

Materials List for:

Isolation and Culture of Rodent Microglia to Promote a Dynamic Ramified Morphology in Serum-free Medium

Hannah Y. Collins¹, Christopher J. Bohlen¹

¹Department of Neurobiology, Stanford University

Correspondence to: Christopher J. Bohlen at cjbohlen@gmail.com

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Materials

Name	Company	Catalog Number	Comments
Rat: Sprague-Dawley	Charles River	Cat# 400	
mouse anti-rat CD11b monoclonal (clone OX42)	Bio-Rad	Cat# MCA275R	Panning: 1:1,000; Staining: 1:500
Goat polyclonal anti-Iba1	Abcam	Cat# AB5076	Staining: 1:500
Rabbit polyclonal anti-Ki67	Abcam	Cat# AB15580	Staining: 1:500
Alexa Fluor Donkey anti-mouse 594	Invitrogen	Cat# 11055	Staining: 1:500
Alexa Fluor Donkey anti-goat 488	Invitrogen	Cat# A-21203	Staining: 1:500
Alexa Fluor Donkey anti-Rabbit 647	Invitrogen	Cat# A-31573	Staining: 1:500
Triton-X (detergent in ICC staining)	Thermo Fisher	Cat# 28313	
Heparan sulfate	Galen Laboratory Supplies	Cat# GAG-HS01	
Heparin	Sigma	Cat# M3149	
Peptone from milk solids	Sigma	Cat# P6838	
TGF-β2	Peprtech	Cat# 100-35B	
Murine IL-34	R&D Systems	Cat# 5195-ML/CF	
Ovine wool cholesterol	Avanti Polar Lipids	Cat# 700000P	
Collagen IV	Corning	Cat# 354233	
Oleic acid	Cayman Chemicals	Cat# 90260	
11(Z)Eicosadienoic (Gondoic) Acid	Cayman Chemicals	Cat# 20606	
Calcein AM dye	Invitrogen	Cat# C3100MP	
Ethidium homodimer-1	Invitrogen	Cat# E1169	
DNaseI	Worthington	Cat# DPRFS	
Percoll PLUS	GE Healthcare	Cat# 17-5445-02	
Trypsin	Sigma	Cat# T9935	
DMEM/F12	Gibco	Cat# 21041-02	
Penicillin/ Streptomycin	Gibco	Cat# 15140-122	
Glutamine	Gibco	Cat# 25030-081	
N-acetyl cysteine	Sigma	Cat# A9165	
Insulin	Sigma	Cat# 16634	
Apo-transferrin	Sigma	Cat# T1147	
Sodium selenite	Sigma	Cat# S-5261	
DMEM (high glucose)	Gibco	Cat# 11960-044	
Dapi Fluoromount-G	Southern Biotech	Cat# 0100-20	
Poly-D-Lysine	Sigma	Cat# A-003-E	

Primaria Plates	VWR	Cat# 62406-456	
Name	Company	Catalog Number	Comments
Stock reagents			
Apo-transferrin			Reconstitution: 10 mg/mL in PBS Concentration used: 1:100 Storage: -20°C
N-acetyl cysteine			Reconstitution: 5 mg/mL in H2O Concentration used: 1:1,000 Storage: -20°C
Sodium selenite			Reconstitution: 2.5 mg/mL in H2O Concentration used: 1:25,000 Storage: -20°C
Collagen IV			Reconstitution: 200 µg/mL in PBS Concentration used: 1:100 Storage: -80°C
TGF-b2			Reconstitution: 2 mg/mL in PBS Concentration used: 1:1,000 Storage: -20°C
IL-34			Reconstitution: 200 µg/mL in PBS Concentration used: 1:1,000 Storage: -80°C
Ovine wool cholesterol			Reconstitution: 1.5 mg/mL in 100% ethanol Concentration used: 1:1,000 Storage: -20°C
Heparan sulfate			Reconstitution: 1 mg/mL in H2O Concentration used: 1:1,000 Storage: -20°C
Oleic acid/Gondoic acid			Reconstitution: Gondoic: 0.001 mg/mL; Oleic: 0.1 mg/mL in 100% ethanol Concentration used: 1:1,000 Storage: -20°C
Heparin			Reconstitution: 50 mg/mL in PBS Concentration used: 1:100 Storage: -20°C
Name	Company	Catalog Number	Comments
Solutions			
Perfusion Buffer			Recipe: 50 µg/mL heparin in DPBS++ (PBS with Ca++ and Mg+ +) Comments: Use when ice-cold
Douncing Buffer			Recipe: 200 µL of 0.4% DNaseI in 50 mL of DPBS++ Comments: Use when ice-cold
Panning Buffer			Recipe: 2 mg/mL of peptone from milk solids in DPBS++
Microglia Growth Medium (MGM)			Recipe: DMEM/F12 containing 100 units/mL penicillin, 100 µg/mL streptomycin, 2 mM glutamine, 5 µg/mL N-acetyl cysteine, 5 µg/mL insulin, 100 µg/mL apo-transferrin, and 100 ng/mL sodium selenite Comments: Use ice-cold MGM to pan microglia off of immunopanning dish.
Collagen IV Coating			Recipe: MGM containing 2 µg/mL collagen IV

Myelin Separation Buffer			<p>Recipe: 9 mL Percoll PLUS, 1 mL 10x PBS without Ca⁺⁺ and Mg⁺⁺, 9 μL 1 M CaCl₂, 5 μL 1 M MgCl₂</p> <p>Comments: Mix well after the addition of CaCl₂ and MgCl₂</p>
TGF-b2/IL-34/Cholesterol containing growth media (TIC)			<p>Recipe: MGM containing human 2 ng/mL TGF-b2, 100 ng/mL murine IL-34, 1.5 μg/mL ovine wool cholesterol, 10 μg/mL heparan sulfate, 0.1 μg/ml oleic acid, and 0.001 μg/ml gondoic acid</p> <p>Comments: Make sure to add cholesterol to media warmed to 37 °C and do not add more than 1.5 μg/mL or it will precipitate out. Do not filter cholesterol-containing media. Equilibrate TIC media with 10% CO₂ for 30 min- 1 hr before adding to cells to insure optimal pH.</p>