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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<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® 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

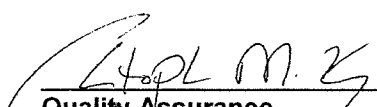
- 1) Prepare neutralized isotonic Corning Collagen I, bovine, solution by mixing 8 parts chilled Corning Collagen I, bovine, solution to one part 0.01 M NaOH and 1 part 10X phosphate buffered saline or 10X buffered serum-free cell culture medium.
- 2) Adjust the pH of the solution to  $7.4 \pm 0.2$ 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