Materials List for:

Rapid and Specific Immunomagnetic Isolation of Mouse Primary Oligodendrocytes

Rafael E. Flores-Obando[1], Mona M. Freidin[2], Charles K. Abrams[2]

[1]Program in Molecular and Cellular Biology, State University of New York Downstate Medical Center

[2]Department of Neurology and Rehabilitation, University of Illinois at Chicago

Correspondence to: Charles K. Abrams at cabrams1@uic.edu

URL: https://www.jove.com/video/57543
DOI: doi:10.3791/57543

Materials

Name	Company	Catalog Number	Comments
10ml serological pipets	Fisher Scientific	13-676-10J	
10ml syringe Luer-Loc tip	BD, Becton Dickinson	309604	
15ml conical tubes	Falcon	352097	
24-well tissue culture plates	Falcon	353935	
40µm cell strainer	Fisher Scientific	22368547	
50ml conical tubes	Falcon	352098	
5ml serological pipets	Fisher Scientific	13-676-10H	
60mm tissue culture plates	Falcon	353002	
70µm cell strainer	Fisher Scientific	22363548	
Alexa Fluor 488 goat anti-mouse IgG (H+L) secondary antibody	Invitrogen	A11001	
Alexa Fluor 488 goat anti-rabbit IgM (H+L) secondary antibody	Invitrogen	A21042	
Alexa Fluor 488 goat anti-rabbit IgM (H+L) secondary antibody	Invitrogen	A11008	
Alexa Fluor 594 goat anti-chicken IgG (H+L) secondary antibody	Invitrogen	A11042	
Anti-O4 beads- Anti-O4MicroBeads	Miltenyi Biotec	130-094-543	
Apo-Transferrin human	Sigma	T1147	
Autofil complete bottle top filter assembly, 0.22um filter, 250ml	USA Scientific	6032-1101	
Autofil complete bottle top filter assembly, 0.22um filter, 250ml	USA Scientific	6032-1102	
B27 Supplement	Invitrogen	17504-044	
Boric acid	Sigma	B7660	
Bovine Growth Serum (BGS)	GE Healthcare Life Sciences	SH30541.03	
BSA	Fisher Scientific	BP-1600-100	
CNTF	Peprotech	450-50	
d-Biotin	Sigma	B4639	
Desoxyribonuclease I (DNAse I)	Worthington	LS002007	
EDTA	Fisher Scientific	S311	
Epifluorescence microscope with an Olympus DP70 camera	Olympus	Bx51	
Feather disposable scalpels	Andwin Scientific	EF7281C	
Forskolin	Sigma	F6886	

German glass coverslips, #1 thickness, 12mm diameter round	NeuVitro	GG-12-oz	
GFAP antibody	Aves	GFAP	
Glucose	Fisher Scientific	D16-1	
GlutaMAX	Invitrogen	35050-61	
Insulin	Invitrogen	12585-014	
Magnetic separator stand - MACS multistand	Miltenyi Biotec	130-042-303	
Magnetic separator-MiniMACS separator	Miltenyi Biotec	130-042-302	
Millex PES 0.22µm filter unit	Millipore	SLG033RS	
Mounting media- Prolong Gold with DAPI	Thermo Fisher	P36930	
N-acetyl-cysteine (NAC)	Sigma	A8199	
Natural mouse laminin	Invitrogen	23017-015	
Neurobasal Medium A	Invitrogen	10888-022	
Neurotrophin-3 (NT-3)	Peprotech	450-03	
NG2 antibody	Millipore	AB5320	
Papain	Worthington	LS003126	
PBS without $Ca2^{+}$ and $Mg2^{+}$	Sigma	D5652	
PDGF	Peprotech	100-13A	
Petri dishes	Falcon	351029	
Poly-D-Lysine	Sigma	P6407	
Primocin	Invivogen	ant-pm-2	
Progesterone	Sigma	P8783	
Putrescine	Sigma	P5780	
Selection column-LS columns	Miltenyi Biotec	130-042-401	
Sodium Selenite	Sigma	S5261	
Trace elements B	Corning	25-000-CI	
Triiodothyronine (T3)	Sigma	T6397	
Triton-X	Sigma	T8787	
Trypan Blue Solution	Corning	25-900-CI	
Tween 20	Sigma	P1379	
B27NBMA			487.75 mL Neurobasal Medium A; 10 mL B27 Supplement; 1 mL Primocin; 1.25 mL Glutamax; Filter sterilize and store at 4 °C until use.
B27NBMA + 10% BGS			27 mL B27NBMA; 3 mL Bovine growth serum
CNTF solution stock (10 µg/ml; 1000X)			Order from Peprotech (450-50). Make up at 0.1 to 1 mg/ml according to Manufacturer's instruction (may vary from lot to lot) in buffer (e.g. DPBS + 0.2% BSA). Store at -80 °C. Working solution (10 µg/ml, 1000X) 1. Make on 0.2% BSA (Fisher scientific BP-1600-100) in DPBS solution and filter sterilize. 2. Dilute master stock aliquot to 10µg/ml in sterile, chilled 0.2% BSA/DPBS.

			3. Aliquot (20µl/tube) and snap freeze in liquid nitrogen. 4. Store aliquots at -80 °C.
d-Biotin stock solution (50 µg/ml; 5000X)			Resuspend d-Biotin (Sigma-B4639) in double-distilled H2O at 50 µg/ml (e.g. 2.5 mg in 50 ml of ddH2O). Resuspension might take fair amount of agitation/ vortexing, or mild warming briefly at 37°C. If the d-Biotin still will not solubilize, it is fine to make up a less concentrated (e.g. 10µg/ml), and to add a higher volume to the B27NBMA (1/1000), instead of 1/5000). Store at 4°C.
DNase I stock solution			1. Dissolve at 12,500 U Deoxyribonuclease I / ml in HBSS chilled on ice. 2. Filter sterilize on ice 3. Aliquot at 200 µl and freeze overnight at -20°C. 4. Store aliquots at -20 to -30°C.
Dulbecco's Phosphate Buffered Saline (w/o Ca2+ and Mg2+)			Dissolve pouch in 1 Liter of water to yield 1 liter of medium at 9.6 grams of powder per liter of medium. Store at 2-8 °C.
Forskolin stock solution (4.2 mg/ml; 1000X)			Add 1 ml of sterile DMSO to 50 mg Forskolin in bottle (Sigma-F6886) and pipette until resuspended. Transfer to a 15 ml centrifuge tube and add 11 ml of sterile DMSO to bring to 4.2 mg/ml. Aliquot (e.g. 20 µl) and store at -20°C.
Hank's balanced salts (HBSS) (Sigma			1. Measure 900 ml of water (temperature 15-20 °C) in a cylinder and stir gently. 2. Add the power and stir until dissolved. 3. Rinse original package with a small amount of water to remove all traces of the powder. 4. Add to the solution in step 2. 5. Add 0.35 gr of sodium bicarbonate (7.5% w/v) for each liter of final volume. 6. Keep stirring until dissolved. 7. Adjust the pH of the buffer while stirring to 0.1-0.3 units below pH= 7.4 since it may rise during filtration. The use of 1N HCl or 1N NaOH is recommended to adjust the pH. 8. Add additional water to bring the final volume to 1L. 9. Sterilize by filtration using a membrane with a porosity of 0.22 microns. 10. Store at 2-8 °C.
Insulin stock solution (4000 µg/ml)			Thaw the bottle and aliquot 25 µl per microcentrifuge tube and store at -20°C.
Laminin solution			Slowly thaw laminin in the cold (2°C to 8°C) to avoid gel formation. Then, aliquot into polypropylene tubes. Store at 5° C to -20° C in aliquots (e.g. 20 µl) and do

			not freeze/thaw repeatedly. Laminin may be stored at these temperatures for up to six months.
Magnetic Cell Sorting (MCS) Buffer			Prepare the solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5% bovine serum albumin (BSA), 0.5 mM EDTA, 5µg/ml Insulin, 1 g/L Glucose. Sterilize and degas by filtration the buffer by passing it through a 0.22 µm Millex filter. Store the buffer at 4°C until use
N-Acetyl-L-cysteine (NAC) stock solution (5mg/ml; 1000X)			Dissolve N-Acetyl-L-cysteine (Sigma-A8199) at 5 mg/ml in DMEM (e.g. 50 mg NAC in 10 ml B27NBMA). Filter sterilize and aliquot (e.g. 20 µl). Store at -20°C.
NT3 stock solution (1 µg/ml; 1000X)			Master stock: Order from Peprotech (450-03). Make up at 0.1 to 1 mg/ml according to manufacturer's instructions (may vary from lot to lot), in buffer (e.g. DPBS + 0.2% BSA). Store at -80°C. Working stock (1µg/ml; 1000X): 1. Make on 0.2% BSA in DPBS solution and filter sterilize. 2. Dilute master stock aliquot to 1 µg/ml in sterile, chilled 0.2% BSA/ DPBS. 3. Aliquot (e.g. 20µl/tube) and snap freeze in liquid nitrogen. 4. Store aliquots at -80°C.
PDGF stock solution (10 µg/ml; 1000X)			Master stock: Order from Peprotech (100-13A). Make up at 0.1 to 1 mg/ml according to manufacturer's instructions (may vary from lot to lot) in buffer (e.g. DPBS) + 0.2% BSA). Store at -80°C. Working stock (1µg/ml; 1000X): 1. Make on 0.2% BSA in DPBS solution and filter sterilize. 2. Dilute master stock aliquot to 1µg/ml in sterile, chilled 0.2% BSA/ DPBS. 3. Aliquot (e.g. 20µl/tube) and snap freeze in liquid nitrogen. 4. Store aliquots at -80°C.
Poly-D-lysine (1mg/ml; 100X)			Resuspend poly-D-lysine, molecular weight 70-150 kD (Sigma P6407) at 0.5mg/ml in 0.15M boric acid pH 8.4 (e.g. 50mg in 50ml borate buffer). Filter sterilize and aliquot (e.g. 100µl/ tube). Store at -20°C. Prior to use, dilute the 100X stock (1mg/ml) to 50 µg/ml in sterile water.
Oligodendrocyte proliferation media			see Supplementary Table 1
Oligodendrocyte differentiation media			see Supplementary Table 1
Sato supplement (100X)			see Supplementary Table 1
References: the list of reagents and recipes were adopted from			

the protocols previously described by Emery et. al. 2013 (Emery, B. & Dugas, J. C. Purification of oligodendrocyte lineage cells from mouse cortices by immunopanning. Cold Spring Harb Protoc. 2013 (9), 854-868, doi:10.1101/pdb.prot073973, (2013)) and Dincman et. al. (Dincman, T. A., Beare, J. E., Ohri, S. S. & Whittemore, S. R. Isolation of cortical mouse oligodendrocyte precursor cells. J Neurosci Methods. 209 (1), 219-226, doi:10.1016/ j.jneumeth.2012.06.017, (2012))			