



FULL MEDIA NAMING: Neurosphere Media

Reagent	Manufacturer	Catalogue #	Type Annotation	Volume	Final Concentration
Neurocult NS-A Basal Medium	Stemcell Technologies	05751	Basal Medium	450 mL	89%
Neurocult NS-A Proliferation Supplement	Stemcell Technologies	05751	Supplement	50 mL	10%
<i>Pen/Strep (100x)*</i>	<i>Invitrogen</i>	<i>15140-163</i>	<i>Antibiotics</i>	<i>5 mL</i>	<i>1%</i>
<i>Fungizone (250 µg/mL)*</i>	<i>Invitrogen</i>	<i>15290018</i>	<i>Antibiotics</i>	<i>0.5 mL</i>	<i>250 ng/mL</i>
EGF (10 µg/mL)	Life Technologies	PHG0314	Supplement	1 mL	20 ng/mL
Heparin (2 mg/mL)	Stemcell Technologies	0780	Supplement	500 µL	2 µg/mL
bFGF (40 µg/mL)	Peptotech	100-19	Supplement	250 µL	20 ng/mL
<i>Gentamicin (50 mg/mL)*</i>	<i>Life Technologies</i>	<i>15750-060</i>	<i>Antibiotics</i>	<i>100 µL</i>	<i>20 µg/mL</i>

* Antibiotics must be added for lines in nursery. Established cultures do not need antibiotics.

1. Aseptically add the proliferation supplement, *Pen/Strep*, *Fungizone*, EGF, heparin, bFGF, and *Gentamicin* to the basal medium.
2. Media should be made in fresh batches every 90 days. Any media older than 2 months must be discarded.

Stock Preparation and Storage for Media Reagents

Reagent	Stock preparation	Stock concentration	Stock storage
Neurocult NS-A Basal Medium	-	-	4C
Neurocult NS-A Proliferation Supplement	-	-	-20C
<i>Pen/Strep</i>	<i>Aliquot 10 mL into 15 mL conical tubes.</i>	<i>100x</i>	<i>-20C</i>
<i>Fungizone</i>	<i>Aliquot 1 mL into 1.5 mL Eppendorf tubes.</i>	<i>250 µg/mL</i>	<i>-20C</i>
EGF	Resuspend lyophilized EGF in 1 mL PBS.	10 µg/mL	4C
bFGF	Reconstitute lyophilized bFGF in 2.5 mL nuclease-free water. Aliquot 250 µL into 1.5 mL Eppendorf tubes.	40 µg/mL	-20C
Heparin	-	2 mg/mL	4C
<i>Gentamicin</i>	-	<i>50 mg/mL</i>	<i>RT</i>

* External orders

SOP #	SOP Name	Date	Version	SOP Owner
CELL307	NSA	2020/09/10	1	Rebecca Deasy