Materials List for:

A Galvanotaxis Assay for Analysis of Neural Precursor Cell Migration Kinetics in an Externally Applied Direct Current Electric Field

Robart Babona-Pilipos[1], Milos R. Popovic[2], Cindi M. Morshead[3]

[1]Institute for Biomaterials and Biomedical Engineering, University of Toronto

[2]Lyndhurst Centre, Toronto Rehabilitation Institute

[3]Department of Surgery, University of Toronto

Correspondence to: Cindi M. Morshead at cindi.morshead@utoronto.ca

URL: https://www.jove.com/video/4193
DOI: doi:10.3791/4193

Materials

Name	Company	Catalog Number	Comments
Neural Precursor Cell Isolation			
2M NaCl	Sigma	S5886	11.688 g dissolved in 100 ml dH_2O
1M KCl	Sigma	P5405	7.456 g dissolved in 100 ml dH_2O
1M $MgCl_2$	Sigma	M2393	20.33 g dissolved in 100 ml dH_2O
155 mM $NaHCO_3$	Sigma	S5761	1.302 g dissolved in 100 ml dH_2O
0.5M Glucose	Sigma	G6152	9.01 g dissolved in 100 ml dH_2O
108 mM $CaCl_2$	Sigma	C7902	1.59 g dissolved in 100 ml dH_2O
Penicillin-streptomycin	Gibco	15070	
Bovine pancreas trypsin	Sigma	T1005	
Sheep testes hyaluronidase	Sigma	H6254	
Kynurenic acid	Sigma	K3375	
Ovomucoid trypsin inhibitor	Worthington	LS003086	
DMEM	Invitrogen	12100046	
F12	Invitrogen	21700075	
30% Glucose	Sigma	G6152	
7.5% $NaHCO_3$	Sigma	S5761	
1M HEPES	Sigma	H3375	23.83 g dissolved in 100 ml dH_2O
L-glutamine	Gibco	25030	
EGF	Invitrogen	PMG8041	Reconstitute in 1 ml of hormone mix and aliquot into 20 µl units.
FGF	Invitrogen	PHG0226	Reconstitute in 0.5 ml of hormone mix and aliquot into 20 µl units.
Heparin	Sigma	H3149	
Apo-transferrin	R&D Systems	3188-AT	0.1 g dissolved into 4 ml dH_20
Putrescine	Sigma	P7505	Dissolve 9.61 mg into Apo-transferrin solution
Insulin	Sigma	I5500	Dissolve 25 mg into 0.5 ml of 0.1N HCl and add to 3.5 ml of dH_20
Selenium	Sigma	S9133	
Progesterone	Sigma	P6149	
Standard Dissection Tools	Fine Science Tools		
Dissection microscope	Zeiss	Stemi 2000	
Galvanotaxis Chamber Preparation			

Square glass cover slides	VWR	16004	
6N Hydrochloric Acid	VWR	BDH3204-1	
High vacuum grease	Dow Corning		
60 mm Petri dishes	Fisher Scientific	0875713A	
Poly-L-lysine	Sigma	P4707	
Matrigel	BD Biosciences	354234	Thaw and aliquot into 150 µl units
FBS	Invitrogen	10082139	Only use if inducing NPC differentiation, otherwise use SFM + EFH culture media as indicated above
Counting microscope	Olympus	CKX41	
Live Cell Time-Lapse Imaging			
Silver wire	Alfa Aesar	11434	
UltraPure Agarose	Invitrogen	15510-027	
Heat Inactivated FBS	Sigma	16140071	
PVC tubing	Fisher Scientific	80000006	3/32"ID x 5/32"OD
Bleach	Clorox		
10 cc syringe	BD	309604	
18 gauge needle	BD	305195	
Dremel drill	Dremel	Model 750	
Inverted microscope equipped with humidified, incubated chamber	Zeiss	Axiovert-200M	

Recipes

Item	**Volume**
2M NaCl	6.2 ml
1M KCl	0.5 ml
1M $MgCl_2$	0.32 ml
155mM $NaHCO_3$	16.9 ml
1M Glucose	1 ml
108 mM $CaCl_2$	0.09256 ml
Penicillin-streptomycin	1 ml
Autoclaved water	74 ml

Artificial cerebrospinal fluid

Item	**Volume or Mass**
Artificial cerebrospinal fluid	30 ml
Bovine pancreas trypsin	40 mg
Sheep testes hyaluronidase	22.8 mg
Kynurenic acid	5 mg

Trypsin Solution

Item	**Volume or Mass**
SFM	15 ml
Ovomucoid trypsin inhibitor	10 mg

Trypsin Inhibitor Solution

Item	**Volume**
Autoclaved water	37 ml

10X DMEM/F12	10 ml
30% Glucose	2 ml
7.5% $NaHCO_3$	1.5 ml
1M HEPES	0.5 ml
Transferrin, Putrescine solution	4 ml
25 mg insulin solution	4 ml
Selenium	100 µl
Progesterone	100 µl

Hormone Mix (100 ml total, store at -20 °C)

Item	**Volume**
Autoclaved water	37.5 ml
10X DMEM/F12 (3:1)	5 ml
30% Glucose	1 ml
7.5% $NaHCO_3$	0.75 ml
1M HEPES	0.25 ml
Hormone mix	5 ml
L-glutamine	0.5 ml
Penicillin-streptomycin	0.5 ml

Serum Free Media EFH-SFM: add 10 µl of EGF, 10 µl of FGF, and 3.66 µl of Heparin FBS-SFM: add 0.5 ml FBS

Item	**Volume**
Matrigel	150 µl
SFM	3.6 ml

Matrigel Solution Matrigel aliquot should be placed in a box of ice and allowed to thaw slowly over 4-5 hours to form a viscous liquid before mixing with SFM. This will ensure the formation of a smooth layer of Matrigel substrate. If not thawed slowly, the resulting substrate will contain clumps of Matrigel, possibly hindering cell migration.

Item	**Volume or Mass**
UltraPure Agarose	300 mg in 10 ml ddH_20
SFM Heat Inactivated FBS	8 ml 2 ml

Matrigel Solution Mix 8 ml of SFM with 2 ml heat inactivated FBS in a 15 cc falcon tube. Mix agarose with 10 ml ddH_20 in an Erlenmeyer flask, and heat in a microwave for 30 sec in 10-sec intervals, ensuring to remove the solution from the microwave after each 10-sec interval and thoroughly mix. Following the final 10-sec microwave period, mix the agarose solution with the SFM/FBS solution and store in a 57 °C water bath.