HOST-PATHOGEN INTERACTIONS LAB
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Collagen coating of coverslips, or Transwells , or plastic plates

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Materials

1. Collagen Type I, Rat Tail, 100 mg. Corning #354236. Store at 2-8ºC.

Each stock bottle of "Collagen Type I" can be with a different collagen concentration.

Our stock bottle has collagen concentration of 3.36 mg/ml.

2. 0.1 M Acetic acid (sterile filtered). Store at 2-8ºC.

3. 50 mM HEPES pH=8.5 (sterile filtered). Store at 2-8 ºC.

These materials are kept in Tissue Culture (TC) room 4 ºC, at the bottom of the door.

Procedure

All the procedure is done in the hood of the TC in a sterile manner.

The concentration of the collagen working solution is 0.2 mg/ml.

In general, the working solution is prepared by mixing the collagen stock 1:1 with 0.1M Acetic acid in a tube and completing the volume with 50mM HEPES pH=8.5 (for details see below).

Solution preparation

1. 0.1M Acetic acid.

   Acetic acid Glacial AR stock is in concentration of 17.4M, so dilute it 174-fold with sterile Cell Culture Grade Water (03-055-1A, Biological Industries).

   In a 50 ml sterile TC tube mix 0.25 ml of Acetic acid Glacial AR stock and 43.25 ml of Cell Culture Grade Water. Sterilize with 0.22mm filtration.

2. 50mM HEPES pH=8.5.

   HEPES (H3375-25G, Sigma) MW=238.3. For preparing 100 ml of 50mM HEPES solution, take 1.1915g HEPES and dissolve it with Cell Culture Grade Water, and bring it to **pH=8.5** accurately. The pH is very important for the process. Sterilize with 0.22mm filtration.

Coating procedure

The amount of collagen working solution to be placed:

24-well or 12mm Transwell → 0.3-0.5 ml

12-well → 0.75 ml

6-well or 24mm Transwell → 1.0-1.5 ml

For example, if I want to prepare a collagen working solution (WS) in the concentration of 0.2 mg/ml for 10 wells, and I place 0.5 ml WS in each well, the calculations are as follows:

10 wells x 0.5 ml/well = **5 ml** -- this is the amount of collagen WS to prepare

5 ml x 0.2 mg/ml = **1 mg** -- this is the amount of collagen to take from the stock bottle (collagen concentration: 3.36 mg/ml)

1 mg/3.36 mg/ml = 0.2976 ml -- take from the stock bottle 0.3 ml of collagen and mix it with 0.3 ml of 0.1M Acetic acid in a tube, and complete to 5 ml with 50mM HEPES pH=8.5 – it is 4.4 ml. (5 ml - (0.3 ml+0.3 ml)) =4.4 ml

Mix the collagen working solution by pipetting up and down.

- The coverslips often come with dirt. Therefore, before adding the collagen WS wash them twice with DPBS.

After adding the collagen WS put the plate in humified 37 ºC TC- incubator from 2 hrs to overnight. Then wash twice with DPBS. Add 0.5 ml DPBS and place the plate opened under UV light in the hood for 20 minutes. Aspirate the DPBS and add fresh DPBS and store at 4 ºC for up to 2-3 weeks. Before cell seeding, wash twice with Growth Medium.