Title	Passaging Neural Stem Cells (Adherent Culture)
Date Submitted	May 5, 2012
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Adapted from -	Gibco Protocol
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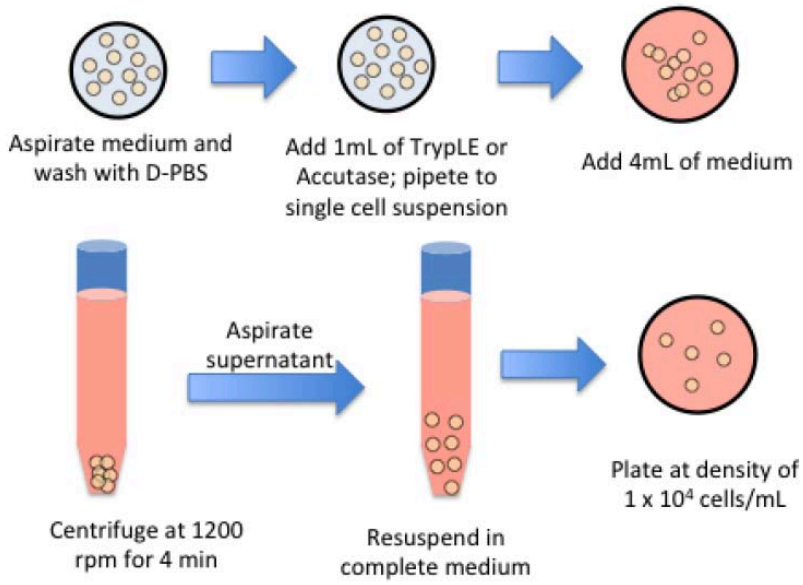
❖ Introduction:



❖ Protocol:

1. Aspirate the medium and wash with D- PBS without Ca2+ and Mg2+.
2. Add 1 mL of TrypLE Express or StemPro Accutase to the culture vessel.
   Note: The monolayer lifts off from the culture dish within 30 seconds of application of TrypLE Express or StemPro Accutase.
3. Gently pipette to loosen monolayer into a single cell suspension. Neutralize the treatment by adding 4 mL of medium. Do not treat the cells for longer than 3 minutes after addition of TrypLE Express or StemPro Accutase.
4. Spin down the cells by centrifugation at 1,200 rpm for 4 minutes. Aspirate and discard the supernatant.
5. Resuspend the cells in StemPro NSC SFM complete medium.
6. Count the cell number
7. Plate cells in fresh medium on a CELLstart CTS - or Fibronectin- coated plate at a density of 1 × 10^4 to 1 × 10^5 cells/cm2, or split the cells at a 1:4 ratio.

❖ Materials:

Neurospheres		
D-PBS without Ca++ and Mg++		
TrypLE Express or StemPro Accutase		
StemPro NSC SFM complete medium		
CELLstart CTS or Fibronectin-coated plate		
StemPro NSC SFM complete medium		
Component	Final concentration	Amount
KnockOutTM D-MEM/F-12	1X	97 mL
GlutaMAXTM-I Supplement	2 mM	01 mL
bFGF	20 ng/mL	20 µg
EGF	20 ng/mL	20 µg
StemPro® Neural Supplement	2%	2 mL

- Troubleshooting:

❖ References: