COLLAGEN COATING OF TISSUE CULTURE DISHES

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1. OBJECTIVE

To coat tissue culture dishes with collagen.

2. DESCRIPTION

2.1 Human placental collagen type IV (Sigma Chemical Co., Cat. # C-7521; Sigma call this product as `Sigma Type VI') is dissolved in 0.2% acetic acid at a concentration of 2 mg/ml. This takes approximately 1 hr.

2.2 The collagen solution is then filtered through a 5 μm syringe filter.

2.3 The above filtrate is then filtered again through a, 0. 45 μm cellulose acetate filter apparatus.

2.4 This filtrate is stored as stock solution at 4^{o}C until needed.

2.5 Dilute the stock collagen solution 1:5 with tissue culture grade water (1 part stock collagen and 4 parts water). Make sure to dilute only the amount needed as this cannot be stored for later use.

2.6 Dispense the following amounts into the desired tissue culture dishes and allow to sit overnight in the tissue culture hood (12-18 hrs under UV exposure).

2 ml/35 mm petri dish
3 ml/60 mm petri dish
5 ml/100 mm petri dish
4 ml/T25 flask
12 ml/T75 flask
0.5 ml/24 mm transwell

2.7 The following day, aspirate the diluted collagen solution from the dishes and allow the dishes to air-dry.

2.8 When dry, the dishes are thoroughly rinsed 2X with sterile, D-PBS. Use the following amounts of PBS/dish.

1 ml/35 mm petri dish
1.5 ml/60 mm petri dish
2.5 ml/100 mm petri dish
2 ml/T25 flask
4 ml/T75 flask
0.5 ml/24 mm transwell

2.9 Air dry the dishes inside the hood, seal with parafilm and store at 4^{o}C until needed.

Note: Dishes used for experimental purposes only need to be collagen coated.

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ProductInformation

Collagens for Cell Culture

Product Number	Description	Source	Storage	Target Cells For Attachment	Concentration For Use	Refs.
C1809	COLLAGEN TYPE I Acid soluble powder	kangaroo tail	2-8 °C	muscle cells, hepatocytes, spinal ganglion, embryonic lung cells, schwann cells. Mediate the attachment of many cell types	6-10 µg/cm^2	33
C7661		rat tail				1,2,5,7, 8,11,17
C9791		calf skin				
C8919	COLLAGEN TYPE I 0.1% Solution Sterile-filtered (Not suitable for 3D gel formation)					
C9301	COLLAGEN TYPE II Powder	chicken sternal cartilage		chondrocytes		18,19
C0543	COLLAGEN TYPE IV Powder	Engelbreth-Holm-Swarm mouse sarcoma	-20 °C; store solubilized product at 2-8 °C	epithelial cells, endothelial cells, muscle cells, nerve cells		5,12,13, 14,15, 16,17
C5533	COLLAGEN TYPE IV Lyophilized	human placenta	-20 °C			34

Product Use

- **Collagen Type I (Product Nos. C1809, C7661, C9791, and C8919)**

Optimal conditions for attachment must be determined for each cell line and application.

1. Add collagen to 0.1 M acetic acid to obtain 0.1% (w/v) collagen solution. Stir at room temperature 1-3 hours until dissolved. (C8919 is prepared as a 0.1% solution, step 1 is not necessary for this product.)
2. We recommend transferring the collagen solution to a glass bottle with a screw cap and carefully layering chloroform at the bottom. The amount of chloroform to use should be ~10% of the volume of collagen solution. DO NOT SHAKE OR STIR. Allow to stand overnight at 2–8 °C. Aseptically remove the top layer containing the collagen solution. We do not recommend sterilizing the collagen solution by membrane filtration. We have found substantial protein loss by this method. (C8919 is a sterile solution, step 2 is not necessary for this product.)
3. Dilute desired volume (according to surface area to be treated) of sterile stock solution in step 2 or C8919 10-fold to a working concentration of 0.01% for coating surfaces.
4. Coat dishes with 6-10 µg/cm^2. Allow the protein to bind for several hours at room temperature or 37 °C, or overnight at 2–8 °C.
5. Remove excess fluid from the coated surface, and allow it to dry overnight. If the collagen solution is not sterile, the dried, coated surface can be sterilized easily by overnight exposure to UV light in a sterile tissue culture hood.
6. Rinse with sterile tissue culture grade water or a balanced salt solution before introducing cells and medium.

- **Collagen Type II and Type IV (Product Nos. C9301, C0543, and C5533)**

Optimal conditions for attachment must be determined for each cell line and application.

1. Collagen Types II and IV may be reconstituted to a concentration of 0.5-2.0 mg/ml in 0.25% acetic acid. **Dissolve for several hours at 2–8 °C, occasionally swirling.**
2. Coating of tissue culture plastic dishes may be performed by air drying the above protein solution or by preincubating the same solution overnight at 2–8 °C (or several hours at 37 °C) without air drying.
3. Dried coated dishes can be sterilized overnight by exposure to UV light in a sterile tissue culture hood or by rinsing with 70% ethanol. Alternatively, the collagen solution may be sterilized by dialysis in a 0.25% acetic acid and 0.5% chloroform solution.

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