated. Thickness may be varied as desired. 50-100 µl of Corning Collagen I, rat tail, is sufficient to coat a 22 mm coverslip. For dishes of 100 mm diameter, add approximately 6.0 mL per dish; for 60 mm dishes add approximately 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 dH<sub>2</sub>O 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 dH<sub>2</sub>O and let sit overnight lidded in a laminar flow hood.
- 5) Aspirate the dH<sub>2</sub>O and replace with serum supplemented balanced salt solution and store at 2-8°C.

#### Alternate Gelation Procedure for Corning® Collagen I, Rat tail

- 1.0 Place on ice the following:
  - 1.1 Corning Collagen I, rat tail
  - 1.2 Sterile 10X phosphate buffered saline (10X PBS)
  - 1.3 Sterile dH<sub>2</sub>O
  - 1.4 Sterile 1 N NaOH
- 2.0 Determine the final volume of Corning Collagen I, 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, 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, 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 dH<sub>2</sub>O:
 
$$(\text{Final volume}) - (\text{Volume collagen}) - (\text{Volume 10X PBS}) - (\text{Volume 1 N NaOH}) = \text{Volume dH}_2\text{O to add}$$
  - 4.5 Mix the contents of tube and hold in ice.
  - 4.6 Add the calculated volume of Corning Collagen I, rat tail, and mix. Leave on ice until ready for use.

- 5.0 The Corning Collagen I, 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](http://www.corning.com/lifesciences)

  
Quality Assurance

September 04, 2018  
Date