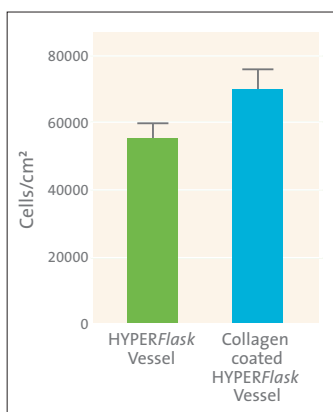


Method for Coating Corning® HYPERFlask® Vessels with Collagen or Gelatin

Protocol

CORNING



HEK293 cell yields after 4 days of growth on coated and uncoated 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. Check with the collagen or gelatin supplier for additional coating protocols.

Collagen

Materials

- ▶ Type I Collagen Solution from Rat Tail (BD Cat. No. C354236)
- ▶ Phosphate buffered saline (PBS) (Mediatech Cat. No. 21-030-CM)
- ▶ HYPERFlask vessel (Corning Cat. No. 10034)

Procedure

NOTE: Due to the viscosity of most collagen it is recommended to make a diluted stock solution first and then use this solution to make a working solution at the final desired concentration. If the stock is made at a high concentration such as 2 mg/mL, it can be stored at -20°C for long term storage or 4°C for short term storage.

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

1. Dilute collagen solution to desired concentration with PBS.

Example of calculations to coat one HYPERFlask vessel at 10 µg/cm² from a 2 mg/mL working solution:

Convert desired coating density (collagen/cm²) to collagen concentration (collagen/mL):

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

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

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

Calculate volume of stock solution needed:

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

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: In order 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 (we typically coat for 2 hours).

NOTE: If a 37°C incubation is preferred the HYPERFlask vessel must be filled completely to prevent pressure buildup within the vessel from the airspace heating up and expanding.

4. After incubation aspirate or pour collagen solution from HYPERFlask vessel and wash once with 50 mL of PBS. The vessel is now ready for use or can be stored at 4°C until needed.

Gelatin

Materials

- ▶ Porcine gelatin (Sigma Cat. No. G1890-100G)
- ▶ Sterile distilled deionized water (Mediatech Cat. No. 25-055-CV)
- ▶ HYPERFlask® vessel (Corning Cat. No. 10034)

Procedure

NOTE: Different cell lines may require different gelatin coating concentrations in order 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 (1g/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 water, gently heating the solution will help the gelatin to dissolve.
3. Autoclave gelatin solution to sterilize.
4. Cool 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 HYPERFlask vessel at 37°C for at least 30 minutes.

NOTE: In order 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 gelatin solution from HYPERFlask vessel and use.

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