

Study of the Functions and Activities of Neuronal K-Cl Co-Transporter KCC2 Using Western Blotting

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Materials

Name	Company	Catalog Number	Comments
40% acrylamide	Sigma-Aldrich	A2917	Used to make separating and stacking gel for SDS-PAGE
Ammonium Per Sulfate	Sigma-Aldrich	248614	Used to make separating and stacking gel for SDS-PAGE
anti pSPAK	Dundee University	S670B	Used as primary antibody for western blotting
anti-KCC2	Dundee University	S700C	Used as primary antibody for western blotting
anti-KCC2 pSer940	Thermo Fisher Scientific	PA5-95678	Used as primary antibody for western blotting
anti-KCC2 pThr1007	Dundee University	S961C	Used as primary antibody for western blotting
anti-KCC2 pThr906	Dundee University	S959C	Used as primary antibody for western blotting
anti-mouse	Cell Signalling technology	66002	Used as secondary antibody for western blotting
anti-NKCC1	Dundee University	S841B	Used as primary antibody for western blotting
anti-NKCC1 pThr203/207/212	Dundee University	S763B	Used as primary antibody for western blotting
anti-rabbit	Cell Signalling technology	C29F4	Used as secondary antibody for western blotting
anti-sheep	abcam	ab6900	Used as secondary antibody for western blotting
anti-SPAK	Dundee University	S669D	Used as primary antibody for western blotting
anti- β -Tubulin III	Sigma-Aldrich	T8578	Used as primary antibody for western blotting
Benzamine	Merck UK	135828	Used as component of lysis buffer
Beta-mercaptoethanol	Sigma-Aldrich	M3148	Used as component of loading buffer and lysis buffer
Bradford Coomassie	Thermo Scientific	1856209	Used for lysate protein quantification

Casting apparatus	Atto	WSE-1165W	Used to run SDS-page electrophoresis
Centrifuge	Eppendorf	5804	Used in lysate preparation
Centrifuge	VWR	MicroStar 17R	Used for spinning samples
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D2650-100ML	Used for cell culture experiment
Dried Skimmed Milk	Marvel	N/A	Used to make blocking buffer
Dulbecco's Modified Eagle's Medium - high glucose	Sigma-Aldrich	D6429	Used for cell culture
ECL reagent	Perkin Elmer	ORTT755/2655	Used to develop image for western blotting
EDTA	Fisher Scientific	D/0700/53	Used as component of lysis buffer
EGTA	Sigma-Aldrich	e4378	Used as component of lysis buffer
Electrophoresis Power Supply	BioRad	PowerPAC HC	To supply power to run SDS-page electrophoresis
Ethanol	ThermoFisher	E/0650DF/17	Used for preparing sterilized equipments and environment
Fetal Bovine Serum - heat inactivated	Merck Life Sciences UK	F9665	Used for cell culture
Fumehood	Walker	A7277	Used for cell culture
Gel Blotting - Whatman	GE Healthcare	10426981	Used in western blotting to make transfer sandwich
Glycine	Sigma-Aldrich	15527	Used to make buffers
GraphPad Prism Software	GraphPad Software, Inc., USA	Version 6.0	Used for plotting graphs and analysing data for western blotting
HCl	Acros Organics	10647282	Used to make separating and stacking gel for SDS-PAGE
Heating block	Grant	QBT1	Used to heat WB loading samples
HEK293 cells	Merck UK	12022001-1VL	Cell line for culture experiment
ImageJ Software	Wayne Rasband and Contributors; NIH, USA	ImageJ 1.53e	Used to measure band intensities from western blotting images
Imaging system	BioRad	ChemiDoc MP	Used to take western blotting images
Incubator	LEEC	LEEC precision 190D	Used for cell culture
Isopropanol	Honeywell	24137	Used in casting gel for electrophoresis
L-glutamine solution	Sigma-Aldrich	G7513	Used for cell culture
Lithium dodecyl sulfate (LDS)	Novex	NP0008	Used as loading buffer for western blotting
MEM Non-essential amino acid	Merck Life Sciences UK	M7145	Used for cell culture
Microcentrifuge	Eppendorf	5418	Used for preparing lysates for WB
Microplate reader	BioRad	iMark	Used for lysate protein concentration readout
Microsoft Powerpoint	Microsoft, USA	PowerPoint2016	Used to edit western blotting images
Molecular Weight Marker	BioRad	1610373	Used for western blotting
N-ethylmaleimide	Thermo Fisher Scientific	23030	Used for cell culture experiment
Nitrocellulose membrane	Fisher Scientific	45004091	Used for western blotting
Penicillin-Streptomycin	Gibco	15140122	Used for cell culture
pH Meter	Mettler Toledo	Seven compact s210	Used to monitor pH of buffer solutions
Phenylmethylsulfonylfluoride (PMSF)	Sigma-Aldrich	P7626	Used as component of lysis buffer
Phosphate Buffer Saline	Sigma-Aldrich	D8537	Used for cell culture
PKC δ pThr505	Cell Signalling technology	9374	Used as primary antibody for western blotting
Sepharose Protein G	Generon	PG50-00-0002	Used for immunoprecipitation

Sodium chloride	Sigma-Aldrich	S7653	Used as component of wash buffer
Sodium Chloride	Sigma-Aldrich	S7653	Used to prepare TBS-T buffer
Sodium Dodecyl Sulfate	Sigma-Aldrich	L5750	Used to make separating and stacking gel for SDS-PAGE
sodium orthovanadate	Sigma-Aldrich	S6508	Used as component of lysis buffer
Sodium Pyruvate	Sigma-Aldrich	S8636	Used for cell culture
sodium- β -glycerophosphate	Merck UK	G9422	Used as component of lysis buffer
Staurosporine (from <i>Streptomyces</i> sp.)	Scientific Laboratory Supplies, UK	S4400-1MG	Used for cell culture experiment
Sucrose	Scientific Laboratory Supplies	S0389	Used as component of lysis buffer
TEMED	Sigma-Aldrich	T7024	Used to make separating and stacking gel for SDS-PAGE
Transfer Chamber	BioRad	1658005EDU	Used in western blotting to transfer protein on membrane
Tris	Sigma-Aldrich	T6066	Used to make separating and stacking gel for SDS-PAGE
Triton-X100	Sigma-Aldrich	T8787	Used as component of lysis buffer
Trypsin-EDTA Solution	Merck Life Sciences UK	T4049	Used for cell culture
Tween-20	Sigma-Aldrich	P3179	Used as make TBS-T buffer
Vacuum pump	Charles Austen	Dymax 5	Used for cell culture
Vortex	Scientific Industries	K-550-GE	Used in sample preparation
Vortex mixer	Scientific Industries Ltd	Vortex-Genie K-550-GE	Used of mixing resolved sample
Water bath	Grant Instruments Ltd. (JB Academy)	JBA5	Used to incubate solutions