

## Certificate of Analysis

### CORNING® COLLAGEN I, BOVINE

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:	354231	LOT NUMBER: 6300001
SOURCE:	Bovine dermis	
QUANTITY:	30 milligrams	
CONCENTRATION:	3 . 1 mg/mL	
FORMULATION:	0.01 N Hydrochloric acid (HCl)	
USE:	Corning Collagen I, bovine, may be used as a gel or as a thin coating. Please see reverse for coating procedures. These are guidelines only - we recommend that each laboratory empirically determine the optimal conditions for their unique applications.	
PURITY:	> 95% by SDS PAGE.	
QUALITY CONTROL:	Corning Collagen I, bovine, has been successfully gelled by exposure to ammonia vapors from solutions containing as little as 0.5 mg/mL. Gel stability however diminishes with decreasing collagen concentration, and a 0.5 mg/mL gel is fragile.	
	<p><b>NOTE:</b> The collagen in this vial is the native molecule. It has been pepsin treated, however, and contains a small amount of nicked or shortened sequences.</p> <p>Tested and found negative for the presence of bacteria, fungi and mycoplasma.</p>	
STORAGE:	Stable when stored at 2-8°C. <b>DO NOT FREEZE.</b>	
EXPIRATION DATE:	January 9, 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, T.F., 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. Vandenburgh, 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, bovine, 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, bovine, as a thin coating at 5-10 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 0.01 N HCl.
- 2) Add enough diluted material to coat dishes with 5-10 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 milliliters 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 air dried. They may then be stored at 2-8°C for up to one week under sterile conditions.

Gelling Procedure - Corning Collagen I, bovine, will gel when its pH is brought to neutrality using the procedure outlined 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) Using aseptic technique, add sufficient volume of Corning Collagen I, bovine, to sterile glass or polystyrene culture dishes, spreading with sterile pipette to evenly cover entire growth surface. For

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dishes of 100 mm diameter add approximately 1.0 milliliter per dish; for 60 mm dishes add approximately 0.5 milliliter, and for 35 mm dishes add approximately 0.2 milliliter.

- 3) Expose Corning Collagen I, bovine, coated dishes to ammonia vapor by placing the coated dishes with their lids off inside the 150 mm dish.
- 4) Expose for two minutes, and remove Corning<sup>®</sup> Collagen I, bovine, coated dishes from chamber. Do not allow dishes to dry out at any point during this process.
- 5) Rinse dishes twice to remove the ammonium hydroxide, using phosphate buffered saline or sterile serum-free medium. Be careful not to dislodge the Corning Collagen I, bovine, coating.
- 6) Dishes are now ready for use.

#### An Alternative to the Ammonium Hydroxide Method

- 1) Prepare neutralized isotonic Corning Collagen I, bovine, solution by mixing 8 parts chilled Corning Collagen I, bovine, solution to one part 0.01 M NaOH and 1 part 10X phosphate buffered saline or 10X buffered serum-free cell culture medium.
- 2) Adjust the pH of the solution to  $7.4 \pm 0.2$  using 0.1 N HCl or 0.1 M NaOH. Use either pH paper or phenol red to monitor the pH. Add the phenol red to the 10X PBS to a concentration of 5 µg/mL.
- 3) This diluted material may be used right away or stored at 2-8°C for several hours.
- 4) When ready for gelation, place desired amount of Corning Collagen I, bovine, in appropriate vessel and incubate at 37°C for 10-20 minutes. Corning Collagen I, bovine, should gel within this time frame and is ready to use.

#### Fibrillar Collagen Gel Preparation

- 1) Add neutralized Corning Collagen I, bovine, solution as prepared above to a thickness of 1.0-2.0 mm.
- 2) Incubate at 37°C for 10-20 minutes to promote gelation.
- 3) Leave dish uncovered in the laminar flow hood overnight or until dry.
- 4) Rinse remaining film with dH<sub>2</sub>O to remove excess salt and to rehydrate the Corning Collagen I, bovine, gel.
- 5) Plates may be used immediately or dried again and stored up to two weeks at 2-8°C.

**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)

**Use restriction for Europe and the United Kingdom:** This product may only be used as *in vitro* laboratory reagent. This product and its residue must not be allowed to come into contact with ruminating animals or swine.

#### Quality Assurance

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