TECHNICAL MANUAL

Enzymatic Dissociation of Adult Mouse and Rat CNS Tissue Using NeuroCult™ Enzymatic Dissociation Kit

STEMCELL™ TECHNOLOGIES

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1.0 Introduction

Neural stem and progenitor cells have been shown to exist in discrete regions of the adult mouse and rat brain, namely the subventricular zone (SVZ)[1-3], the hippocampus[4], the olfactory bulb[5], and the cortex[6]. These cells can be isolated by microdissecting the tissue from the adult brain regions of interest and then used in further applications, such as generating neurospheres with serum-free medium containing growth factors. However, before the cells from adult central nervous system (CNS) tissues can be used for experiments, the tissue needs to be pre-treated with appropriately buffered solutions containing tissue digestion enzymes and must undergo mechanical dissociation in order to obtain a cell suspension that is free of cell clumps, debris, myelin, dead cells, and undissociated tissue.

STEMCELL Technologies has developed a “ready-to-use” NeuroCult™ Enzymatic Dissociation Kit for Adult CNS Tissue (Mouse & Rat) and protocols for enzymatically pre-treating adult mouse or rat CNS tissue to obtain a clean cell suspension with high cell viability for further experiments. NeuroCult™ Enzymatic Dissociation Kit for Adult CNS Tissue (Mouse & Rat) is composed of four optimized solutions:

(1) Tissue Collection Solution is an optimized HEPES/EDTA-buffered solution for collecting dissected CNS tissues and maintaining high cell viability.

(2) Dissociation Solution contains tissue culture-grade enzymes for digesting tissue.

(3) Inhibition Solution is for inactivating the enzyme digestion reaction.

(4) Resuspension Solution is for washing and resuspending the final cell suspension.

The NeuroCult™ enzymatic dissociation procedure has been optimized so that it is reproducible, fast, and yields high cell numbers and viabilities. The resulting single-cell suspension is ready for immediate use in downstream applications.

2.0 Materials and Equipment Required

2.1 NeuroCult™ Enzymatic Dissociation Kit for Adult CNS Tissue (Mouse & Rat; Catalog #05715)

COMPONENT #	COMPONENT NAME	SIZE
05715A	Tissue Collection Solution	500 mL
05715B	Dissociation Solution*	30 mL
05715C	Inhibition Solution	30 mL
05715D	Resuspension Solution	500 mL

*Aliquot into 3 mL volumes and store at -20°C. Each 3 mL aliquot is sufficient for SVZ tissue isolated from up to 8 adult mouse brains or 6 adult rat brains in the NeuroCult™ enzymatic dissociation procedure.

2.2 Complete NeuroCult™ Proliferation Medium

Complete NeuroCult™ Proliferation Medium is necessary for the culture and subculture of mouse and rat neurospheres. The medium consists of the following components:

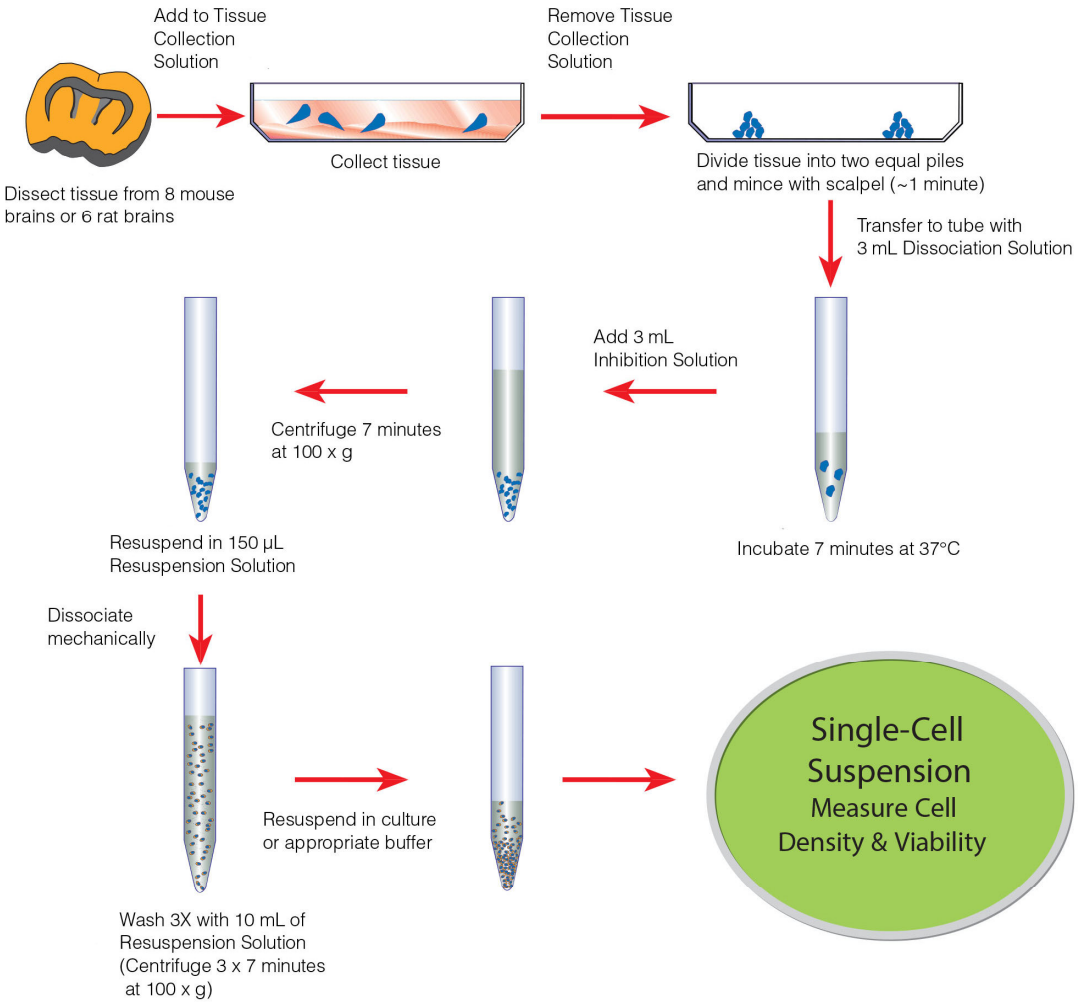
- NeuroCult™ Basal Medium (Mouse & Rat; Catalog #05700)
- NeuroCult™ Proliferation Supplement (Mouse & Rat; Catalog #05701)
- 20 µL of 10 µg/mL Human Recombinant EGF (Catalog #78006.1; to give a final concentration of 20 ng/mL)
- 10 µL of 10 µg/mL Human Recombinant bFGF (Catalog #78003.1; to give a final concentration of 10 ng/mL)
- 10 µL of Heparin Solution (Catalog #07980; to give a final concentration of 0.0002% Heparin (w/v); equals 2 µg/mL)

For additional details refer to the Technical Manual: In Vitro Proliferation and Differentiation of Mouse and Rat Neural Stem and Progenitor Cells Using NeuroCult™ (Document #28704), available at www.stemcell.com or contact us to request a copy.

2.3 Equipment

- Biohazard safety cabinet certified for Level II handling of biological materials
- 37°C incubator with humidity and gas control to maintain > 95% humidity and an atmosphere of 5% CO_2 in air
- Low-speed centrifuge equipped with biohazard containers
- Pipettors: 10 µL, 200 µL, and 1 mL with sterile pipette tips
- Vortex
- Pipette-aid
- Stopwatch
- Scalpel
- 40 µm Cell Strainer (Catalog #27305)
- 100 mm Dish, Tissue Culture-Treated (Catalog #38046); for collection of dissected tissue in Tissue Collection Solution
- Falcon® Round-Bottom Tubes, 14 mL (Catalog #38008)
- Falcon® Conical Tube, 50 mL (Catalog #38010)
- Hemocytometer
- Routine light microscope for hemocytometer cell counts
- Inverted microscope with flatfield objectives and eyepieces for magnification of approximately 20 - 30X, 80X, and 125X

3.0 Procedure Diagram



4.0 Enzymatic Dissociation of Adult Mouse or Rat CNS Tissue

Important notes when performing the NeuroCult™ enzymatic dissociation procedure:

- This procedure is optimized for processing the subventricular tissue isolated from up to 8 adult mouse or 6 adult rat brains. If more brains are used, set up more tubes for the additional tissue and thaw additional aliquots of Dissociation Solution.
- The incubation times outlined in the procedure are crucial for performance; it is important to observe the incubation times precisely and to use an accurate lab timer.
- The mechanical dissociation of tissue involves pipetting the tissue suspension in a consistent rhythm up and down gently through the plastic disposable tip pressed against the bottom of the tube without introducing any air bubbles. Air bubbles will result in high cell death.
- The microdissection of different regions of the adult mouse or rat brain is not outlined in this protocol. Perform microdissections according to Gritti et al.[7] or use routine dissection procedures recommended by your institution.

1. Add 10 mL **Tissue Collection Solution** to a 100 mm tissue culture-treated dish.
2. Thaw a 3 mL aliquot of **Dissociation Solution** at room temperature (15 - 25°C).
3. Perform dissections on the CNS tissue region of interest from adult mouse or rat brains and transfer dissected tissue pieces to the 100 mm dish containing **Tissue Collection Solution**.
4. Remove all the **Tissue Collection Solution** from the dish and mince tissue by chopping in a quick rhythm with a sterile scalpel for approximately 1 minute.

   Note: Divide the tissues into two roughly equal piles in the same 100 mm dish and mince each pile separately. This ensures that the tissue is minced into the smallest pieces possible and can be pipetted through disposable plastic tips.
5. Dispense 1 mL **Dissociation Solution** into the dish containing the minced tissue. Resuspend the tissue pieces, and transfer the suspension into a 14 mL round-bottom tube. Repeat twice, using the entire 3 mL of **Dissociation Solution**.

   Note: It is very important that no air bubbles are introduced into the tissue suspension during the transfer steps.
6. Incubate the minced tissue at 37°C for 7 minutes, preferably in a water bath (or in a beaker containing pre-warmed water which is placed in a 37°C incubator).
7. Remove the tube containing the minced tissue from the water bath or incubator.
8. Add 3 mL of **Inhibition Solution** and mix the tissue suspension gently, avoiding air bubbles.
9. Centrifuge the suspension at 100 x *g* for 7 minutes.
10. Discard the supernatant and resuspend the pellet with 150 µL **Resuspension Solution**.
11. Mechanically dissociate (triturate) the digested tissue using a plastic disposable tip attached to a 200 µL pipettor which has been set to 180 µL, by pipetting up and down ~5 - 10 times until a smooth and "creamy" suspension is achieved. The suspension should be able to pass through the plastic tip bore without getting lodged in the tip.
12. Once a smooth suspension has been achieved, add 100 µL **Resuspension Solution** to bring the volume to approximately 300 µL.
13. Pipette the suspension approximately 5 more times with a disposable plastic tip attached to a 1 mL pipettor (set to 280 µL) to achieve a homogeneous cell suspension without any clumps of tissue.
14. Add **Resuspension Solution** to the cell suspension to a final volume of 10 mL. Mix thoroughly.
15. Centrifuge at 100 x *g* for 7 minutes. Discard the supernatant.

16. Add 200 µL **Resuspension Solution** and resuspend the pellet by pipetting up and down 5 times using a plastic disposable tip attached to a 200 µL pipettor.
17. Add **Resuspension Solution** to a final volume of 10 mL. Mix thoroughly. Centrifuge at 100 x *g* for 7 minutes. Discard the supernatant.
18. Repeat steps 16 & 17 for a total of 3 washes with **Resuspension Solution**.
19. Resuspend the final pellet in 0.5 - 1 mL of medium (volume dependent on the size of the pellet and number of brains dissected) appropriate for subsequent experiments:
    - For experiments involving antibody labeling for FACS or cell separation, resuspend the pellet in Resuspension Solution.[8]
    - For generating neurospheres from adult mouse or rat CNS cells, resuspend the pellet in Complete NeuroCult™ Proliferation Medium.
20. Place a sterile 40 µm Cell Strainer over the mouth of a sterile 50 mL conical tube. Gently dispense the cell suspension from step 19 through the strainer. Allow the cell suspension to flow through the strainer by gravity (do not force the cells through).
21. Measure the volume of the flow-through and count cells using Trypan Blue (Catalog #07050) dye exclusion (1 in 5 or 1 in 10 dilution) and a hemocytometer.

    Note: Counting cells can be difficult because of the small pieces of debris, damaged blood vessels, and myelin. However, the viable cell yield should be approximately 5 x 10^4 cells from the SVZ of one mouse brain.[7]
22. To generate neurospheres, seed primary adult cells into Complete NeuroCult™ Proliferation Medium (section 2.2) at the following densities:

    2 x 10^4 cells/cm^2 (1.9 x 10^5 total cells) in a 6-well tissue culture plate (e.g. Catalog #38015)

    OR

    2 x 10^4 cells/cm^2 (5 x 10^5 total cells) in a T-25 cm^2 flask

    For the neurosphere assay, refer to the Technical Manual: In Vitro Proliferation and Differentiation of Mouse and Rat Neural Stem and Progenitor Cells Using NeuroCult™ (Document #28704), available at www.stemcell.com or contact us to request a copy.

5.0 References

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