Title	Dopaminergic Neuron Differentiation
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Adapted from -	Gibco Protocol
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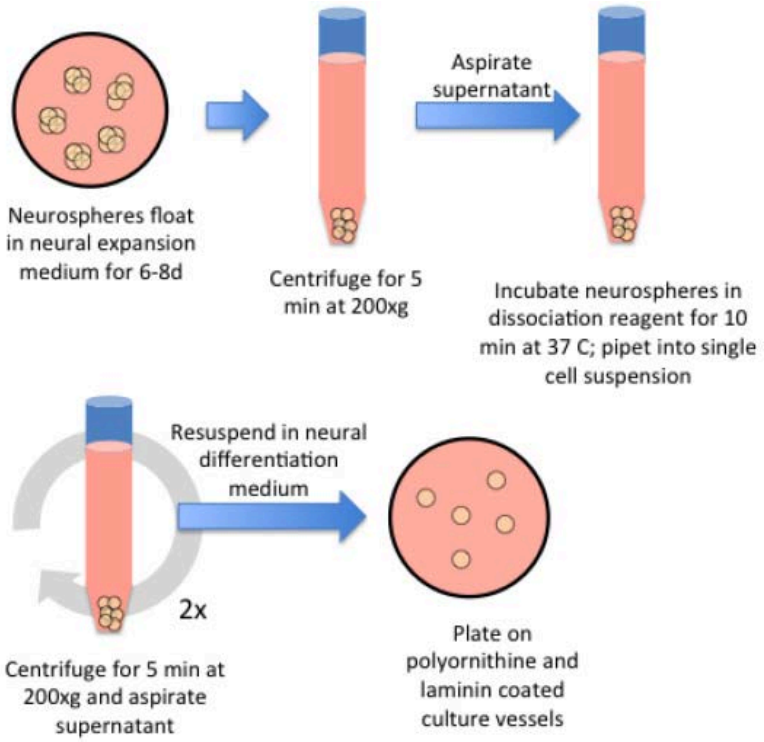
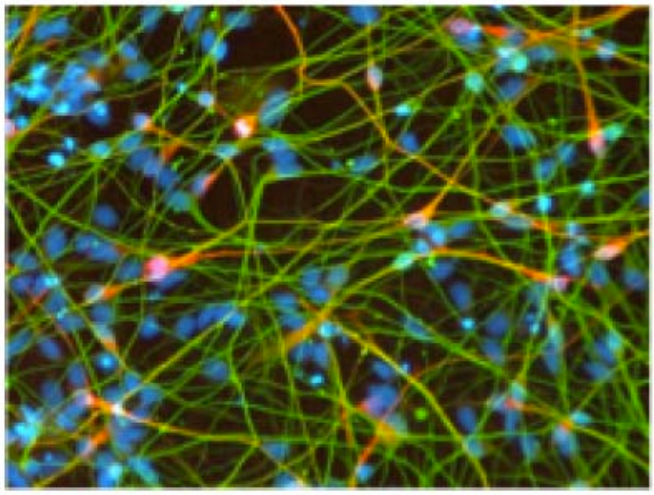
❖ Introduction:





Dopaminergic neurons expressing Tuj1 (green) and dopamineric neuron marker TH (red). - taken from ReproCELL, 2012

❖ Protocol:

1. Coat the surface of the culture vessel (with or without cover slips) with poly- L- ornithine working solution at 20 ug/mL in distilled water (14 mL for T- 75, 7 mL for T- 25, 3.5 mL for 60- mm dish, 2 mL for 35- mm dish) and incubate the vessel overnight at room temperature.
2. Wash the poly- L- ornithine- coated vessel 4 times with distilled water, and then coat it with laminin working solution at 10 ug/mL in D- PBS without calcium or magnesium (14 mL for T- 75, 7 mL for T- 25, 3.5 mL for 60- mm dish, 2 mL for 35- mm dish). Incubate the culture vessel for 3 hours at 37 C. Note: You may coat the culture vessels in advance, replace the laminin solution with D- PBS without calcium or magnesium, and store them wrapped tightly in Parafilm for up to 1 week. Make sure that the culture vessels do not dry out.
3. After the neurospheres float in neural expansion medium for 6-8 days, transfer them into a 15- mL tube and centrifuge for 5 minutes at 200 × g.
4. Aspirate the supernatant and incubate the neurospheres in pre- warmed StemPro Accutase Cell Dissociation Reagent for 10 minutes at 37 C.
5. Gently pipet the cell clumps up and down to break the larger clumps into a single cell suspension.
6. Centrifuge the cells for 5 minutes at 200 × g and aspirate the supernatant.
7. Resuspend the cells in 10 mL of pre- warmed neural differentiation medium.
8. Repeat steps 6 and 7.
9. Aspirate the laminin from the coated culture vessels and plate the dissociated DA progenitors.
10. Incubate the cells in a 37 C incubator with a humidified atmosphere of 5% CO2 and replace the spent medium with fresh neural differentiation medium every other day.
11. You can evaluate DA neuron differentiation 3-4 weeks after plating.

❖ Materials:

poly-L-ornithine coated culture vessel
distilled water
laminin
D-PBS
neural expansion medium
StemPro Accutase Cell Dissociation Reagent
neural expansion medium
neural differentiation medium
Neural Expansion Medium
Component Amount
D-MEM/F-12 96 mL

N-2 Supplement	1 mL
B-27® Supplement	2 mL
NEAA	1 mL
Basic FGF Solution	200 µL
Heparin Solution	100 µL
DA Neuronal Differentiation Medium	
Component	Amount
Neurobasal® Medium	96 mL
L-Glutamine	1 mL
B-27® Supplement	2 mL
NEAA	1 mL
GDNF Solution*	100 µL
BDNF Solution*	100 µL
Ascorbic Acid Solution*	100 µL
dcAMP Solution*	100 µM

*Add GDNF, BDNF, ascorbic acid, and dcAMP at the time of medium change

- Troubleshooting:

References: