



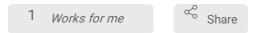


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Preparation of primary rat cortical neuron and astrocyte co-culture

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ABSTRACT

This protocol describes how to prepare primary rat cortical neuron and astrocyte coculture.

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MATERIALS TEXT

- Dissecting buffer: Hanks' Balanced Salt solution (HBSS) supplemented with
 [M]10 millimolar (mM) HEPES and [M]20 % volume Fetal bovine serum (FBS)
- Washing buffer: HBSS supplemented with 10mM HEPES
- Disgesting buffer: [M] 0.5 Mass / % volume EDTA-trypsin supplemented with DNAse
- Neurobasal completed medium: Neurobasal A medium supplemented with B27,
 [M]2 millimolar (mM) Glutamax, Pen/Strep
- 1 1-3 days postpartum Sprague Dawley rats (University College London breeding colony) are used.
 Experimental procedures are performed according to the United Kingdom Animal (Scientific Procedures) Act of 1986.
- 2 Rat cortices are placed in an ice-cold Dissecting buffer (described in Materials).
- 3 Wash five times with Washing buffer (described in Materials).
- Tissues are digested with a Disgesting buffer (described in Materials) for **© 00:15:00**
- 5 Digested tissues are neutralized with a dissecting buffer.
- 6 Washing twice with a Washing buffer,
- 7 Dissociate with a Washing buffer supplemented with DNAse.
- 8 Dissociated pellets are collected in Neurobasal completed medium.

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9	Approximately 600,000 cells are plated on → 25 mm	Poly-D-Lysin (PDL) coated coverslips and
	200,000 cells for 8-well ibidi chambers (PDL coated).	

- The cultures are maintained at § 37 °C ([M]5 % volume CO2), and the media are changed every 4-5 days.
- 11 Cells can be used at 12-16 days.