

## Fibronectin Coverslips

### Day One (night before explants)

1. Flame polish 22mm sq. glass coverslips by immersing in 100% EtOH and flaming.
2. Place a glass plate on top of Whatman paper in large Petri dish, moisten Whatman paper.
3. Cut small spacer pieces from plastic.
4. Place coverslips on glass plate, place 2-3 spacers on top of coverslip.
5. Resuspend fibronectin in PBS at 20-25 $\mu$ g/ml (stock is 1 $\mu$ g/ $\mu$ l)
6. Add approximately 100 $\mu$ l fibronectin to glass coverslip.
7. Place 2<sup>nd</sup> coverslip on top of first with spacers separating them.
8. Add fibronectin to the side of coverslip sandwich until entire area of both coverslips is exposed to fibronectin.
9. Leave on bench for approximately 30-45 minutes then move to 4°C overnight (room temp incubation optional).

### Day Two

1. Place coverslips in small Petri dishes fibronectin coated side up
2. Wash coverslips 5' in 1X PBS
3. Block coverslips one hour at room temp in 1X PBS + 1% BSA
4. Wash coverslips 5' in 1X PBS
5. Fill Petri dish with Danilchik's + .1% BSA
6. Explant and culture explants in Danilchik's + .1% BSA