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Maintenance & Differentiation: Embryonic Mouse Hippocampal Cells (CLU198)

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We use this protocol and it's working

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Abstract

This protocol details the maintenance & differentiation of Embryonic Mouse Hippocampal Cells (CLU198).

Materials

Media:

1. **Maintenance Media:** DMEM full media containing (DMEM/10% FBS/1% Pen-Strep).

2. **Differentiation Media:** Complete Neurobasal media containing

I. Neurobasal-A (1x) Media Gibco
II. Glutamax (100x) Gibco-(1x final)
III. Pen-Strep (1% final)
IV. B27-Supple (50x) Gibco-(**0.5x** final)

For differentiation:

- 12 Well plate (1 ml/well)
- 6 Well plate/35 mm dish (9.5 cm^2-2 ml/well)
- 60 mm dish (21 cm^2-3.5 ml/dish)
- 100 mm dish (56 cm^2-10 ml/dish)
- 250 ml Flask for sub-culture/maintenance (10 ml) [Tissue culture flask-Greiner bio-one- Cat.No.-658 170]

  CELL CULTURE FLASK, 250 ML, 75 CM², PS, RED STANDARD SCREW CAP, CLEAR, CELLSTAR® TC, STERILE, 5 PCS. **greiner bio-one Catalog #**658170

Maintenance

1 For regular maintenance of CLU cells, use DMEM full media.

Differentiation (CLU 198): Takes about a week of differentiation. 33m

2 30m

> **Note**
>
> No need to add Retinoic acid.

Day-01 (Mon): Plating with DMEM full media.

- Warm DMEM full media, PBS, and Trypsin in the 37 °C bead bath for 00:30:00. Clean the working area by using 70% ethanol.

2.1 Sup out old media without touching cells.

2.2 Wash by adding 5 mL PBS slowly, rinse, and rock back and forth.

2.3 Add 2 mL - 3 mL trypsin (0.25%); keep in incubator for 00:03:00. 3m

2.4 Check under microscope if cells are detached, add 5 mL media and transfer to a tube.

2.5 Spin 300 x g, 00:03:00.

2.6 Sup out and add 10 mL fresh media & re-suspend cells gently and carefully.

2.7 Count cells density and split accordingly. 15,000 cells/ml for maintenance

- (i) Usually **1.5**x10^4/ml cells for Biochem, and

- (ii) **0.5**x10^4/ml cells for IF.

3 **Day-02 (Tue):**

Replace with Complete Neurobasal Media.

4 **Day-03 (Wed):**

Rest.

5 **Day-04 (Thu):**

Rest.

6 **Day-05 (Fri):**

Replace with Complete Neurobasal Media/ (Start drug treat if necessary).

7 **Day-06 (Sat):**

Rest.

8 **Day-07 (Sun):**

Rest.

9 **Day-08 (Mon):**

Replace with Neurobasal Media/Drug treat.

10 **Day-09 (Tue):**

Drug treat if necessary /Harvesting.

11 **Day-10 (Wed):**

Drug treat if necessary /Harvesting.

Cells harvesting:

20m 2s

12 Wash once with cold PBS.

13 Add cold lysis buffer.

14 Keep On ice & scrap immediately in Eppendorf tube.

15 Sonicate (10 S on 00:00:02 off 20% Amplitude, 2 Pulses). 2s

16 Boil (100 °C , 00:10:00). 10m

17 Centrifuge 13.000 rpm, 4°C, 00:10:00 / Collect sup. 10m

18 Keep in -80 °C Freezer.

19 BCA to measure protein concentration.

20 Prepare with sample buffer and run WB analysis.