Title	Differentiating Neural Stem Cells into Astrocytes
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Adapted from -	Gibco Protocol
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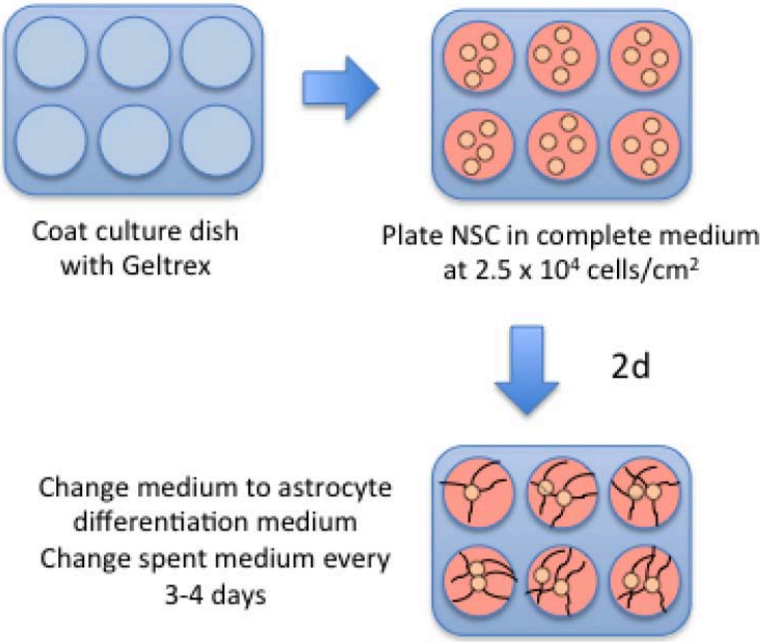
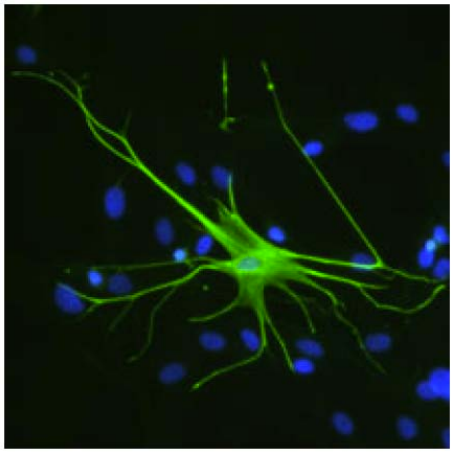
❖ Introduction:





Human astrocyte, courtesy of Dr. Riccardo Cassiani-Ingoni, NIH NINDS

❖ Protocol:

Neural stem cells (NSCs) will proliferate as progenitors a few times even after the complete growth medium is replaced with the appropriate differentiation

medium. If the cells reach 90% confluency, it might be necessary to split the cells at a 1:2 ratio. However, do not split the cells once they reach day 9-10 of differentiation when they can get damaged during the passaging process.

1. Plate the NSCs on a Geltrex - coated culture dish in complete StemPro NSC SFM at 2.5 × 10^4 cells/cm2.
2. After 2 days, change medium to astrocyte differentiation medium. Change the spent medium every 3 to 4 days.

❖ Materials:

Geltrex-coated culture dish		
StemPro NSC SFM complete Medium		
astrocyte differentiation medium		
StemPro NSC SFM Complete Media		
Component	Final concentration	Amount
KnockOutTM D-MEM/F-12	1X	97 mL
GlutaMAXTM-I Supplement	2 mM	1 mL
bFGF (prep as 100 µg/mL stock)	20 ng/mL	20 µL
EGF (prep as 100 µg/mL stock)	20 ng/mL	20 µL
StemPro® Neural Supplement	2%	2 mL
Astrocyte Differentiation Medium		
Component	Final concentration	Amount
D-MEM	1X	97 mL
N-2 Supplement	1%	1 mL
GlutaMAXTM-I Supplement	2 mM	1 mL
FBS	1%	1 mL

❖ Troubleshooting:

❖ References: