

# An Improved Protocol to Purify and Directly Mono-Biotinylate Recombinant BDNF in a Tube for Cellular Trafficking Studies in Neurons

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## Materials

Name	Company	Catalog Number	Comments
2 way stopcock	BioRad	7328102	Chromatography apparatus component
2-mercaptoethanol	Sigma	M6250	BDNF elution buffer
Acrylamide/Bisacrylamide	BioRad	1610154	SDS-PAGE gel preparation
Amicon Ultra-15 10K	Millipore	UFC901024	BDNF concentration
Ammonium Persulfate	Sigma	A9164	SDS-PAGE gel preparation
anti B-III-Tubulin antibody	Sigma	T8578	Western blot assays for BDNF biological activity detection
anti BDNF antibody	Alomone	AGP-021	Western blot assays for BDNF quantification
anti BDNF antibody	Alomone	ANT-010	Western blot assays for BDNF quantification
Anti ERK antibody	Cell Signaling	9102	Western blot assays for BDNF biological activity detection
anti pCREB antibody (S133)	Cell Signaling	9198	Western blot assays for BDNF biological activity detection
anti pERK antibody (T202, Y204)	Cell Signaling	4370	Western blot assays for BDNF biological activity detection
anti pTrkB antibody (Y515)	Abcam	ab109684	Western blot assays for BDNF biological activity detection
Antibiotic/Antimycotic	Gibco	15240-062	HEK293 maintenance
ATP	Sigma	A26209	BDNF monobiotinylation buffer
B-27 Supplement	Gibco	17504-044	Neuron maintenance
Bicine	Sigma	B3876	BDNF monobiotinylation buffer
BirA-GST	BPS Bioscience	70031	Enzyme for BDNF AviTag monobiotinylation

Bovine Fetal Serum	HyClone	HC.SH30396.02	HEK293 maintenance
Bovine Serum Albumin	Jackson ImmunoResearch	001-000-162	BDNF buffer modification component, blocking buffer for western blot and immunofluorescence
D-Biotin	Sigma	B4639	BDNF monobiotinylation buffer
DMEM High Glucose Medium	Gibco	11965-092	Neuron seeding
DMEM Medium	Gibco	11995-081	HEK293 maintenance
Econo Column Funnel	BioRad	7310003	Chromatography apparatus component
EDTA	Merck	108418	
EZ-ECL Kit	Biological Industries	1633664	Protein detection by western blotting
Glutamax	Gibco	35050-061	Neuron and HEK293 maintenance
Glycerol	Merck	104094	BDNF elution buffer, lysis buffer for western blot assays
Hettich Rotina 46R Centrifuge	Hettich	Discontinued	Centrifuge used for clearing the medium of debris
Hettich Universal 32R Centrifuge	Hettich	Discontinued	Centrifuge used for protein concentrator centrifugation
Horse Serum	Gibco	16050-122	Neuron seeding
ImageQuant LAS 500	GE Healthcare Life Sciences	29005063	Western blot image acquisition
Imidazole	Sigma	I55513	BDNF buffer modification component
KCl	Winkler	BM-1370	PBS component
KH <sub>2</sub> PO <sub>4</sub>	Merck	104873	PBS component
Laminin	Invitrogen	23017-015	Cover coating for compartmentalized neurons
Luer Tubing Adaptor	BioRad	7323245	Chromatography apparatus component
Luminata™ Forte Western HRP Substrate	Millipore	WBLUF0100	Protein detection by western blotting
Mg(CH <sub>3</sub> COO) <sub>2</sub>	Merck	105819	BDNF monobiotinylation buffer
Mowiol 4-88	Calbiochem	475904	Mounting reagent for immunofluorescence assays
MyOne C1 Streptavidin Magnetic Beads	Invitrogen	65001	Biotinylation verification
Na <sub>2</sub> HPO <sub>4</sub>	Merck	106586	BDNF buffer modification component
NaCl	Winkler	BM-1630	PBS component, BDNF buffer modification component
NaH <sub>2</sub> PO <sub>4</sub>	Merck	106346	BDNF buffer modification component
Neurobasal Medium	Gibco	21103-049	Neuron maintenance
Ni-NTA Agarose Beads	Qiagen	30210	BDNF AviTag purification
Nikon Ti2-E	Nikon		Microscope for fluorescence imaging
Nitrocellulose Membrane	BioRad	1620115	Protein transfer for western blotting
ORCA-Flash4.0 V3 Digital CMOS camera	Hamamatsu	C13440-20CU	Camera for epifluorescence imaging
P8340 Protease Inhibitor Cocktail	Sigma	P8340	BDNF buffer modification component
Paraformaldehyde	Merck	104005	Fixative for immunofluorescence assays
Penicillin/Streptomycin	Gibco	15140-122	Neuron maintenance
Poli-D-Lysine	Corning	DLW354210	Cover coating for compartmentalized neurons
Poli-L-Lysine	Millipore	P2363	Cover coating for non-compartmentalized neurons

Poly-Prep Chromatography Column	BioRad	7311550	Chromatography apparatus component
Polyethyleneimine 25K	Polysciences Inc.	PLY-0296	HEK293 transfection
Quantum Dots 655 streptavidin conjugate	Invitrogen	Q10121MP	Monobiotinylated BDNF AviTag label for live and fixed cell experiments
Saponin	Sigma	S4521	Detergent for immunofluorescence assays
Syldgard 184 silicone elastomer base	Poirot	4019862	Microfluidic chamber preparation
TEMED	Sigma	T9281	SDS-PAGE gel preparation
Tris	Winkler	BM-2000	Lysis buffer component
Triton X100	Merck	108603	Cell permeabilization in immunofluorescence and western blot assays
Trypsin-EDTA 0.5%	Gibco	15400-054	HEK293 passaging