GC 9/4/12

Poly-ornithine/Laminin substrate for neurons and neural progenitors

1. Dissolve unopened vial of Poly-Ornithine in Borate Buffer at 1mg/ml (P-Orn)
2. Filter sterilize and store at 4 degrees for 3 months
3. Pretreatment for coverslips:
    a. Sterilize forceps in 100% EtOH
    b. Dip 15mm coverslip generously in 100%EtOh, place in 24wp or 4wp wells
    c. Wash wells 3x with TC H2O
    d. Aspirate completely and Dry in hood
4. Add P-Ornithine solution to cover well
    a. ~0.5ml/24wp well or 8 well slide well
    b. Note: if using coverslip, make sure to tamp down coverslip with sterile tip
    c. Incubate 2-6 hrs to overnight in incubator
5. Aspirate and save P-Orn for reuse (mark reuse number and store at 4 degrees)
6. Wash wells 2-3x, 0-2 min each with ≥coating volume of TC grade $H_2O$
7. Add Laminin in L15+ NaBicarbonate solution: final 10ug laminin/ml, same volume and incubate ≥ 12 hours in TC incubator
    a. As long as laminin solution does not dry, substrate is good for several weeks in incubator, add water each week.
    b. Alternatively, desalt wash 2-3x with H2O and dry (see Drying Protocol below).
8. When ready to seed cells, aspirate laminin (can save for reuse) and seed cell solution directly without drying substrate

Reagents and Notes

Poly-L-ornithine hydrobromide,mol wt 30,000-70,000, Sigma-Aldrich P3655-50MG extremely hygroscopic, do not attempt to weigh

Borate Buffer: Boric Acid to 0.15M in ddH20, pH to 8.4 with NaOH, filter and store at room temperature

P-Orn Stock solution: store at 4 degrees for 3 months

Natural Mouse Laminin, Invitrogen, 23017015

L15+Bicarbonate: add 12.5ml sterile Sodium Bicarbonate (7.5g/L) to 500ml L15 with phenol red.

Under the substrate: plastic vs glass. Tissue culture treated plastic is much stickier (hydrophillic) and much softer (stiffness(kPa)) than glass and I prefer it for growth and imaging. High ornithine can compensate for this deficit a bit but not completely and cannot soften the glass. If using glass, “german” borosilicate (decksglasser) glass grade is preferred and acid etching prior to coating is recommended. Neuvitro makes excellent No.1.5 german glass coverslips and they offer pre-extched, pre-coated coverslips. Optical plastic cultureware is available from many suppliers, just make sure it is No1.5 glass equivalent and optically clear and tissue culture treated. I have had good experience with Greiner, 96wp

black plates e.g., Ibidi chamber slides and multiwell plates, and Mattek. Coverslips: German glass #1.5 coverslips are recommended. Nunc is stickier and more even than most commercial manufacturers.

Commercial sources: Precoated lysine/laminin coverslips from NeuVitro work nicely; Becton Dickinson precoated slides and coverslips are usually good but are inferior to NueVitro or home-coated

Poly-Ethyl-Imine in Borate buffer may be used in place of laminin at the same concentration

Drying: coated cultureware can be dried out for storage at 4 degrees and future use or for drop-seeding. Adhesion may be slightly less than freshly prepared (wet) substrate but this has not been methodically tested. Rule of thumb in lab is use fresh for very sensitive applications here consistency and maximal adhesion are crucial

1. Best to dry freshly prepared substrate, rather than one that has been incubating for some time
2. Aspirate laminin
3. Wash 3x with TC water
4. Completely aspirate water without scratching surface
5. Dry dishes open in the hood for 15-20 min.
6. Parafilm and store at 4 degrees for 6 months