Title	Passaging Neural Stem Cells (Suspension Culture)
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Adapted from -	Gibco Protocol
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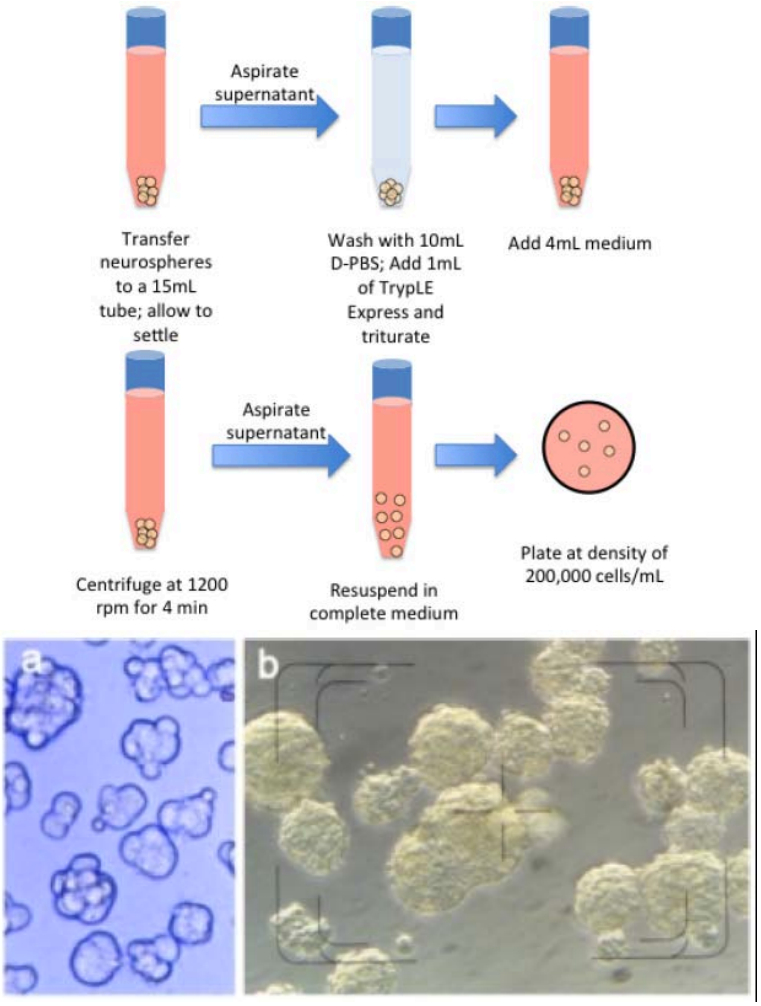
❖ Introduction:



Early phase neurosphere formation (a) and high density neurosphere culture (b), phase contrast microscopy[1]

❖ Protocol:

1. Transfer medium containing neurospheres into a 15- or 50- mL conical tube.

2. Leave the tube at room temperature and allow the neurosphere to settle to the bottom of tube. Alternatively, spin down the cells by centrifugation at 500 rpm (200 × g) for 2 minutes.
3. Aspirate the supernatant carefully, and leave the neurospheres in a minimum volume of medium.
4. Wash the neurospheres with 10 mL D- PBS without Ca2+ and Mg2+, aspirate the D- PBS supernatant carefully, and leave the neurospheres in a minimum volume of D- PBS.
5. Add 1 mL of TrypLE Express to the spheres and gently triturate neurospheres using a Pasteur pipette to create a single cell suspension.
6. Neutralize the treatment by adding 4 mL of medium.
7. Spin down the cells by centrifugation at 1,200 rpm for 4 minutes. Aspirate and discard the supernatant.
8. Resuspend the cells in StemPro NSC SFM complete medium.
9. Count cell number using hemacytometer.
10. Seed the cells in fresh medium in a suspension dish (a non- coated flask can be used) at a density of 200,000 cells/mL.

❖ Materials:

Neurospheres		
D-PBS without calcium and magnesium		
TrypLE Express		
StemPro NSC SFM complete 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

❖ Troubleshooting:

❖ References:

1. Laura Pacey KK, Shelley Stead, et al. Neural Stem Cell Culture: Neurosphere generation, microscopical analysis and cryopreservation. Protocol Exchange. (2006).