Title	Cryopreserving Neural Stem Cells
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Adapted from -	Gibco Protocol
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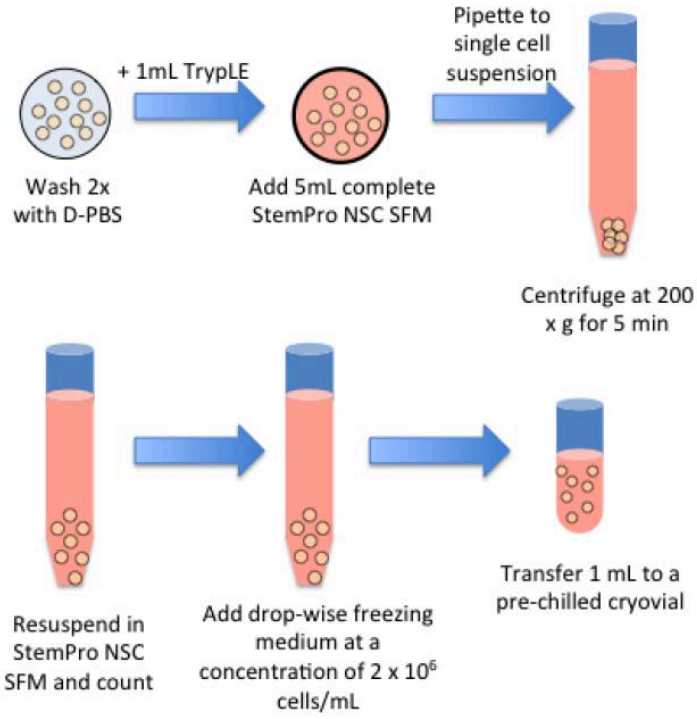
❖ Introduction:



❖ Protocol:

1. When NSCs are 80-90% confluent (2-4 days after seeding), aspirate the complete StemPro NSC SFM from the culture vessel.
2. Wash the cells twice with D- PBS. Aspirate the D- PBS and discard.
3. Add 1 mL of pre- warmed TrypLE Select to the culture vessel and incubate at 37 C for 2 minutes.
   Note: Do not incubate the NSCs in TrypLE Select for more than 2 minutes to avoid cell death. Neutralize TrypLE Select by adding complete StemPro NSC SFM immediately after the incubation period (see below).
4. Detach the NSCs from the culture vessel by pipetting off the cells or by tapping the culture vessel against the heel of your hand.
5. Stop the TrypLE Select treatment by adding 5 mL of complete StemPro NSC SFM.

6. Gently pipet the NSCs up and down to get a single cell suspension and transfer the cell suspension into a sterile 15- mL conical tube.
7. Centrifuge the NSCs at 200 × g for 5 minutes. Aspirate the supernatant and discard.
8. Resuspend the cell pellet in a minimal volume of pre- warmed complete StemPro NSC SFM and remove a sample for counting.
9. Determine the total number of cells using your method of choice.
10. Gently aspirate the medium from the conical tube and drop- wise add pre- chilled (4 C) freezing medium to resuspend the cells at a concentration of 2 × 10^6.
11. Transfer 1 mL of the NSC suspension in freezing medium into each pre- labeled, pre- chilled (4 C) cryovial.
12. Transfer the cryovials to the Cryo 1 C Freezing Container and place the container into a 80 C freezer. This procedure ensures that the cells freeze slowly.
13. The next day, transfer the cells into a liquid nitrogen.

❖ Materials:

Neural Stem Cells		
KnockOut DMEM/F-12		
StemPro NSC SFM		
FGF basic, Recombinant Human (bFGF)		
EGF, Recombinant Human		
TrypLE Select (1X)		
D-PBS		
DMSO		
StemPro NSC SFM complete medium		
Component	Final concentration	Amount
KnockOutTM D-MEM/F-12	1X	48.5 mL
GlutaMAXTM-I Supplement	2 mM	0.5 mL
bFGF	20 ng/mL	1 µg
EGF	20 ng/mL	1 µg
StemPro® Neural Supplement	2%	1 mL
Freezing medium		
Component	Final concentration	Amount
StemPro NSC SFM CM	90%	9mL
without bFGF and EGF		
DMSO	10%	

❖ Troubleshooting:

❖ References: