

Materials List for:

Regeneration of Arrayed Gold Microelectrodes Equipped for a Real-Time Cell Analyzer

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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	Peptotech	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	
Stock reagents	reconstitution	Concentration used	Storage
Apo-transferrin	10 mg/mL in PBS	1:100	-20°C
N-acetyl cysteine	5 mg/mL in H ₂ O	1:1,000	-20°C
Sodium selenite	2.5 mg/mL in H ₂ O	1:25,000	-20°C
Collagen IV	200 µg/mL in PBS	1:100	-80°C
TGF-b2	20 µg/mL in PBS	1:10,000	-20°C
IL-34	100 µg/mL in PBS	1:1,000	-80°C
Ovine wool cholesterol	1.5 mg/mL in 100% ethanol	1:1,000	-20°C
Heparan sulfate	1 mg/mL in H ₂ O	1:1,000	-20°C
Oleic acid/Gondoic acid	Gondoic: 0.001 mg/mL; Oleic: 0.1 mg/mL in 100% ethanol	1:1,000	-20°C
Heparin	50 mg/mL in PBS	1:100	-20°C
Solutions	Recipe		Comments
Perfusion Buffer	50 µg/mL heparin in DPBS ⁺⁺ (PBS with Ca ⁺⁺ and Mg ⁺⁺)		Use when ice-cold
Douncing Buffer	200 µL of 0.4% DNaseI in 50 mL of DPBS ⁺⁺		Use when ice-cold
Panning Buffer	2 mg/mL of peptone from milk solids in DPBS ⁺⁺		
Microglia Growth Medium (MGM)	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		Use ice-cold MGM to pan microglia off of immunopanning dish.
Collagen IV Coating	MGM containing 2 µg/mL collagen IV		
Myelin Separation Buffer	9 mL Percoll PLUS, 1 mL 10x PBS without Ca ⁺⁺ and Mg ⁺⁺ , 9 µL 1 M CaCl ₂ , 5 µL 1 M MgCl ₂		Mix well after the addition of CaCl ₂ and MgCl ₂
TGF-b2/IL-34/Cholesterol containing growth media (TIC)	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		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% CO ₂ for 30 min- 1 hr before adding to cells to insure optimal pH.