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Human Primary Astrocyte (hPA) Culture

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We use this protocol and it's working

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Abstract

This protocols describes methods for culturing human primary astrocytes.

Materials

Ordering information

- Human Astrocytes, SciencCell 1800
- Astrocyte media (complete kit), ScienCell 1801
- Poly-L-Lysine, 10mg/ml, ScienCell 403
- Poly-D-Lysine, 5mg, Sigma P6407

Astrocyte Growth Media

For standard culture use Astrocyte media (ScienCell 1801) supplemented with 10% FBS.

To prepare:

1. Remove 60ml basal media from 500mml bottle
2. Add thawed AGS, FBS, and P/S supplements (ScienCell 1801)
3. Add additional 40ml FBS to bring concentration from 2% to 10%
4. Filter

- Media is stable for one month when stored in the dark at 4C
- Media should not be warmed in 37C water bath, but instead should be warmed to RT

Cell counts and size

1. 1 confluent 10cm holds approx. 10 million ScienCell 1800 hPAs
2. Cells range from approx. 10-24um in suspension (via Countess), average size: 15um

Neurogenic media recipe

	A	B	C	D	E
	NDM (Guo 2014)	**unit**	**100 mL**	**250 mL**	**500 mL**
	DMEM/F12 (GIBCO)	mL	97.5	243.75	487.5
	0.5% FBS (GIBCO)	mL	0.5	1.25	2.5
	3.5 mM glucose (Sigma)*	mg	63.056	157.64	315.28
	penicillin/streptomycin (GIBCO)	mL	1	2.5	5
	N2 supplement (GIBCO)	mL	1	2.5	5
	Filter sterilize				

	A	B	C	D	E
	after combining				

Aliquot preparation

Aliquot prep

BDNF (Peprotech 450-02-50UG)

- Dilute 50ug in 250ul H2O (final conc 200 ug/ml)
- Add 2.25ml 0.1% BSA (final: 2.5ml @ 20 ug/ml).
  - This should be 1000x of what most protocols call for (20 ng/ml)
- Make 50ul aliquots, store at -80C

NT-3 (Peprotech 450-03)

- Dilute 10ug in 100ul H2O (final conc. 100 ug/ml)
- Add 900ul sterile PBS + 0.1% BSA (final: 1ml @ 10ug/ml).
  - This should be 1000x of what most protocols call for (10 ng/ml)
  - Make 50ul aliquots, store at -80C

- To make BSA PBS
  - 0.1g BSA in 10ml PBS, filter sterilize (0.22um filter)

PDL aliquot preparation

1. Add 5 ml ddH2O in a 5mg bottle of PDL (Sigma P6407)
2. Filter through 0.45um filter. Make 100ul or 400ul aliquots; store at -20C
   - label aliquot size
3. Stocks are 100x, dilute to 1x before coating
   - For 100ul aliquots: dilute to 10ml
   - For 400ul aliquots: dilute to 40ml

Troubleshooting

Coating plates (PDL)

1 All plates need to be coated with ply-D-Lysine and rinsed before plating cells

2 Dilute PDL stock (10x) to 1x with sterile molecular biology grade water

3 Add to plate (enough volume to cover bottom)

4 Incubate at 37C for minimum 1hr

5 Before plating, rinse 2x with water or dPBS

5.1 after second rinse, aspirate carefully to remove all liquid

6 Add cell culture media

Thawing cells

7 Prepare plates and prepare a 15ml tube with >=2ml culture media

8 Remove vial from liquid nitrogen and thaw quickly in water bath until only small ice crystals remain

9 Carefully pipette cells from seal into 15ml tube

9.1 Rinse cryovial once with media

9.2 Minimize pipetting, cells are fragile

9.3 Add thawed cells directly into TC plate. NO NOT SPIN! Leave DMSO in media

9.4 Record the lot #

10 The next day, change media to remove DMSO

Maintaining cells

11 Change the medium every three days, until the culture is approx. 70% confluent

12 One the culture riches 70% confluence, change medium every other day until the culture is approx. 90% confluent

Passaging cells

13 Passage when culture reaches 90-95% cofluency

14 Prepare ploy-D-lysine coated plate (see steps 1-6, coating plates)

15 Warm the following reagents to ROOM TEMPERATURE: media, 0.025% Trypsin/EDTA (note, this is a 1:10 dilution from standard 0.25% Trypsin/EDTA), dPBS, FBS

16 Rinse cells with dPBS

17 Add 10ml 0.025% Trypsin/EDTA to cells (10cm dish-scale volume to plate)

17.1 While incubating at 37C, add 5ml FBS to 50ml conical tube

18 Once cells completely round up, transfer Trypsin solution from plate to the 50ml centrifuge tube (a small percentage of cell may detach), and continue to incubate at 37C

for another minute (no solution in the dish)

19 At the end of the incubation, gently tap the side of the dish to dislodge cells from the surface. Check under a microscope to make sure that all cells detach

20 Add 5ml of media to collect cells and transfer detached cells to the 50ml centrifuge tube.

20.1 Rinse the dish with another 5ml of media to collect the residual cells if needed

20.2 Check flask to make sure not too many cells were left behind

21 Centrifuge the cell in the 50ml centrifuge tube at 1000 rpm for 5 minute

22 Gently resuspend cells in culture media

23 Plate at desired density

23.1 1:6-1:8 for routine split

23.2 If transducing for reprogramming: Cells should be 75-90% confluent at transduction