Materials List for:

How to Culture, Record and Stimulate Neuronal Networks on Micro-electrode Arrays (MEAs)

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Materials

Supplies

- Disposable bottle top filter (150ml, 0.2µm pore size; Nalgene #565-0020)
- Embryonic day 18 timed-pregnant female rat
- Micr–lectrode arrays (see description in protocol, B1)
- Micr–lectrode array lids (see description in protocol, B3)
- Nylon cell strainer (40µm, BD Falcon, #352340)
- Polypropylene conical vials (sterile, 15ml)
- Polystyrene Petri dishes (sterile, 35mm and 100x15mm)
- Pipet tips (sterile, 20µl, 200µl and 1000µl)
- Serological pipets (5ml)

Equipment:

- Biological safety cabinet/laminar flow hood
- Cell counter
- Centrifuge
- Container of ice
- Dissecting scissors and forceps (sterilized)
- Dissecting microscope in a laminar flow hood
- Handheld electric pipetman
- Hemacytometer
- Isofluorane and gas chamber
- Light microscope
- Handheld pipetman (P-20 (20µl), P-200 (200µl), P-1000 (1000µl))
- MEA recording system (see description in protocol, D1)
- Rodent Guillotine
- Tri-gas incubator (see description in protocol, B15)
- Vacuum flask with aspirator attached in the hood
- Vortex
- Water bath (35-37°C)

Solutions and Reagents:

BSA solution:
Make 5% BSA (Sigma A-9418) in phosphate buffered saline pH 7.4 and filtered at 0.2µm. This solution can be prepared ahead of time and stored at 4°C for several months.

Cell Medium:
Combine 90 ml Dulbecco's modified Eagle's medium (Irvine Scientific 9024), 10 ml horse serum (Gibco 16050), 1ml sodium pyruvate (100mM, Gibco 11360), 250 µl GlutaMAX (Gibco 35050), and insulin (final concentration 2.5 µg/ml, Sigma I-5500) then pass through a 0.2 µm filter.22 Warm and equilibrate the cell medium in the incubator prior to use.

Dissection solution for preparing papain solution:[23]

Dissection solution (100ml stock)	Combine in order, filter 0.22 µm and store at 4°C.	
Component	Volume ml	Final Concentration (mM)
Double-distilled water	91.175	
1 M $MgCl_2$	0.58	5.8 mM

1 M $CaCl_2$	0.025	0.25 mM
0.5 M HEPES	0.32	1.6 mM
0.5% Phenol red	0.2	(0.001%)
0.1 N NaOH	0.2	0.2 mM
2 M Na2SO4	4.5	90 mM
1 M K2SO4	3.0	30 mM
0.1 M Kynurenic acid	1.0	1 mM
5 mM APV	1.0	0.05 mM

This solution can be prepared ahead of time and stored at 4°C for several months.

DNAse I:
Invitrogen (#18047019). 50µl aliquots are flash frozen and stored at -80°C. An aliquot is thawed in the laminar flow hood prior to starting the cell dissociation process.

Hank's balanced salt solution:
Sigma (#4891). A 1X solution is prepared in deionized water, sterile filtered at 0.2µm and stored at 4°C.

Hibernate solution:
Prepare on day of culturing. Combine 98 ml L-15 medium (Invitrogen 11415) with 2 ml B-27 supplement (50X, Invitrogen #17504) and filter at 0.2 µm.

Laminin solution:
Prepare on day of culturing. Dilute 20µl of laminin (1mg/ml, Sigma L-2020) in 980 µl cell medium for a final concentration of 0.02mg/ml. 20 µl aliquots of the laminin (1mg/ml) are stored at -80°C and thawed just prior to use. Thawing should occur on the bench top and not in the water bath as rapid thawing can sometimes lead to gelling of the laminin.

Papain solution:[23]
Warm 10 ml of dissection solution (above) to 30-32°C. Add 200 units papain (Sigma P-4762) and 1.6 mg L-cysteine (Sigma C-7880). Adjust pH to 7.4 with about 14 µl of 0.1 N NaOH. Incubate for about 30 minutes until the solution clears, filter 0.2 µm, flash freeze with liquid nitrogen in 2 ml aliquots in 15 ml polypropylene sterile conical vials and store at -80°C. Thaw an aliquot at 35-37°C prior to starting protocol.

Polyethyleneimine (PEI) solution:[13]
100 µl of PEI (50% w/v, Sigma P-3143) is diluted in 100 ml sodium borate buffer (0.1 M pH 8.4, Sigma B-9876) for a final concentration of 0.05% w/v. Sterile filter at 0.2 µm. This can be prepared in advance and stored at 4°C for months.

Sterile de-ionized water:
Autoclave and allow to cool to room temperature prior to use.

Trypsin (0.25% with EDTA, Gibco 25200):
1ml aliquots are flash frozen and stored at -80°C. Aliquots are thawed in the 35-37°C water bath prior to use.