

2 MATERIALS

2.1 ANTIBODIES

2.1.1 Primary antibodies (monoclonal)

phosphotyrosine	Boehringer Mannheim (Germany) Calbiochem (San Diego, CA, USA) Sigma (St. Louis, MO, USA)
SNAP 25	Sternberger Monoclonals (Baltimore, MD, USA)
v-src	Calbiochem (San Diego, CA, USA)
synaptobrevin I	R. Jahn (Göttingen, Germany) (Baumert et al., 1989)
synaptobrevin II (Cl 69.1)	R. Jahn (Göttingen, Germany) (Edelmann et al., 1995)
synaptophysin (Cl 7.2)	R. Jahn (Göttingen, Germany) (Jahn et al., 1985)
synaptotagmin (Cl 41.1)	R. Jahn (Göttingen, Germany) (Brose et al., 1992)
syntaxin	Sigma (St. Louis, MO, USA)

2.1.2 Primary antisera (polyclonal)

α -subunit of the Kv1.6 channel	R. Veh (Berlin, Germany) (Veh et al., 1995)
munc 18	Synaptic Systems (Göttingen, Germany)
proton pump	Synaptic Systems (Göttingen, Germany)
synaptobrevin II (Cl 106.5)	R. Jahn (Göttingen, Germany) (Edelmann et al., 1995)
synaptophysin (G96)	R. Jahn (Göttingen, Germany) (Jahn et al., 1985)
synaptoporin	Synaptic Systems (Göttingen, Germany)

2.1.3 Secondary antibodies

horse anti-mouse IgG, peroxidase-labelled	Vector Laboratories (Burlingame, CA, USA)
horse anti-mouse IgG, phosphatase-labelled	Vector Laboratories (Burlingame, CA, USA)
goat anti-rabbit IgG, peroxidase- labelled	Vector Laboratories (Burlingame, CA, USA)
goat anti-rabbit IgG, phosphatase-labelled	Vector Laboratories (Burlingame, CA, USA)
goat anti-rat IgG, peroxidase- labelled	Calbiochem (San Diego, CA, USA)

2.1.4 Recombinant antibodies

Anti-phosphotyrosine RC 20: HRPO	Transduction Laboratories (Lexington, CA, USA)
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2.2 CHEMICALS AND REAGENTS

2-mercaptoethanol	Sigma (St. Louis, MO, USA)
acrylamide stock solution (30 %)	Roth (Karlsruhe, Germany)
BCA (sodium bicinchoninate-4,4-dicarboxy-2,2'- bichinoline)	Sigma (St. Louis, MO, USA)
BCIP (5-bromo-4-chloro-3-indoyl-phosphate)	Sigma (St. Louis, MO, USA)
bisacrylamide stock solution (30 %)	Roth (Karlsruhe, Germany)
BSA (bovine serum albumin)	Sigma (St. Louis, MO, USA)
CHAPS (3-([3-cholamidopropyl]-dimethylammonio)-1- propanesulfonate)	Sigma (St. Louis, MO, USA)

cholic acid	Sigma (St. Louis, MO, USA)
DSS (disuccinimidyl suberate)	Sigma (St. Louis, MO, USA)
DMSO (dimethyl sulfoxide)	Roth (Karlsruhe, Germany)
DNase I	Boehringer Mannheim (Germany)
ECL™ (enhanced chemiluminescence)	Amersham (Buckinghamshire, UK)
EDTA (ethylenediaminetetraacetic acid)	Roth (Karlsruhe, Germany)
Eupergit C1Z beads	Roehm Pharma (Weiterstadt, Germany)
Ficoll® 400	Pharmacia Biotech (Uppsala, Sweden)
HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)	Sigma (Munich, Germany)
Hybond C nitro-cellulose membrane for Western blotting	Amersham (Buckinghamshire, UK)
iPTG isopropyl-β-D-thiogalactoside	Roth (Karlsruhe, Germany)
LB Broth base	Life Technologies (Paisley, Scotland)
LB agar	Life Technologies (Paisley, Scotland)
lysozyme	Sigma (St. Louis, MO, USA)
LMW (low molecular weight marker)	Pharmacia Biotech (Uppsala, Sweden)
NBT (nitro blue tetrazolium chloride)	Sigma (St. Louis, MO, USA)
Ni-NTA Superflow (nickel-beads)	Quiagen (Santa Clarita, CA, USA)
Polyprep chromatography column	Bio-Rad (Hercules, CA, USA)
Ponceau S	Sigma (St. Louis, MO, USA)

protease inhibitors:

aprotinin	Boehringer Mannheim (Germany)
leupeptin	Boehringer Mannheim (Germany)
pepstatin A	Boehringer Mannheim (Germany)
PMSF (phenylmethylsulfonyl fluoride)	Roth (Karlsruhe, Germany)

Protein G Sepharose 4 Fast Flow Pharmacia Biotech (Uppsala, Sweden)

Roti-quant kit Roth (Karlsruhe, Germany)
for determination of protein concentration

SDS Bohringer Mannheim (Germany)
(sodium dodecyl sulphate)

Triton X-100 Roth (Karlsruhe, Germany)

2.3 BUFFERS AND SOLUTIONS

Antibody solution

1.5 % bovine serum albumin in TS buffer

AP-buffer (for immunodetection on Western Blots)

1 ml 1.0 M Tris, pH 9.5
1 ml 0.5 M MgCl₂
1 ml 1.5 M NaCl
7 ml H₂O

AP-developing solution (for immunodetection on Western Blots)

50.0 µl NBT (75 mg NBT in 700 µl dimethylformamide)
37.5 µl BCIP (50 mg BCIP in 1 ml dimethylformamide)

Bacterial extraction buffer

500 mM NaCl
100 mM Tris pH 7.4
8 mM imidazole

Blocking solution

5.0 % low fat milk powder
0.1 % Tween 20 in TS buffer

Colour development solution

2 ml 3 mg / ml α -chloronaphthol in DMSO
10 ml TS
10 μ l hydrogen peroxide (30 %)

Coomassie blue solution

1 g Coomassie brilliant blue R-250 in 0.5 l methanol and 0.5 l acetic acid

Dialysing buffer

120 mM NaCl
20 mM Tris pH 7.4
1,5 % cholate

Electrophoresis buffer (10 X)

30 g Tris
144 g glycine
10 g SDS make up to 1 l using dH₂O (no need to adjust pH)

Extraction buffer

140 mM KCl
20 mM HEPES-KOH, pH 7.3
2 mM EDTA
1 % Triton X-100

Krebs Ringer buffer

140.0 mM NaCl
5.0 mM NaHCO₃
1.0 mM MgCl₂
1.2 mM Na₂HPO₄
10.0 mM glucose
20.0 mM Hepes, pH 7.4

LB agar37 g in 1 l dH₂O

autoclave

LB medium25 g in 1 l dH₂O

autoclave

Phosphate-buffered saline

140.0 mM NaCl

2.7 mM KCl

10.0 mM Na₂HPO₄1.8 mM KH₂PO₄

pH 7.4

Ponceau solution

0.5 % w/v Ponceau S

3.0 % v/v trichloroacetic acid

Sample buffer (3 X)

12.48 ml Stacking gel buffer (4 X)

1.50 ml 0.1 M EDTA (Na-salt) pH 6.8-7.0

15 g sucrose

5.00 ml 1.5 M DTT make up to 50 ml using dH₂O**Separating gel buffer (4 X)**

181.7 g Tris pH 8.8

4.0 g SDS make up to 1 l using dH₂O, and filter

Sodium buffer

10.0 mM	Glucose
5.0 mM	KCl
140.0 mM	NaCl
5.0 mM	NaHCO ₃
1.0 mM	MgCl ₂
1.2 mM	Na ₂ HPO ₄
20.0 mM	HEPES, pH 7.4

Solution A (for BCA assay)

1.00 %	BCA-disodium	
1.70 %	Na ₂ CO ₃	
0.16 %	Na ₂ -tartrate	
0.40 %	NaOH	
0.95 %	NaHCO ₃	in dH ₂ O, pH 11.25

Solution B (for BCA assay)

4 %	CuSO ₄ .5H ₂ O
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Stacking gel buffer (4 X)

60.5 g	Tris	pH 6.8	
4.0 g	SDS		make up to 1 l using dH ₂ O, and filter

Sucrose solution

0.32 M	sucrose in dH ₂ O
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TB medium ("terrific" medium for bacterial culture)

6 g	bacto tryptone
12 g	bacto yeast extract
2 ml	glycerol
450 ml	H ₂ O

autoclave for 20 minutes and add 100 ml sterile TB-salts

TB salts (salts for "terrific" medium for bacterial culture)

2.4.2 Centrifugal filter units

Centricon-10 (molecular weight cut-off of 10 kDa)

Centricon-3 (molecular weight cut-off of 3 kDa) (Millipore, Bedford, MA, U.S.A.)

2.4.3 Electrophoresis equipment

Trans-Blot[®] SD semi-dry electrophoretic transfer cell

Bio-Rad (Hercules, CA, USA)

Power/Pac 200 power supply

Bio-Rad (Hercules, CA, USA)

Mini-PROTEAN[®] II electrophoresis cell

Bio-Rad (Hercules, CA, USA)

2.4.4 Spectrophotometers

Dynatech MR 5000 Elisa reader

Dynatech (Ashford, United Kingdom)

UV-1202 UV-VIS spectrophotometer

Shimadzu (Kyoto, Japan)