

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 $\alpha_1 \text{Beta}_1$, $\alpha_2 \text{Beta}_1$, and $\alpha_3 \text{Beta}_1$.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 epithelium¹² and growth patterns of normal and neoplastic mammary cells.^{13,14}

CATALOG NUMBER:	354236	LOT NUMBER: 7247004
SOURCE:	Rat tail tendon	
QUANTITY:	100 milligrams protein (measured by Pyrochemiluminescence)	
CONCENTRATION:	4.0 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:	<p>≥90 % by SDS PAGE.</p> <p>This product has been tested for its ability to promote the attachment and spreading of HT-1080 Human Fibrosarcoma cells.</p> <p>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.</p>	
STORAGE:	<p>Stable when stored at 2-8°C. DO NOT FREEZE.</p> <p>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.</p>	
EXPIRATION DATE:	October 17, 2019	

REFERENCES:

1. Kuhn, K. The Classical Collagens: Type I, II and III in Structure and Function of Collagen Types (R. Mayne and R. E. Burgeson, eds.) pp 1-42, Academic Press, NY (1987).
2. Linsenmayer, TF. Collagen, in Cell Biology of Extracellular Matrix (ed., E.D. Hay) pp 5-37, Plenum Press, NY (1991).
3. Chan, B.M., and Hemler, M.E., *J. Cell Biol.*, **120**:537 (1993).
4. De Wever, O., et.al., *Int. J. Dev. Biol.*, **54**:887 (2010).
5. Baker, E.L., et.al., *PLoS One.*, **6**:e20355 (2011).
6. Gobeaux, F., et.al., *J. Mol. Biol.*, **376**:1509 (2008).
7. Wesley, R.B. II., et.al., *Arterioscler. Thromb. Vasc. Biol.*, **18**:432 (1998).
8. Izumi, T., et.al., *J. Cell. Physiol.*, **126**:155 (1986)
9. Sidhu, J.S., et.al., *Arch. Biochem. Biophys.*, **301**:103 (1993).
10. Gómez-Lechón, M.J., *J. Cell Physiol.*, **177**:553 (1998).
11. Vandeburgh, H.H., et.al., *In Vitro Cell Dev. Biol.*, **24**:166 (1988).
12. Hall, H.G., and Bissell, M.J., *Exp. Cell Res.*, **162**:379 (1986).
13. Azzam, H.S., and Thompson, E.W., *Cancer Res.*, **52**:4540 (1992).
14. Streuli, C.H., et.al., *J. Cell. Biol.*, **120**:253 (1993).

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/cm². 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/cm².

For example: A 35 mm dish has a surface area of approximately 10 cm². 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 μ l 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 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, 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 dH₂O
 - 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):

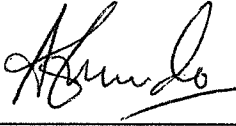
$$\frac{\text{Final volume} \times \text{Final collagen concentration in mg/mL}}{\text{Concentration in bottle (see lot specific spec. sheet)}} = \text{volume collagen to be added}$$
 - 4.3 Add to the 10X PBS the following volume of sterile ice cold 1 N NaOH:

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

October 16, 2017
Date