Instructions Surface coating microcell arrays

Protocol for poly-l-lysine coating

Materials

Either poly-lysine or poly-D-lysine with MW between 30,000 - 150,000 can be used for the coating.

Prepare 1 mg/mL stock solution by dissolving 100 mg of poly-D-lysine hydrobromide in 100 mL of PBS. The stock solution can be stored at -20°C.

The working solution is obtained by diluting the stock solution with PBS to around 0.1 mg/mL.

Procedure

1. Insert the microcell array into the chamber to be used for imaging.
2. Using a 1,000 µL pipette slowly add 500 µL of 100% ethanol on top of the microcell array, then cycle the ethanol over the microcell array to assist in removing any trapped air bubbles.
3. Slowly add media to one corner of the imaging chamber until almost full.
4. Draw out the media/ethanol mixture from the opposite corner of the imaging chamber, **taking care that the microcell array is always covered with a small amount of liquid**. Repeat steps 2 and 3 at least five times to make sure that the ethanol is completely diluted out.
5. Add the working solution of poly-lysine to the microcell array.
6. Incubate for 1 hour at room temperature in a hood.
7. Carefully aspirate the poly-lysine solution from the imaging chamber, **taking care that the microcell array is always covered with a small amount of liquid**. Note that the poly-lysine working solution can be re-used a few times.
8. Rinse 3 times with PBS, **taking care that the microcell array is always covered with a small amount of liquid**.
9. The poly-lysine coated microcell array should be used immediately.

Protocol for collagen coating

Materials

Rat tail collagen (type 1) can be used for the coating. It is recommended that you purchase collagen solutions.

Dilute the purchased solution to 50 µg/mL with 0.02M acetic acid to produce the working solution.

Procedure

1. Insert the microcell array into the chamber to be used for imaging.
2. Using a 1,000 µL pipette slowly add 500 µL of 100% ethanol on top of the microcell array, then cycle the ethanol over the microcell array to assist in removing any trapped air bubbles.
3. Slowly add media to one corner of the imaging chamber until almost full.
4. Draw out the media/ethanol mixture from the opposite corner of the imaging chamber, **taking care that the microcell array is always covered with a small amount of liquid**. Repeat steps 2 and 3 at least five times to make sure that the ethanol is completely diluted out.
5. Add the working solution of collagen (50 µg/mL) to the microcell array at 5 µg/cm^2.
6. Incubate for 1 hour at room temperature in a hood.
7. Carefully aspirate the collagen solution from the imaging chamber, **taking care that the microcell array is always covered with a small amount of liquid**.
8. Rinse 3 times with PBS, **taking care that the microcell array is always covered with a small amount of liquid**.
9. The collagen coated microcell array should be used immediately.

Protocol for fibronectin coating

Materials

Human fibronectin is diluted to 10 - 50 µg/mL with Ca++ and Mg++ free PBS to produce the working solution. Note that the concentration should be proportional to the coating concentration. For example, make 10 µg/mL solution for 1 µg/cm^2 coating and 50 µg/mL solution for 5 µg/cm^2 coating.

Make appropriate aliqouts and store at -20°C and avoid multiple freeze/thaw cycles.

Procedure

1. Insert the microcell array into the chamber to be used for imaging.
2. Using a 1,000 µL pipette slowly add 500 µL of 100% ethanol on top of the microcell array, then cycle the ethanol over the microcell array to assist in removing any trapped air bubbles.
3. Slowly add media to one corner of the imaging chamber until almost full.
4. Draw out the media/ethanol mixture from the opposite corner of the imaging chamber, **taking care that the microcell array is always covered with a small amount of liquid**. Repeat steps 2 and 3 at least five times to make sure that the ethanol is completely diluted out.
5. Add the appropriate amount of fibronectin solution to the microcell array.
6. Incubate for 1 hour at room temperature in a hood.
7. Carefully aspirate the fibronectin solution from the imaging chamber, **taking care that the microcell array is always covered with a small amount of liquid**.
8. Rinse 3 times with PBS, **taking care that the microcell array is always covered with a small amount of liquid**.
9. The fibronectin coated microcell array should be used immediately.

Protocol for laminin coating

Materials

Laminin is diluted to 5 µg/mL in PBS. The concentration should be proportional to the coating concentration. For example, make 10 µg/mL solution for 1 µg/cm^2 coating and 50 µg/mL solution for 5 µg/cm^2 coating.

Make appropriate aliqouts and store at -20°C and avoid multiple freeze/thaw cycles.

Procedure

1. Insert the microcell array into the chamber to be used for imaging.
2. Using a 1,000 µL pipette slowly add 500 µL of 100% ethanol on top of the microcell array, then cycle the ethanol over the microcell array to assist in removing any trapped air bubbles.
3. Slowly add media to one corner of the imaging chamber until almost full.
4. Draw out the media/ethanol mixture from the opposite corner of the imaging chamber, **taking care that the microcell array is always covered with a small amount of liquid**. Repeat steps 2 and 3 at least five times to make sure that the ethanol is completely diluted out.
5. Add the appropriate amount of laminin solution to the microcell array.
6. Incubate for 1 hour at room temperature in a hood.
7. Carefully aspirate the laminin solution from the imaging chamber, **taking care that the microcell array is always covered with a small amount of liquid**.
8. Rinse 3 times with PBS, **taking care that the microcell array is always covered with a small amount of liquid**.
9. The laminin coated microcell array should be used immediately.