

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.^{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:	354231	LOT NUMBER: 0069001
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.	
	NOTE: 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.	
	Tested and found negative for the presence of bacteria, fungi and mycoplasma.	
STORAGE:	Stable when stored at 2-8°C. DO NOT FREEZE.	
EXPIRATION DATE:	March 15, 2022	

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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/cm². 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/cm².

For example: A 35 mm dish has a surface area of approximately 10 cm². 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® 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

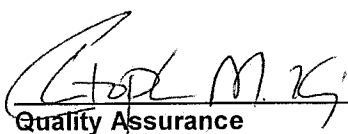
- 1) Prepare neutralized isotonic Corning Collagen I, bovine, solution by mixing 8 parts chilled Corning Collagen I, bovine, solution to one part 0.01 M NaOH and 1 part 10X phosphate buffered saline or 10X buffered serum-free cell culture medium.
- 2) Adjust the pH of the solution to 7.4 ± 0.2 using 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 dH₂O 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

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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February 13, 2020
Date

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