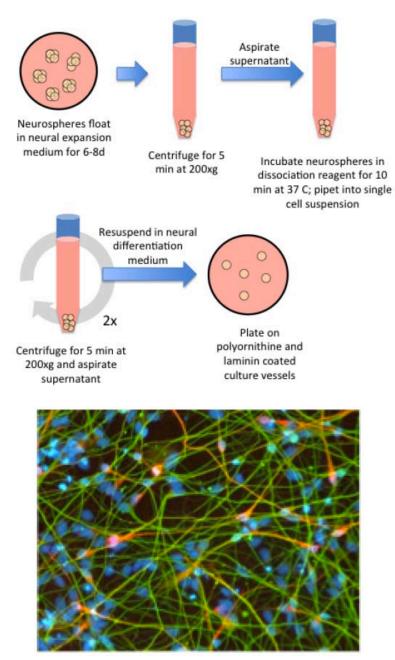
Title	Dopaminergic Neuron Differentiation		
Date Submitted	May 5, 2012		
Submitted by -	Efthymiou, Anastasia - anastasia.efthymiou@nih.gov		
Adapted from -	Gibco Protocol		
Contributors -	Efthymiou, Anastasia		
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### **\*** Introduction:



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  $200 \times g$ .
- 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		

1 mL				
2 mL				
1 mL				
200 μL				
100 μL				
DA Neuronal Differentiation Medium				
Amount				
96 mL				
1 mL				
2 mL				
1 mL				
100 μL				
100 μL				
100 μL				
100 μΜ				
	2 mL 1 mL 200 μL 100 μL ion Medium Amount 96 mL 1 mL 2 mL 1 mL 100 μL 100 μL			

<sup>\*</sup>Add GDNF, BDNF, ascorbic acid, and dcAMP at the time of medium change

# Troubleshooting:

## **\*** References: