Companion SOP:

Micropatterning Matrigel in the Microfluidic channels for Invasion or Angiogenesis Assays

✓Read the whole protocol before beginning. Prepare everything listed before attempting. Note that you will have to thaw the Matrigel overnight before proceeding.

Required

- Matrigel (BD Biosciences, cat # 356234, Standard Matrigel or #356230, Reduced Growth Factor Matrigel)

RECONSTITUTION AND USE (from BD Matrigel Manual): Color variations may occur in frozen or thawed vials of BD Matrigel Basement Membrane Matrix, ranging from straw yellow to dark red due to the interaction of carbon dioxide with the bicarbonate buffer and phenol red. Variation in color is normal, does not affect product efficacy, and will disappear upon equilibration with 5% CO_2.

a) Thaw overnight at 4°C on ice (Matrigel may gel at slightly elevated temperatures in a refrigerator).
b) Once Matrigel Basement Membrane Matrix is thawed, swirl vial to be sure that material is evenly dispersed. Handle using sterile technique.
c) Place thawed vial of Matrigel Basement Membrane Matrix in sterile area in wet ice
d) Spray top of vial with 70% ETOH and air dry.
e) Matrigel Basement Membrane Matrix may be gently pipetted using a pre-cooled pipette to ensure homogeneity into appropriate aliquots (~250 ul), using pre-cooled tubes, and refreeze immediately. Avoid multiple freeze thaws. **DO NOT STORE IN FROST-FREE FREEZER.**

- CO2-independent Media (Invitrogen, cat #18045088) supplemented as needed with Glutamax, 1%v/v FBS (and Pen/Strep if desired)
- BioFlux 24-well plate (Fluxion, cat # 900-???? "V4" plates)
- Heater plate (Fluxion, cat # 900-0047)- preheated to 38°C
- Access to a -20°C freezer, wet ice in a bucket and a defrosted, cold gel ice pack
- Timer

Special notes:

CAUTION (from BD Matrigel Manual):
"BD Matrigel Basement Membrane Matrix will gel rapidly at 22°C to 35°C. Thaw overnight at 4°C on ice (Matrigel may gel at slightly elevated temperatures in a refrigerator). Keep product on ice before use, and use pre-cooled pipettes, tips, and tubes when preparing BD Matrigel Basement Membrane Matrix for use. Gelled BD Matrigel Basement Membrane Matrix may be re-liquified if placed at 4°C on ice for 24-48 hours. "

1. Do not attempt this assay if the Matrigel cannot be easily aspirated into a p100 pipet tip- it is gelled and will not work.

2. *Matrigel is very temperature sensitive*. It will only remain in a liquid form when below 4°C. It is absolutely crucial to thaw Matrigel as directed (overnight at 4°), otherwise it might gel or crystallize in an inconsistent manner and the protocol will not work properly.

3. A BioFlux glass heating plate P/N 900-0047 must be used – preheated to 38°C- for the gelling step

4. Balance well volumes on all sides of the channel.

5. When placing the Bioflux plate in the freezer and on ice be ***extremely*** careful not to push the glass down hard onto any hard surface. Because the experiment shifts temperatures, the glass can be easily broken by an impact.

6. Use the parallel flow option in the 24-well user interface.

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1. Remove from freezer. Prime device from the outlet well with room temperature (22°C) 100 µl CO_2-independent media plus 1% FBS (100ul per channel)– device is to be placed on a room temperature surface, not on ice pack at this point (because it is cold enough to freeze the media in the channels).

Only prime until there is ≤5µl of fluid in the inlet wells.- use 5 dyn/cm^2 for 10sec, while watching the inner punch of the inlet wells for a tiny bead of fluid.

Pre-clear the Matrigel aliquots (of aggregated material) by centrifuging in a micro-centrifuge at max 4000g for a few seconds before using- keep cold. Then only remove fluid from the top of the tube (as if there was an invisible pellet present- there might be an actual pellet).

2. Prepare Matrigel with compounds on ice in pre-iced eppendorf tubes using pipet tips (set in a box of ice, dry ice works well, but wet ice is fine if dry ice is not available) to mix the compounds into the gel. Take care to prepare stock solutions of compound at a high enough concentration such that the Matrigel is not significantly diluted (plan on adding less than 10µl of compound to 500 µl of Matrigel).

3. Place Bioflux plate on a slightly thawed ice pack for a few minutes (you want the plate to be at or close to 4*C). Add 100 µl of room temperature or colder CO_2 independent media as above to all inlet 'A's

4. Add prepared Matrigel mixtures to all inlet 'B's – 100 µl only

5. Begin flow immediately for 2 minutes on the partially defrosted ice pack Flow inlet A at 1 dyn/cm^2 and inlet B at 1.5 dyn/cm^2 .

6. Continue flow for exactly 1 min off the ice pack and then exactly at the 1 min mark, place the plate on the preheated glass heater (38°C)– continue flow for 30 sec and STOP FLOW immediately at 30seconds.

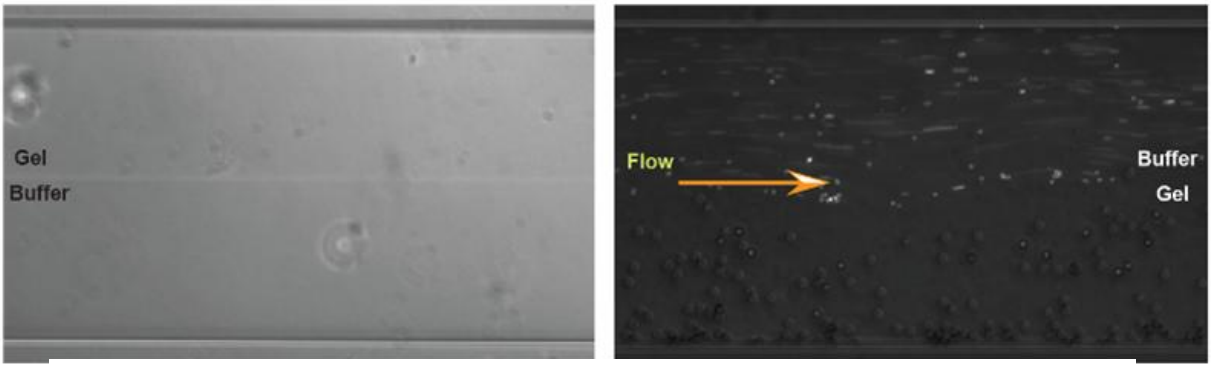

Figure 1. You can observe the gel: fluid interface with a microscope during the room temperature incubation- it is easiest to see at the convergence point of inlet A and B.

7. Leave the BioFlux plate on heater for 1 minute without touching it.

8. After the 1 min, remove plate from heater and place on room temperature bench for an additional 20 min.

Once gel has formed, avoid perfusion from outlet well and avoid fast perfusion above 2dyn/cm^2.Gel and fluid compartments of the channel should be observable using phase contrast as a slight line along the middle of the channel. Do not use ice cold fluid – it will result in bubble formation in the channels.

9. Before adding cells, be sure to coat with an experiment specific adhesion molecule in the fluid side of the channel. Example, 1/40 v/v diluted matrigel, perfused from inlet A as directed (not from outlet) – ALL SUBSEQUENT PERFUSION STEPS SHOULD BE PERFORMED ONLY FROM INLET A with room temperature or warmer buffer.

Following this protocol, one should expect the 900-0014 plates to have gel coverage in about 40-50% of the channel. If desired, experiment with the force ratio between the inlet streams to customize the percentage of gel coverage.

Also note that the shear will be roughly double the number entered in the software due to the reduction in channel width.