

Isolation of Targeted Hypothalamic Neurons for Studies of Hormonal, Metabolic, and Electrical Regulation

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jove.com/video/65674

Materials

Name	Company	Catalog Number	Comments
Embryo extraction			
1 curved point forceps	Fine Science Tools	11270-20	Dumont
1 fine surgical scissor	Fine Science Tools	14058-11	Dumont
100 mm Petri dish	Corning	430167	
2 straight fine forceps	Fine Science Tools	11254-20	Dumont
60 mm Petri dish	Corning	430196	
70% ethanol	Decon Laboratories, INC.	2801	Ethanol 190 Proof
Anti-Biotin MicroBeads 1mL	Miltenyi Biotec	130-115-390	
Anti-MAP2 antibody	Abcam	ab5392	1 : 800
Bench pads			
Bovine Serum Albumin	Sigma-Aldrich	A9418-50G	
Buffer Y	Miltenyi Biotec	130-094-802	
Buffer Z	Miltenyi Biotec	130-094-802	
Cell Culture			
Anti-Biotin MicroBeads 1mL	Miltenyi Biotec	130-115-390	
Bovine Serum Albumin	Sigma-Aldrich	A9418-50G	
Buffer Y	Miltenyi Biotec	130-094-802	
Buffer Z	Miltenyi Biotec	130-094-802	
Enzyme A	Miltenyi Biotec	130-094-802	
Enzyme P	Miltenyi Biotec	130-094-802	
GG-12-1.5, 12 mm dia.#1.5 thick 100 pc cell culture tested German coverglasses	Neuvitro Corporation	GG-12-15	
Gibco B-27 Supplement 10 mL	ThermoFisher	17504-044	
Gibco Basal Medium Eagle (BME) 500 mL	ThermoFisher	21010046	(+) Earle's Salts, (-) L-Glutamine
Gibco HBBS (1x) Hanks' Balanced Salt Solution 500 mL	ThermoFisher	14025092	Calcium, Magnesium, No phenol red
Gibco HI FBS 100 mL	ThermoFisher	16140-063	

Gibco L-Glutamine 200 mM (100x)	ThermoFisher	25030-081	
Gibco Penicilline/Streptomycin	ThermoFisher	15140-122	10,000 U/mL
Gibco Sodium Pyruvate (100 mM) 100 mL	ThermoFisher	11360070	
MiniMACS Separator and Starting Kit	Miltenyi Biotec	130-042-102	
Mouse Leptin R Biotinylated Antibody	R&D Systems	ABAF497	0.25 µg/10 ⁶ cells
MS Column	Miltenyi Biotec	130-042-201	
Neabeaur-Improved Brightline 100 µm Chamber	Hausser Scientific	3120	
Neural Tissue Dissociation Kit - Postnatal Neurons	Miltenyi Biotec	130-094-802	
Neuronal Culture Medium 500 mL	ThermoFisher	88283	
Non-Neuronal Cell Biotin-Antibody Cocktail mouse 1 mL	Miltenyi Biotec	130-115-389	
Olympus SZ61 Zoom Stereomicroscope	Olympus Life Science	SZ61/SZ51	
Pierce Primary Neuron Isolation Kit	ThermoFisher	88280Y	
Staining			
Anti-MAP2 antibody	Abcam	ab5392	1 : 800
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488	ThermoFisher	A32766	1 : 500
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488	ThermoFisher	A32790	1 : 500
Dulbecco's Phosphate Buffered Saline (DPBS)	Sigma Aldrich	MFCD00131855	
Goat anti-Chicken IgY (H+L) Cross- Adsorbed Secondary Antibody, Alexa Fluor Plus 647	ThermoFisher	A32933	1 : 500
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	ThermoFisher	A11037	1 : 200
Invitrogen Leptin Receptor Recombinant Rabbit Monoclonal Antibody (JA73-01)	ThermoFisher	MA5-32685	1 : 500
Mouse Leptin R Biotinylated Antibody	R&D Systems	ABAF497	1 : 500
POMC Rabbit mAb	Cell Signaling Technology	D3R1U	1 : 500
PSD95 (D74D3) XP Rabbit mAb	Cell Signaling Technology	D74D3#3409	1 : 500
Streptavidin, Alexa Fluor 594 conjugate	ThermoFisher	S11227	1 : 500
Synapsin 1 Monoclonal Antibody (7H10G6)	ThermoFisher	MA5-31919	1 : 500
Vectashield Plus Antifade Mountina Medium with DAPI 10 mL	Vector Laboratories	H-2000	