

Title

Collagen type I coating of culture wares

Protocol

Materials:

- Glacial Acetic Acid (GAA)
- Phosphate Buffered Saline (PBS), Sterile
- Water, Sterile
- Cultrex Rat Collagen I, 5 mg/ml (Catalog : 3440-100-01, R&D System)

Procedure:

Prepare 0,02 M GAA → Dilute GAA 1:700 into sterile water (14µl GAA/ 10ml water). To prepare a stock solution of 40ml: 56µl GAA + 40 ml sterile water in 50 ml falcon.

- Prepare coating solution as prescribed in manual, to a final concentration of 50µg/ml:
- $C1 = 5\text{mg/ml}$ $C2 = 50\text{ µg/ml}$ $V2 = 5\text{ml}$ $V1?$
- $V1 = C2 \cdot V2 / C1 = 0,05\text{ ml}$ Collagen I → 4,95 ml of Acetic Acid + 0,05 ml Collagen I
- Add solution to T25 plate, 5 ml (to have a final concentration of 10µg/cm²).
- Incubate 60 min at 37 °C.
- Carefully aspirate solution from plate.
- Rinse 3 times with 1x PBS.
- Plates may be used immediately or air dried for future use. Unused flasks should be capped and can be stored at 4 °C for up to 2 weeks.

Volume for Coating of Petri dishes/T-flasks/Transwells:

- Procedure for a 10 µg/cm² coating: add the according volume of rat collagen type I working solution (50 µg/mL) to Petri dishes/T-flasks.
 1. 6-well plate (BD 353046) = 9.6 cm² → 2.1 mL
 2. 12-well plate (TPP) = 3.66 cm² → 732 µL
 3. 24-well plate (TPP) = 1.91 cm² → 382 µL
 4. Dish 60 x 10 mm (BD 353004) = 19.6 cm² → 3.9 mL
 5. T-25 flask (TPP/BD) = 25 cm² → 5 mL
 6. T-75 flask (TPP/BD) = 75 cm² → 15 mL
 7. T-150 flask (TPP/BD) = 150 cm² → 30.0 mL

8. 24-well Bio-one inserts 3.0 μm pore size= $0,336 \text{ cm}^2 \rightarrow 67,2 \mu\text{l}$
9. 12mm diameter insert, 12 well= $1,12 \text{ cm}^2 \rightarrow 224 \mu\text{l}$
10. Falcon 6 cm dish(REF 353004)= $19,5 \text{ cm}^2 \rightarrow 3,9 \text{ ml}$
11. Falcon 100x20 mm dish (REF353003)= $58,1 \text{ cm}^2 \rightarrow 11,62 \text{ ml}$
12. Tissue culture dish, ($\varnothing$ xH): 100 x 20 mm (REF83.3902)= $58,8 \text{ cm}^2 \rightarrow 11,76 \text{ ml}$
13. Nunc™ Lab-Tek™ Chamber Slide System (177372PK)= $9,4 \text{ cm}^2 \rightarrow 1,88 \text{ ml}$