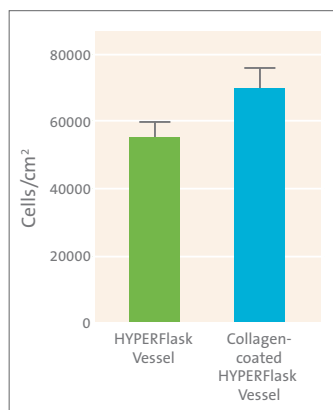


# Method for Coating Corning® HYPERFlask® Vessels with Collagen or Gelatin

CORNING

## Protocol



HEK293 cell yields after 4 days of growth on coated and uncoated Corning HYPERFlask vessels. Error bars are the result of 3 separate studies (n = 9).

## Introduction

There are many procedures that can be used to coat flasks with collagen, gelatin, or other biological coatings. The following protocols are designed to produce thin coatings to aid in cell attachment and spreading. We recommend using a higher volume of diluted coating solution to make sure the HYPERFlask is completely coated. The final  $\mu\text{g}/\text{cm}^2$  will be the same as a standard vessel but a larger volume of more dilute coating solution will be required.

## Collagen

### Materials

- ▶ Collagen I, rat tail (Corning 354236)
- ▶ Phosphate buffered saline (PBS) (Corning 21-030-CM)
- ▶ Corning HYPERFlask vessel (Corning 10034)

### Procedure

**NOTE:** Due to the viscosity of most collagen, it is recommended to make a diluted stock solution first then use this solution (listed below) to make a working solution at the final desired concentration.

**NOTE:** Different cell lines will require different collagen coating densities to obtain the desired results. Optimizing the collagen concentration and coating time for your cell line and experimental needs are recommended.

1. Dilute collagen solution to desired concentration with PBS.

Example of calculations to coat one HYPERFlask vessel at  $10 \mu\text{g}/\text{cm}^2$  from a 2 mg/mL working solution:

Convert desired coating density (collagen/ $\text{cm}^2$ ) to collagen concentration (collagen/mL):

$$\frac{1720 \text{ cm}^2 \times 10 \mu\text{g}/\text{cm}^2}{560 \text{ mL}} = 30.71 \mu\text{g}/\text{mL}$$

Calculate total collagen needed to make 560 mL of coating solution:

$$560 \text{ mL} \times 30.71 \mu\text{g}/\text{mL} = 17200 \mu\text{g of collagen}$$

Calculate volume of stock solution needed:

$$\frac{17.2 \text{ mg}}{2 \text{ mg/mL}} = 8.6 \text{ mL of 2 mg/mL stock solution}$$

Combine: 8.6 mL stock solution with 551.4 mL PBS = 560 mL.

2. Add 560 mL of diluted collagen solution to the HYPERFlask vessel to fill it completely.

**NOTE:** To achieve a uniform coating, the HYPERFlask vessel must be filled completely.

3. Incubate the HYPERFlask vessel at room temperature for the desired length of time (typically 2 hours).

**NOTE:** If a  $37^\circ\text{C}$  incubation is preferred, the HYPERFlask vessel must be filled completely to prevent pressure buildup within the vessel from the air heating and expanding.

4. After incubation, aspirate or pour collagen solution from the HYPERFlask vessel and wash once with 50 mL of PBS, making sure that all layers of the HYPERFlask are washed appropriately. The vessel is now ready for use or storage at  $4^\circ\text{C}$  until needed.

## Gelatin

### Materials

- ▶ Porcine gelatin (MilliporeSigma G1890-100G)
- ▶ Cell culture grade water (Corning 25-055-CV)
- ▶ Corning® HYPERFlask® vessel (Corning 10034)

### Procedure

**NOTE:** Different cell lines may require different gelatin coating concentrations to obtain the desired results. Optimizing the concentration and coating time for your cell line and experimental needs is recommended.

1. Make gelatin solution to desired concentration with water.

Example of calculations to coat one HYPERFlask vessel with a 0.1% gelatin solution (1 g/1L):

Calculate total gelatin needed to make 560 mL of coating solution:

$$560 \text{ mL} \times 1 \text{ g}/1000 \text{ mL} = 0.56 \text{ g of gelatin}$$

2. Add gelatin to the water. Gently heating the solution will help the gelatin to dissolve.
3. Autoclave the gelatin solution to sterilize.
4. Cool the gelatin to room temperature before use or store at 4°C until ready to use.
5. Add 560 mL of room temperature gelatin solution to the HYPERFlask vessel to fill it completely.
6. Incubate the HYPERFlask vessel at 37°C for at least 30 minutes.

**NOTE:** To achieve a uniform coating and to prevent pressure buildup within the vessel, the HYPERFlask vessel must be filled completely during the 30-minute or longer incubation.

7. After incubation, aspirate or pour out the gelatin solution from the HYPERFlask vessel and use.

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