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Poly-ornithine/laminin substrate for neural cell culture V.2

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Takazawa T, Croft GF, Amoroso MW, Studer L, Wichterle H, Macdermott AB. Maturation of spinal motor neurons derived from human embryonic stem cells. PLoS One. 2012;7(7):e40154. doi: 10.1371/journal.pone.0040154. Epub 2012 Jul 3. PMID: 22802953; PMCID: PMC3388990.

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We use this protocol and it's working

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Abstract

This protocol is used to create adhesive and bioactive substrate for neural cell types, low to high density nuerons, astrocytes, or organoids. It is based on standard methods but includes several optimizations, use case recommendations, and alternatives, and advice.

Attachments

PDF

Poly ornithine lamin...

42KB

Guidelines

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. Nunc delta surface is reliably stickier and more even than many alternatives. High-ornithine can compensate for the adhesion defecit of galls, 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-etched, pre-coated coverslips.

Optical plastic cultureware is available from many suppliers. Needs to be No 1.5 glass equivalent and optically clear and tissue culture treated. I have had good experience with Greiner, 96wp black plates, **Ibidi** chamber slides and multiwell plates are excellent optically and TC treatment is great, and **Mattek** is good but mostly glass.

Commercial sources or precoated cultureware: We have found i house coated substrates are best but commercial providers are very useful as an alternative. Precoated lysine/laminin coverslips from NeuVitro are preferred; Becton Dickinson precoated slides and coverslips are usually good as well b 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 where consistency and maximal adhesion are crucial

Materials

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

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.

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

Troubleshooting

Safety warnings

Borate/PLL solution is toxic Aspirate well and wash before proceeding to laminin coating.

Preparing Reagents

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:

3.1 Sterilize forceps in 100% EtOH

3.2 Dip 15mm coverslip generously in 100%EtOh, place in 24wp or 4wp wells

3.3 Wash wells 3x with TC H2O

3.4 Aspirate completely and Dry in hood

Coating

4 Add P-Ornithine solution to cover well

4.1 ~0.5ml/24wp well or 8 well slide well

4.2 Note: if using coverslip, make sure to tamp down coverslip with sterile tip

4.3 Incubate 2-6 hrs to overnight in incubator

5 Add P-Ornithine solution to cover well

6 Wash wells 2-3x, 0-2 min each with ≥coating volume of TC grade H2O

6.1 borate+polyamino acid solution is toxic, aspirate completely during washes and use greater than coating vollume for washes.

7 When ready to seed cells, aspirate laminin (can save for reuse) and seed cell solution directly without drying substrate

8 Add Laminin in L15+ NaBicarbonate solution: final 10ug laminin/ml, same volume and incubate ≥ 12 hours in TC incubator

8.1 As long as laminin solution does not dry, substrate is good for several weeks in incubator, add water each week.

8.2 Alternatively, desalt wash 2-3x with H2O and dry (see Drying Protocol below).

9 Best to dry freshly prepared substrate, rather than one that has been incubating for some time

Drying coated surfaces

10 Aspirate laminin

11 Wash 3x with TC water

12 Completely aspirate water, including residual droplets, without scratching surface

13 Dry dishes open in the hood for 15-20 min.

14 Parafilm and store at 4 degrees for 6 months

Protocol references

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