Coating Protocols for C-Stretch Chambers

C-Stretch chambers as sold are not sterile and provide no cell adhesion. For long-term culture, the chamber should first be autoclaved for 20 minutes at 121° C, and then the membrane must be coated with extracellular matrix before seeding cells.

→ Coating protocol: Fibronectin

1. Prepare a fibronectin solution by dissolving 0.05 mg/ml of fibronectin in PBS.
2. Place the stretch chamber in a culture dish and pour the fibronectin solution into the chamber well so that it completely covers the bottom surface.
3. Incubate the fibronectin-treated chamber in the culture dish at 37° C for at least four hours.
4. Remove the culture dish from incubator and draw up any remaining solution from the chamber using a pipette or other suitable device.

→ Coating Protocol: Collagen

1. Prepare and autoclave a dilution of hydrochloric acid (pH3.0, 1 mM).
2. Dilute type 1 collagen in the autoclaved hydrochloric acid.
3. Place the stretch chamber in a culture dish and pour the collagen solution into the chamber well so that it completely covers the bottom surface.
4. Cover the culture dish with a lid and incubate at 37°C for at least four hours.
5. Remove the culture dish from incubator, and leave to stand for a period. Then draw up the remaining solution from the chamber using a pipette or other suitable device.
6. Rinse the chamber twice with serum-free culture fluid to remove any excess collagen solution that may have remained after the above steps.

→ Coating protocol: Gelatin

1. Prepare a gelatin solution by dissolving 2% gelatin powder in PBS. Autoclave the gelatin solution.
2. Place the stretch chamber in a culture dish and pour the gelatin solution into the chamber well so that it completely covers the bottom surface.
3. Incubate the gelatin-treated chamber in the in the culture dish at 37°C for at least four hours.
4. Remove the culture dish from incubator, and draw up any remaining solution from the chamber using a pipette or other suitable device.

→ Protocol of Fluorescent Staining after Stretching

1. Splice out cell plane from stretch chamber with surgical knife (as large as approx. 0.8×0.5cm) – the size varies according to the size of container to be used since step 2. Splicing to make the longer side get along with the stretching direction makes it easy to find out the stretching direction afterwards.
2. Put the membrane spliced in step 1 into the container with PBS(-). (Using 1×1 chamber (without frame) attached on a cover glass.）
3. PBS(-) wash, 2 times.
4. Fix with 4% formalin solution in PBS(-)->RT, 5 min, shake.
5. PBS(-) wash, RT, 5 min, 3 times.
6. 0.1% TritonX-100 in PBS(-), pierce cell membrane->RT, 5 min, shake.
7. PBS(-) wash->RT, 5 min, 3 times.
8. Block 2% BSA in PBS(-)->RT, 30 min, shake.
9. Primary antibody in 0.1%Tween20 in PBS(-)->RT, 30min, shake.
10. 0.1%Tween20 in PBS(-) wash->RT, 5 min, 3 times.
11. Secondary antibody in 0.1%Tween20 in PBS(-)->RT, 30 min, shake.
12. 0.1%Tween20 in PBS(-) wash->RT, 5 min, 3 times.

13. Put on a slide glass to enclose (Perma Fluor) – put the cell membrane side down.

14. Fluorescent observation.