

Materials List for:

Primary Neuronal Cultures from the Brains of Late Stage Drosophila Pupae

Beatriz Sicaeros¹, Jorge M. Campusano¹, Diane K. O'Dowd¹

¹Department of Development and Cell Biology, Department of Anatomy and Neurobiology, University of California, Irvine (UCI)

Correspondence to: Diane K. O'Dowd at dkodowd@uci.edu

URL: <https://www.jove.com/video/200>

DOI: [doi:10.3791/200](https://doi.org/10.3791/200)

Materials

Name	Type	Company	Catalog Number	Comments
Concanavalin A		Sigma-Aldrich	C-2010	To 2.5 ml DS, add 25 mg Concanavalin A bottle. This is 10 mg/ml concentration. Make aliquots of 90 ul and store at -20 C, no longer than 3 months.
Laminin		Sigma-Aldrich	L-2020	Add 1ml DS to 1mg Laminin bottle. This is 0.5 mg/ml concentration. Make aliquots of 10 ul and store at -20 C, no longer than 3 months.
Coverslips		Bellco Glass	1943-00012	12 mm glass coverslips
ConA + Laminin solution				Stock solution, from Con A stock and laminin stock, for coverslip coating: Add in 5 ml DS, 83.5 ul of ConA (167 ug/ml) and 8.35 ul of Laminin (0.835 ug/ml). Mix. Make aliquots of 100 ul and store at -20 C, not longer than a month.
Dissecting Solution	Buffer			For 500 ml: 400 ml Ultra filtered water + 25 ml Stock Solution A + 14 ml Stock Solution B + 3.0 g (33.3 mM) D (+)-Glucose (Sigma G-8270) + 7.5 g (43.8 mM) Sucrose (Sigma S-0389). Adjust pH to 7.4 with 1N NaOH (around 2 ml). Bring final volume to 500 ml with ultra filtered water. Decant into a clean glass bottle and autoclave. Label "Dissecting Solution" and store at 4°C.
Dissecting Solution	Buffer			For 500 ml: 400 ml Ultra filtered water + 25 ml Stock Solution A + 14 ml Stock Solution B + 3.0 g (33.3 mM) D (+)-Glucose (Sigma G-8270) + 7.5 g (43.8 mM) Sucrose (Sigma S-0389). Adjust pH to 7.4 with 1N NaOH (around 2 ml). Bring final volume to 500 ml with ultra filtered water. Decant into a clean glass bottle and autoclave. Label

				"Dissecting Solution" and store at 4°C.
Solution B: HEPES	Buffer	Sigma-Aldrich	H-3375	20.97g (9.9mM). Add ultra filtered water up to 200 ml. Mix until dissolve and bring final volume to 250 ml. Place in a clean bottle and autoclave. Label "Solution B" and store at 4 C.
Solution A	Buffer			For 500 ml: 80.0g (137 mM) NaCl (Sigma S-9625) + 4.0g (5.4mM) KCl (Sigma P-4504) + 0.24g (0.17mM) Na2HPO4 (Sigma S-0876) + 0.3g (0.22 mM) KH2PO4 (Sigma P-5379). Weigh out all ingredients and mix until dissolved with 400 ml ultra filtered water. Bring final volume to 500 ml. Place in a clean bottle and autoclave. Label "Solution A" and store at 4°C.
<p>Coating Coverslips: Put autoclaved coverslips in a 60 mm petri dish. Pipet 5 ul of Con A/Laminin mix onto center of each coverslip. Place in 37 C incubator for 2 hours. Rinse 3x coverslips with 100 ul of autoclaved water each. Use vacuum attached to a sterile Pasteur pipet. During the last rinse, pick up the coverslip with forceps and dry both sides. Transfer the coverslip to a 35mm Petri dish. Store at room temperature for up to a month.</p>				