Materials List for:

Growing Neural Stem Cells from Conventional and Nonconventional Regions of the Adult Rodent Brain

Steven W. Poser[1], Andreas Androutsellis-Theotokis[1,2]

[1]Department of Medicine, University of Dresden

[2]Center for Regerative Therapies Dresden

Correspondence to: Andreas Androutsellis-Theotokis at Andreas.Theotokis@uniklinikum-dresden.de

URL: https://www.jove.com/video/50880
DOI: doi:10.3791/50880

Materials

Name	Company	Catalog Number	Comments
6-well tissue culture dishes	BD Biosciences	353934	
Poly-L-ornithine	Sigma-Aldrich	P-3655	5 mg/ml stock solution prepared in double distilled water (Stable for several months at -20 °C). Working concentration 0.5 mg/ml in water (Stable for 1 month at 4 °C).
Fibronectin	R&D Systems	1030-Fn	Do not agitate stock solution
Filtration Apparatus	Corning Life Sciences	430769	
DMEM/F-12	Mediatech	10-090-CV	See note below for complete N2 media preparation
Apo-transferrin	Sigma-Aldrich	T-2036	
Insulin	Sigma-Aldrich	I-0516	
Putrescine	Sigma-Aldrich	P-5780	1 M stock solution in ddH_2O (Stable at 20 °C for 6 months)
Sodium Selenite	Sigma-Aldrich	S-5261	500 µM stock solution in ddH_2O (Stable at -20 °C for 6 months)
Progesterone	Sigma-Aldrich	P-8783	100 µM stock solution in ethanol (Stable at -20°C for 6 months)
Penicillin/Streptomycin	Invitrogen	15140-122	
Phosphate Buffered Saline	Mediatech	21-040-CV	
Basic Fibroblast Growth Factor (bFGF)	R&D Systems	233-FB	Working concentration 20 ng/ml
Delta4 (Dll4)	R&D Systems	1389-D4	Working concentration 500 ng/ml
Angiopoetin 2 (Ang2)	R&D Systems	623-AN	Working concentration 500 ng/ml
JAK Inhibitor	Calbiochem	420099	Working concentration 200 nM
Bovine Serum Albumin	Sigma-Aldrich	A-2058	
15- and 50-ml Conical tubes	Corning Life Sciences	430053, 430829	
Other necessary items include: General dissection instruments, including razor blade and forceps, Adult rat (3-6 months old; Sprague-Dawley or Long Evans), CO_2 intoxication chamber, Laminar flow hood for cell culture and incubator Incubator (humidified, 37 °C, 5% CO_2, 5% O_2). Note: For complete N2 media preparation, to one bottle of DMEM/F-12 (500 ml) add 0.05 g of apotransferin, 0.0125 g of insulin			

(freshly predissolved in 1 ml of 10 mM NaOH), 50 µl of putrescine, 30 µl of sodium selenite, 100 µl of progesterone stocks, and 5 ml of penicillin/streptomycin solution. Adjust pH to 7.2, if needed. Filter-sterilize and store at 4 °C for up to 3 weeks and protect from light.			
Immunofluorescence Reagents Table			
Paraformaldehyde	Electron Microscopy Sciences	15719	
Normal Donkey Serum (NDS)	Sigma-Aldrich	D-9663	
Triton X-100	Sigma-Aldrich	T-8787	
4,6-Diamidino-2-phenylindole (DAPI)	Sigma-Aldrich	D-8417	5 mg/ml stock solution in methanol
Primary Antibody Table			
Nestin	Chemicon	MAB353	Dilution Factor: 1:400 Species: Mouse IgG1
Hes3	Santa Cruz	sc-25393	Dilution Factor: 1:100 Species: Rabbit IgG
Sox2	R&D Systems	MAB2018	Dilution Factor: 1:100 Species: Mouse IgG2a
CNPase	Chemicon	MAB326	Dilution Factor: 1:200 Species: Mouse IgG1
β-tubulin III (TUJ1)	R&D Systems	MAB1195	Dilution Factor: 1:500 Species: Mouse IgG2a
Glial Fibrillary Acidic Protein (GFAP)	Dako North America	Z0334	Dilution Factor 1:500 Species: Rabbit
Secondary Antibody Table			
Alexa 568	Invitrogen	A-21124	Dilution Factor: 1:200 Species: Goat anti Mouse IgG1
Alexa 488	Invitrogen	A-21131	Dilution Factor: 1:200 Species: Goat anti Mouse IgG2a
Cy5	Jackson ImmunoResearch	59883	Dilution Factor: 1:200 Species: Goat anti Rabbit