



- 4.4 Add to the 10X PBS/1 N NaOH the following volume of sterile ice-cold dH<sub>2</sub>O:
- (Final volume) - (Volume collagen) – (Volume 10X PBS) - (Volume 1 N NaOH) = Volume dH<sub>2</sub>O to add
- 4.5 Mix the contents of tube and hold in ice.
- 4.6 Add the calculated volume of Corning® Collagen I HC, rat tail, and mix. Leave on ice until ready for use.
- 5.0 The Corning Collagen I HC, 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](http://www.corning.com/lifesciences)

  
Quality Assurance

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Date

### Suggested Coating Procedures

Gelling Procedure- Corning Collagen I HC, rat tail, will gel when its pH is brought to alkalinity using the procedure below. Please use this as a guideline for determining the optimum concentration for your application.

- 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 HC, rat tail, on surface to be coated. Thickness may be varied as desired. 50-100 µl of Corning Collagen I HC, 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 dH<sub>2</sub>O 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 dH<sub>2</sub>O and let sit overnight lidded in a laminar flow hood.
- 5) Aspirate the dH<sub>2</sub>O and replace with serum supplemented balanced salt solution and store at 2-8°C.

### Alternate Gelation Procedure for Corning Collagen I HC, Rat tail

- 1.0 Place on ice the following:
  - 1.1 Corning Collagen I HC, rat tail
  - 1.2 Sterile 10X phosphate buffered saline (10X PBS)
  - 1.3 Sterile dH<sub>2</sub>O
  - 1.4 Sterile 1 N NaOH
- 2.0 Determine the final volume of Corning Collagen I HC, 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 HC, 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 HC, 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:
 
$$(\text{volume collagen to be added}) \times 0.023 \text{ mL} = \text{volume 1 N NaOH}$$