

Hippocampal Slice Culture

(Draft Protocol)

1. Prepare the dissecting tools.

One small scissor
Two small tweezers
Put in sterilization pouch and autoclave

2. Hippocampal slice cultures were prepared from **6-day old mice** by the method of Stoppini et al. (1991).

Culturing slices from older animals for prolonged periods of time has proven difficult

3. Hippocampi were dissected out and **Horizontal** 400 µm slices were cut with a vibratome. Slices were dissected out, separated, placed on 25 mm-diameter Nunc tissue culture inserts (pore size 0.02 µm) in 6-well tissue culture plates.

4. Slices are fed with 1.5 ml **Early Growth Medium**

77% **DMEM/F12** (contains L-glutamine and HEPES buffer)
20% **horse serum**
0.5mM L-ascorbate (from **100X solution**)
1% **Penicillin and Streptomycin mixture**
Supplement with additional 15mM D-glucose (from **1.5M stock**), PH 7.2;

5. At DIV5, replace by half with the **Late Growth Medium**

97% **DMEM/F12** (contains L-glutamine and HEPES buffer)
0.5mM L-ascorbate (from **100X solution**)
1% **Penicillin and Streptomycin mixture**
Supplement with additional 15mM **D-glucose**, and 1% **B27 mixture**. PH 7.2;

6. The cultures were maintained for up to 2 to 3 weeks at 36°C at 5% CO₂ in a humidified incubator. After about a week in culture, hippocampal slices flattened from 400 µm to about 100 µm in thickness. After the first change of medium, **replace the medium by half the volume every 3 days.**

Chemicals, tools, solutions to bring to the culture room

1. Dissecting tools

- One small scissor
- Two small sharp forceps
- Two small scupulae
- Two pieces of filter paper
- Alcohol swap pads
- One tissue chopper (McIlwan type with micrometer)

Put in sterilization pouch and autoclave

2. Culture media

- DMEM/F12 medium
- Horse serum
- Glucose supplement (100X)
- L-ascorbate supplement (100X)

Total medium of 50ml should be more than enough for the slice culture

3. Culture plastics

- One 6 well plate
- Two Tanswell/Millicell inserts
- Two 50ml tube for preparing medium
- Two 60ml syringe for filtering the medium
- Two syringe filters

4. Mice of 6 day old

Bottles of sterilized water if the incubator needs water (CHECK REGULARLY) to make sure humidity is close to 100%

Tools can be found in the culture room:

- Pipette (1ml, 200ul, 20ul)
- Sterilized pipette tips
- Stereo microdissection scope