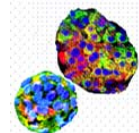


SOP



Title:	Coating cell culture plates				
Protocol #:	1.1	Submitted:	050510	Approved:	200610
Category:	CC	Author(s): [†]	AKR,MVJ	Checked by:	AAH

Reagents:

1. Terg-A-Zyme detergent (Fisher Scientific, Cat No. 04-322-11A)
2. Sterilization pouches (Fisher Scientific, Cat No.01-812-55)
3. 100-mm glass culture dishes (Kimble-Kontes, Cat No.23060-10020)
4. Gelatin (Sigma-Aldrich, Cat No. G1393)

Equipment

1. Autoclave
2. 37°C water bath

Reagent Setup

1. 2% gelatin solution

Procedure:

Glass culture (Petri) dishes coated with gelatin will be used for the differentiation of hIPCs.

Cleaning and sterilization

1. Dissolve Terg-A-Zyme detergent (Fisher Scientific, Cat No. 04-322-11A) in distilled water (1% solution, 10 g per liter). Prepare the appropriate volume of the cleaning solution in the Nalgene Rectangular Tank.
2. Immerse the used 100-mm glass culture dishes (Kimble-Kontes, Cat No.23060-10020) in the cleaning solution. Let soak overnight so that residual cell material is dissolved. Make sure that all of the dishes are covered with solution.
3. Dish covers are normally not as soiled as the bottoms and can be cleaned separately (brief immersion into the cleaning solution and rinse).
4. Next day use the cellulose sponge to remove any cell debris from the dishes. Rinse repeatedly with distilled water. Perform the last rinse with double-distilled water.
5. Invert dishes and dry them upside down on lint-free paper.
6. Assemble dishes (cover and bottom), fix cover to bottom with small squares of autoclave paper and place in sterilization pouches (Fisher Scientific, Cat No.01-812-55) (three dishes per pouch).
7. Leave in autoclave basket on 2nd floor (Use P2 program (dry heat) to autoclave the dishes).
8. Allow dishes to cool before use.

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Note on cleaning: The plastic tank and cellulose sponges are not to be used for anything else than cell culture vessel cleaning. Tap water is to be avoided for rinsing.

Dish coating

Sterilized dishes can be coated with gelatin before use in FIPC culture

1. Warm up (37°C water bath) the stock 2% gelatin solution (Sigma-Aldrich, Cat No. G1393). When solution becomes clear, make up a 0.2% working solution (1 part of stock solution in 10 parts of cell-culture grade water).
2. Add 10 ml of the 0.2% gelatin solution per dish.
3. Incubate at 37°C in a humidified incubator for 1 hour.
4. Removed excess gelatin and let the dishes open in a vertical laminar flow hood to dry (10-15 min).
5. Dishes are now ready for seeding and can be used as any other cell culture vessel.

Anticipated results

N/A

Representative image / picture

N/A

References:

N/A