MLPC™-UCB Clonal Cell Line
Neurogenic Differentiation of MLPC

Reagents and Materials

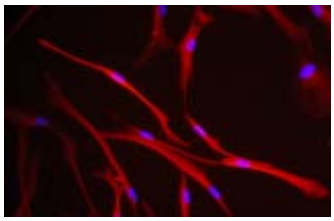
- MSCGM, Lonza Walkersville, Inc., PT-3001
- Neural Progenitor Maintenance Media Kit, Lonza Walkersville, Inc., CC-3209
- Fibroblast growth factor-4, Sigma, F-8424
- GlutaMax I Supplement, Invitrogen, 35050-061
- Penicillin/Streptomycin, Invitrogen, 15140-122
- Laminin coated 35mm dishes, BD Biocoat, 354458
- Hank's Balanced Salt Solution, Sigma, H1641 or equivalent
- Bovine Serum Albumin, Fisher, BP1600-1 or equivalent
- Paraformaldehyde, Sigma, P-6148 or equivalent
- Triton X-100, Sigma, X100 or equivalent
- anti-beta tubulin III [TuJ-1], R&D Systems, MAB1195
- anti-GFAP [GF5], Advanced Immunochemical, HG2b-GF5
- Oligodendrocyte Marker O1, Chemicon, MAB344
- Oligodendrocyte Marker O4, Chemicon, MAB345
- Alexa Fluor 594 GAM, Molecular Probes, A11020
- DAPI Nucleic Acid Stain, Lonza Walkersville, Inc., PA-3013

Neural Procedure

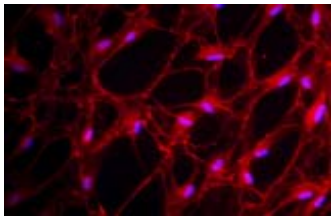
1. Plate MLPCs on laminin coated dishes at 1.25 x 10^5 cells/dish in 2 mL of MSCGM.
2. Incubate the cells in a 5% CO_2 atmosphere at 37°C.
3. Cells are fed every 3-4 days with 2 mL of MSCGM until the cultures reach confluency.
4. At confluency, continue to feed the control dishes with 2 mL of MSCGM. The experimental dishes are fed with 2 mL of Neural Progenitor medium supplemented with 20 ng/mL FGF-4, 200mM GlutaMax I Supplement and 100U penicillin/100 µg streptomycin per mL.
5. Cells are fed every 3-4 days for 21 days.
6. Differentiation of MLPCs toward the neural lineage was confirmed by positive staining of neuron, astrocyte and oligodendrocyte subtypes.

Neural Staining

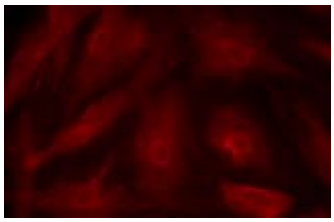
1. Remove the medium from each dish.
2. Wash each dish twice with 1 mL Hank's Balanced Salt Solution-HBSS (with Ca^{+2} and Mg^{+2}) supplemented with 1% BSA .
3. Add 2 mL of 4% Paraformaldehyde to each dish.
4. Incubate for 20 minutes at room temperature.
5. After incubation, remove the 4% Paraformaldehyde and wash each dish twice with 1 mL of HBSS + 1% BSA.
6. Add 2 mL of 0.5% Triton X-100 to each dish.
7. Incubate for 3-5 minutes at room temperature.
8. After the incubation, wash each dish twice as directed above.
9. Add approximately 750µl of HBSS + 1% BSA to each dish.
10. Add the appropriate amount of primary antibody (manufacturer's recommended dose) to the appropriate dish and incubate for 20-30 minutes.
11. If primary antibody is fluorescently labeled, cells are washed twice with HBSS + 1% BSA. Retain some fluid in the dish to keep cells moist while examining under a fluorescent microscope.
12. If the primary antibody is unlabeled, the cells are washed twice with HBSS + 1% BSA then Alexa Fluor 594 GAM is added. Cells are incubated with the secondary antibody for 20 minutes at room temperature.
13. DAPI nuclear counterstain may be added to the dishes during the last 5 minutes of the incubation with the secondary antibody. Dilute the stock DAPI nucleic acid stain using 2 uL DAPI to 10 mL PBS.
14. Add 300 uL of diluted stock to each dish. Incubate for 5 minutes.
15. Cells are washed twice with HBSS + 1% BSA. Retain some fluid in the dish to keep cells moist while examining under fluorescent microscope.



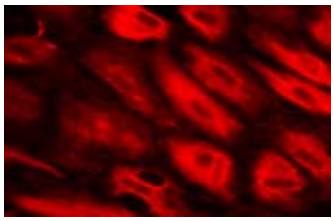
beta-tubulin III



GFAP



Oligodendrocyte-O1



Oligodendrocyte-O4