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Microvessel Plating and Culture

Questions? Email microvesselsupport@advancedsolutions.com

Objective

To thaw and suspend microvessels in collagen gels for culture.

Reagent Preparation

All reagents should be sterilized before use. Proper aseptic techniques should be utilized throughout the procedure.

4X DMEM

- 10g of powdered DMEM (low glucose w/ phenol red; Fisher Scientific #31600-034)
- 3.7g of sodium bicarbonate (Fisher Scientific #S233-3)
- 10ml 1M HEPES (Lonza #17-737E)

Mix components until dissolved and bring to a final volume of 250ml in milliQ water. Sterile filter through 0.22 µm filter. 4x DMEM can be stored at 4°C for up to 7 days.

Recommended Microvessel Culture Medium

Now available from our store! In convenient packs of 5 x 10ml or 5 x 50ml

Medium Component	Product Number	Final Medium Concentration
RPMI	Corning 10-040-CV	
B27	Thermo Fisher A1895601	1x
Vascular Endothelial Growth Factor (VEGF-A_{165})	Peprotech #100-20	50 ng/ml

1) Make up stock solutions of:
    - VEGF: Add 0.1% BSA to UltraPure water and sterile filter. Then reconstitute VEGF with the sterile solution to a concentration of 10 µg/ml. Store aliquots at -80.
2) To make 50 ml of complete medium, add to 50 ml RPMI:
    - 1ml of 50x B27
    - 250 µl of VEGF
3) Store for up to 1 week at 4°C.

Collagen I

- 1 M NaOH
- Rat tail collagen I, high concentration (Corning® #354249).
- UltraPure or MilliQ water (sterilized by autoclaving or sterile filtering)
- 4x DMEM (see above preparation instructions)

Collagen solution will be made during each experiment. Detailed instructions will be found at step 4 of the procedure.

Thawing medium

- RPMI containing 10% fetal bovine serum (FBS)

Procedure

1. Determine total number of microvessels and volume of collagen/microvessel suspension required for your experiment.

- Microvessels are routinely used at a density of **50,000 - 150,000** microvessels/ml for robust angiogenesis. **See Product Information sheets for exact recommended densities for each lot of microvessels.**
- Effective collagen concentrations range from 1.5 mg/ml to 7 mg/ml, with 3 mg/ml being the most frequently used concentration.
- Typically, 100µl of collagen/MV suspension is used for wells of a 96 well plate, and 250µl for a 48 well plate.

2. Quickly thaw microvessels in a 37°C water bath and move them to a centrifuge tube containing approximately 10 ml of thawing medium. Use a micropipette and culture medium to rinse out the vial.

3. Centrifuge the microvessel suspension at 400g for 4 minutes.

- Vessels will be resuspended after centrifugation directly in collagen. If you will be testing multiple collagen or microvessel concentrations, divide your suspension and spin each group down separately.

4. While microvessels are in the centrifuge, prepare the collagen gel:

- Undiluted, collagen stocks can be stored at 4°C for 6 months. Longer storage times can result in poor neovessel growth.
- All components of the collagen mix should be kept on ice throughout the preparation. Chilling pipette tips may also help prevent collagen from gelling.
- Prepare extra collagen (approximately 50% extra) to compensate for tube and pipette wall adhesion of the viscous collagen.
- Once prepared, use the collagen within 30 minutes.

- Collagen is prepared from 4x DMEM, sterile UltraPure water, and high concentration stock collagen.
- Calculate the volume of reagents needed to dilute your stock collagen to the desired concentration using the below equations.

$$Volume\ of\ Stock\ Collagen = \frac{(Desired\ Collagen\ Concentration) * (Desired\ Final\ Volume\ of\ Collagen)}{Concentration\ of\ Stock\ Collagen}$$

$$Volume\ of\ 4X\ DMEM = (Desired\ Volume\ of\ Collagen) * (0.25)$$

$$Vol\ DI\ Water = Desired\ Vol\ Collagen - (Vol\ 4X\ DMEM + Vol\ Stock\ Collagen + 50\mu l\ Microvessel\ Suspension)$$

- These equations assume 50 µl of microvessel suspension will be added to your total volume of collagen (see step 5). This may need to be adjusted if different volumes are used.
- Add all the reagents together in a 15ml centrifuge tube. The collagen stock should be the last reagent added.
- If the color is orange or yellow, add a few microliters of sterile 1N NaOH and mix well. Repeat until the mix turns red/light pink (reflecting pH 7.4), waiting 1 minute between each addition. Magenta means it is too basic (add back 1M HCl), and orange means it is still too acidic (add more NaOH).
  *Do not add NaOH or HCl once the microvessels are in the collagen

5. Aspirate the supernatant from the microvessel pellet so that no more than 50µl media remain above the pellet. Resuspend the microvessels with a micropipette and place the centrifuge tube on ice. Keep the tube on ice for minimum of one minute, to cool the tube to prevent collagen from gelling in it.

6. Dispense the appropriate volume of cold collagen solution into the tube containing the microvessel pellet and resuspend the pellet thoroughly with a micropipette, taking care to avoid introducing bubbles.

7. Dispense the microvessel/collagen mix into the appropriate plate.

8. Place in a 37°C, 5% CO_2 incubator for 1 hour to gel the collagen.

9. Add culture medium to each well, at 1.5 times the volume of collagen in the well. Place well-plate back into incubator. Medium should be changed every 4 days; more frequent medium changes may slow neovessel growth.

- Neovessel sprouting should be visible in 3-5 days