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 $alpha_1$ $Beta_1$, $alpha_2$ $Beta_1$, 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:	354231	LOT NUMBER: 7261003
SOURCE:	Bovine dermis	
QUANTITY:	30 milligrams	
CONCENTRATION:	3.0 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:	October 9, 2019	

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)
CLSTechServ@Corning.com www.corning.com/lifesciences

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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/cm^2. Please use this as a guideline for determining the optimum concentration for your application.

1) Dilute material to 50 µg/mL using 0.01 N HCl.

2) Add enough diluted material to coat dishes with 5-10 µg/cm^2.

   For example: A 35 mm dish has a surface area of approximately 10 cm^2. 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

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 $\pm$ 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_2O 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 **Date** 9/22/17

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