Erratum: One Mouse, Two Cultures: Isolation and Culture of Adult Neural Stem Cells from the Two Neurogenic Zones of Individual Mice

Protocol section 1.1 was changed from:

At least two days prior to commencing the experiment, prepare Poly-D-lysine (PDL)/Laminin coated plates for adherent monolayer cultures. To prepare wells/flasks add enough PDL (10 mg/ml in dH$_2$O) to coat the surface and incubate overnight at room temperature. Remove the solution from the dish and wash the dish three times with dH$_2$O. Allow to air dry. Add Laminin (5 mg/ml in cold DMEM:F12) and incubate at 37 °C overnight. Remove the Laminin and either use the plates immediately or store with the Laminin at -20 °C until required.

to:

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Protocol section 1.3 was changed from:

On the day of dissection, prepare the appropriate amount of culture medium by mixing Neural Basal Medium with 2% B27, 1x GlutaMAX, 2 µg/ml heparin, 50 units/ml Penicillin/Streptomycin, 20 ng/ml purified mouse receptor-grade epidermal growth factor (EGF), and 20 ng/ml recombinant bovine fibroblast growth factor (FGF-2). Warm the culture medium to 37 °C in a water bath.

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Protocol section 3.6 was changed from:

Add growth medium to a total volume of 5 ml and pass the cell suspension through a 40 mm sieve to remove debris and undissociated tissue clumps.

to:

Add growth medium to a total volume of 5 ml and pass the cell suspension through a 40 µm sieve to remove debris and undissociated tissue clumps.

Protocol section 3.7 was changed from:

Centrifuge at 300 x g for 5 min, discard the supernatant and resuspend the resulting pellet in 200 ml growth medium.

to:

Centrifuge at 300 x g for 5 min, discard the supernatant and resuspend the resulting pellet in 200 µl growth medium.

Protocol section 6.2 was changed from:

Add 50 ml Accutase and incubate at 37 °C for 2-3 min (checking to see if the cells are rounded and detached).

A correction was made to One Mouse, Two Cultures: Isolation and Culture of Adult Neural Stem Cells from the Two Neurogenic Zones of Individual Mice. Many micro symbols were changed into milli symbols by accident: In the Protocols, sections 1.1, 1.3, 3.6, 3.7, 6.2, 6.6, 8.1, 9.1.6, 9.2.2, and 9.2.5 need to be fixed, as does Figure 1 description in the Results section.
Add 50 µl Accutase and incubate at 37 °C for 2-3 min (checking to see if the cells are rounded and detached).

Protocol section 6.6 was changed from:

For subsequent passages, resuspend cells in 200 ml growth medium and count using a hemocytometer. Plate at 1 x 10^4 cells/cm^2 in the appropriate sized coated well or flask.

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Protocol section 8.1 was changed from:

Dilute the dissociated SVZ or DG tissue from one animal in 20 ml of culture medium and plate 200 ml/well across a 96-well plate using a 10 ml multidoser pipette.

to:

Dilute the dissociated SVZ or DG tissue from one animal in 20 ml of culture medium and plate 200 µl/well across a 96-well plate using a 10 ml multidoser pipette.

Protocol section 9.1.6 was changed from:

Remove 10 ml of the cell suspension and mix with an equal volume of trypan blue and perform a live cell count using a hemocytometer.

to:

Remove 10 µl of the cell suspension and mix with an equal volume of trypan blue and perform a live cell count using a hemocytometer.

Protocol section 9.2.2 was changed from:

Add 100 ml of 0.05% Trypsin-EDTA to each well to be passaged and incubate at room temperature for 3 min.

to:

Add 100 µl of 0.05% Trypsin-EDTA to each well to be passaged and incubate at room temperature for 3 min.

Protocol section 9.2.5 was changed from:

Transfer the 200 ml containing the dissociated neurosphere to a new well of a 24-well plate containing 1.5 ml of growth medium. Incubate at 37 °C with 5% CO_2 until secondary neurospheres form.

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Transfer the 200 µl containing the dissociated neurosphere to a new well of a 24-well plate containing 1.5 ml of growth medium. Incubate at 37 °C with 5% CO_2 until secondary neurospheres form.

Figure 1 description was updated from:

Figure 1. Adult mouse precursor cells can be cultured as adherent monolayer cultures (A) or as neurospheres (B: SVZ, C: DG). Scale bar is 50 mm.

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Figure 1. Adult mouse precursor cells can be cultured as adherent monolayer cultures (A) or as neurospheres (B: SVZ, C: DG). Scale bar is 50 µm.

Protocol

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Disclosures

No conflicts of interest declared.