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.[1,2] Collagen binding integrin receptors are $alpha_1$ $Beta_1$, $alpha_2$ $Beta_1$, and $alpha_3$ $Beta_1$.[3] 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,[4,5] fibrillogenesis studies,[6] culture and/or differentiation of monocytes and/or macrophages,[7] and autoradiographic studies of granulocytes and macrophages.[8] Collagen I is also used in the maintenance of hepatocyte function, state of differentiation and elevated levels of liver cell gene transcription.[9,10] Collagen gels will maintain the differentiated state of cultured avian skeletal myotubes,[11] and can be used to study secretory epithelium[12] and growth patterns of normal and neoplastic mammary cells.[13,14]

CATALOG NUMBER:	354236	LOT NUMBER: 5064009
SOURCE:	Rat tail tendon	
QUANTITY:	100 milligrams protein (measured by Pyrochemiluminescence)	
CONCENTRATION:	3.26 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:	≥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:	April 25, 2017	

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For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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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^2. 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^2.

   For example: A 35 mm dish has a surface area of approximately 10 cm^2. 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_2O 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_2O and let sit overnight lidded in a laminar flow hood.

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5) Aspirate the dH_2O 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_2O
- 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):

$$\frac{\text{Final volume x Final collagen concentration in mg/mL}}{\text{Concentration in bottle (see lot specific spec. sheet)}} = \text{volume collagen to be added}$$

4.3 Add to the 10X PBS the following volume of sterile ice cold 1 N NaOH:

(volume collagen to be added) x 0.023 mL = volume 1 N NaOH

4.4 Add to the 10X PBS/1 N NaOH the following volume of sterile ice-cold dH_2O:

(Final volume) - (Volume collagen) – (Volume 10X PBS) - (Volume 1 N NaOH) = Volume dH_2O 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

Quality Assurance **Date** 08/31/15

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