NEUROMICS

A Division of CA3 Biosciences, Inc.

Protocol for Human NeuroProgenitors

Recommended Products
NeuroProgenitor Medium (cat# NM42400).

Handling of Arriving Cells
Store in liquid nitrogen until ready to culture.

Note: Handling human derived products is potentially biohazardous. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.
Note: Always work under aseptic conditions.

CULTURE PROTOCOL

1. Prepare a Vitronectin (Gibco A14700) coated 35mm plate with a final concentration of 0.5ug/cm2-1.0ug/cm2.
2. Rapidly thaw (<2 minute) frozen vial of NP-cells in a 37°C water bath. Remove vial from water bath just before the last trace of ice has melted. Spray vial with 70% ethanol to prevent contamination in tissue culture hood.
3. Thaw cells in 37°C water bath with agitation.
4. Wash once with 5 mLs NeuroProgenitor Medium
5. Pellet at 1000 rpm for 2 mins at room temp.
6. Resuspend cells in 2 mLs NeuroProgenitor and add to Vitronectin coated plate (created in step 1).
7. Place cells in 37°C incubator at 5% CO2, replacing half of media every day until confluent (typically 2-3 days).
8. Cells should be passaged using Versene solution (Gibco). Wash cells with PBS (without Calcium and magnesium).
9. Add 1mL of Versene solution to cells (35mm plate). Place cells back into incubator for 2 mins.
10. Remove cells from incubator and place into tissue culture hood. Using a 1mL pipettor, pipette the 1mL solution (step 9) over the plate vigorously until cells are no longer adherent. Pippette solution, with cells, into a 15 mL centrifuge tube. It may be necessary to wash plate with additional PBS or media (NeuroProgenitor) in order to remove all cells. Adherent cell are visible to the eye, however it is recommended to examine the plate microscopically to ensure cells have been collected.
11. Pellet cells at 1000 rpm for 2 mins at room temperature.
12. Carefully remove supernatant without disturbing the cell pellet.
13. Resuspend cell pellet in 1 mL of NeuroProgenitor media and add half of the volume to two 35mm Vitronectine coated plates (previously coated).
14. Place cells in 37°C incubator at 5% CO2, replacing half of media every day until confluent (typically 2-3 days).

Note: Passaging should be performed at 90% confluency or greater. If cells are allowed to remain at 100% confluency for multiple days may inhibit or greatly reduce cell growth after passaging. Cells should be passaged 1:2, and anything greater may be too sparse and cells may not recover, nor grow to confluence.

Note: Should any issues arise while using our cells, our team is here to help troubleshoot any issues. Our cells are backed by our one-time replacement or refund policy. Our recommended protocol including recommended products must be used to be eligible for replacement or refund. Cells that have been refrozen are no longer eligible for refund or replacement.

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