

## **5 Materials**

All standard chemicals for commonly used buffers and solutions were purchased from Sigma-Aldrich, St. Louis, MO, USA, or from Roth, Karlsruhe, Germany. Specific chemicals, solutions, buffers, kits and consumables were purchased from the suppliers listed below.

- Agfa-Gevaert, Mortsel, Belgium
- Ambion, Austin, TX, USA
- Amersham, Buckinghamshire, UK
- ATCC, Manassas, VA, USA
- Bacto Laboratories, Liverpool, NSW, Australia
- BD Biosciences, San Jose, CA, USA
- BD Falcon, Lincoln Park, NJ, USA
- Beckman Coulter Inc., Fullerton, CA, USA
- Biochrom, Berlin, Germany
- BioRad, Munich, Germany
- Biotex, Berlin, Germany
- Biozym GmbH, Hess. Oldendorf, Germany
- BMA, Rockland, ME, USA
- Boehringer, Ingelheim, Germany
- Canteon, Marburg, Germany
- Clontech, Palo Alto, CA, USA
- Dako, Glostrup, Denmark
- Dianova, Hamburg, Germany
- DNA Star Inc., Madison, WI, USA
- DSMZ, Braunschweig, Germany
- Dynal Biotech, Oslo, Norway
- Eurogentec, Seraing, Belgium
- Gibco Invitrogen, Carlsbad, CA, USA
- Heraeus, Hanau, Germany
- Immunotech Coulter Inc., Fullerton, CA, USA
- Immunotools GmbH, Friesoythe, Germany
- Invitex, Berlin, Germany
- Invitrogen, Carlsbad, CA, USA
- Kodak, Rochester, NY, USA
- MBI Fermentas, St. Leon-Rot, Germany
- Micromet AG, Munich, Germany
- Millipore, Bedford, MA, USA
- MJ Research, Waltham, MA, USA
- Molecular Probes, Eugene, OR, USA
- NEN Perkin Elmer, Boston, MA, USA
- Novex, Invitrogen, Carlsbad, CA, USA
- Nunc, Wiesbaden, Germany
- PAA Laboratories, Pasching, Austria
- Packard GMI Inc., Ramsey, MN, USA
- PAN Biotech, Aidenbach, Germany
- Peqlab Biotechnology GmbH, Erlangen, Germany
- Pharmacia Amersham, Buckinghamshire, UK
- Pierce, Rockford, IL, USA
- Promega, Madison, WI, USA
- Qiagen, Hilden, Germany
- Roche, Basel, Switzerland
- Roth, Karlsruhe, Germany
- Santa Cruz Biotechnology, Santa Cruz, CA, USA
- Sartorius, Goettingen, Germany
- Savant GMI Inc., Ramsey, MN, USA
- Sigma-Aldrich, St. Louis, MO, USA
- Stratagene, La Jolla, CA, USA
- TPP, Trasadingen, Switzerland
- TSI, Zeven, Germany
- Vector Laboratories Inc., Burlingame, CA, USA

### **5.1 Kits and consumables**

6cm Ø cell culture dishes

BCA Protein Assay Kit

BD Falcon™ FACS tubes, 5ml

BD Falcon™ 2063 6ml polypropylene tubes

Biotinylated Lectin Kits I-III

No. 93060, TPP

No. 23225, Pierce

No. 352052, BD Falcon

No. 2063, BD Falcon

No. BK-1000; BK-2000 and BK-3000,  
Vector Laboratories

## Materials

---

Bradford Protein Assay	No. 500-0006, BioRad
C10/10 reusable column	No. 19-5001-01, Pharmacia
Calibrite beads	No. 349502, BD
Collection™ Pan Mouse IgG Kit	No. 115.19, Dynal
Disposable mini columns 0.5-2ml	No. 29920, Pierce
Disposable mini columns 1-5ml	No. 29922, Pierce
Dynabeads© CD19 M450	No. 111.04, Dynal
Dynabeads© Pan Mouse IgG	No. 110.23, Dynal
Dynabeads© ProteinA	No. 100.02, Dynal
ECL™ Reagent	No. RPN 2134, Amersham Biosciences
Electroporation cuvettes (0.2cm gap width)	No. P-450-50, Invitrogen
Electroporation cuvettes (0.4cm gap width)	No. P-460-50, Invitrogen
Express [ <sup>35</sup> S] Protein Labelling Mix	No. NEG 072, NEN Perkin Elmer
FACS tubes, 0.6ml	No. 01101, Greiner
HexaLabel Plus DNA Labelling Kit	No. K-0614, MBI Fermentas
Hybond-N membrane	No. RPN 303N, Amersham
IEF Zoom Strip (pH3-10)	No. ZM-0011, Invitrogen
IFN- $\gamma$ -ELISA Set	No. 31333539, Immunotools
Immuno Pure Protein G IgG Plus Orientation Kit	No. 44990, Pierce
Invisorb Spin Plasmid Mini Two Kit	No. 10101403, Invitex
IsoStrip Mouse Monoclonal Antibody Isotyping Kit	No. 1493027, Roche
Maxi Sorb ELISA plates	No. 446469, Nunc
NuPAGE 4-12% Bis-Tris Zoom Gels	No. NP 0330, Invitrogen
pCMV-Script® XR cDNA Library Construction Kit	No. 200465, Stratagene
pGEM® T easy cloning kit	No. A-1360, Promega
PKH 26 Red fluorescent cell linker kit	No. PKH26-GL, Sigma-Aldrich
Plasmid Maxi Kitp	No. 12165, Qiagen
Plasmid Midi Kit	No. 12145, Qiagen
PlusOne™ Silver Staining Kit Protein	No. 17-1150-01 Pharmacia
PolyATract® mRNA Isolation Kit	No. Z-5200, Promega
ProFound™ Label Transfer Sulfo SBED Protein:Protein Interaction Reagent	No. 33033, Pierce
PVDF Immobilon™-P membrane (0.45 $\mu$ m)	No. IPVH-00010, Millipore
QiaEx II Agarose Gel Extraction Kit	No. 20051, Qiagen
Quick spin columns	No. 1273973, Roche
Riboprobe <i>in vitro</i> Transcription Systems	No. P-1450, Promega
RNeasy Mini Kit	No. 74106, Qiagen
Slide-A-Lyzer Dialysis Cassettes MW10.000,	No. 66425, Pierce
Thermo Fast 24-well PCR plates	No. 82-0624, Peqlab Biotechnology
Ultracentrifuge tubes, 6.5ml	No. 355645, Beckman
X-omat LS films, 13x18cm	No. 161 1342, Kodak
X-omat LS films, 18x24cm	No. 868 8681, Kodak
Zenon™ Alexa Fluor® 488 Mouse IgG <sub>1</sub> Labelling Kit	No. Z-25002, Molecular Probes
Zenon™ R-Phycoerythrin Mouse IgG <sub>1</sub> Labelling Kit	No. Z-25055, Molecular Probes
Zoom® IPG Runner™ System	No. ZM-0002, Invitrogen
$\lambda$ -ZAP®-CMV XR Library Construction Kit	No. 200448, Stratagene

## 5.2 Chemicals

### 5.2.1 Protein applications

APS (ammonium peroxodisulfat)	No. 9592.2, Roth
Brilliant Blue G, colloidal concentrate	No. B-2025, Sigma-Aldrich
BSA (bovine serum albumin) fraction V	No. 8076.3, Roth
CHAPS (cholamidopropyl-dimethylammonio propane sulphonate)	No. 1479.2, Roth
DTSSP (dithiobis-sulfosuccinimidylpropionate)	No. 21578, Pierce
DTT (dithiotreitol)	No. 6908.2, Roth
EZ link Sulfo-NHS (N-hydroxysuccinimid) biotin	No. 21217, Pierce
H <sub>2</sub> O <sub>2</sub> 30%	No. 8070.2, Roth
HEPES (hydroxyethyl-piperazine-ethanesulfonic-acid)	No. 9105.3, Roth
HMW (high molecular weight) protein marker	No. M-3788, Sigma-Aldrich
Immobilized NeutrAvidin™	No. 29200, Pierce
LMW (low molecular weight) protein marker	No. M-3913, Sigma-Aldrich
NeutrAvidin™ HRP (horseradish peroxidase) conjugated	No. 31001, Pierce
Non-fat dry milk powder (“Sucofin”)	No. /, TSI
OPD ( <i>o</i> -phenylenediamine) tablets	No. 34006, Pierce
PBS (phosphate buffered saline)	No. L-182-50, Biochrom
PEG (polyethyleneglycol) 1450	No. 25322-68-3, ATCC
PMSF (phenyl-methyl-sulfonyl fluoride)	No. 6367.2, Roth
Poly-L-lysine	No. P-4707, Sigma-Aldrich
Precision Protein Marker, prestained, broad range	No. 1610372, BioRad
Protein A/G-PLUS agarose	No. sc-2003, Santa Cruz Biotechnology
Protein G Plus agarose	No. sc-2002, Santa Cruz Biotechnology
ProteinA Sepharose CL-4B	No. 17-0780-01, Pharmacia Biotech
Rotiphorse™ 40, acrylamide solution,	No. A515.1, Roth
Sigma-Cote® (silane)	No. SL-2, Sigma-Aldrich
Sigma-Fast® ( <i>o</i> -phenylenediamine-dihydrochloride)	No. P-9187, Sigma-Aldrich
Sodium borohydride	No. S-9125, Sigma-Aldrich
Sodium-m-periodate	No. S-1147, Sigma-Aldrich
Streptavidin, FITC (fluorescein isothiocyanate) conjugated	No. 115-116-146, Dianova
Streptavidin, HRP (horseradish peroxidase) conjugated	No. RPM 1231, Amersham
Streptavidin, PE (phycoerythrin) conjugated	No. 016-100-084, Dianova
TEMED (tetramethylethylenedimine)	No. 2367.1, Roth
Triton X-100	No. 14897820, Boehringer
Tween® 20 (polyethyleneglycol sorbitan monolaureate)	No. P-9416, Sigma-Aldrich
Venimmun®N	No. 088/1c, Centeon

### 5.2.2 Nucleic acid applications

Agarose	No. 840004, Biozym
dATP (deoxyadenosine triphosphate)	No. R-0141, MBI Fermentas
dCTP (desoxycytidine triphosphate)	No. R-0151, MBI Fermentas
DEPC (diethylpyrocarbonate)	No. D-5758, Sigma

## Materials

---

dGTP (desoxyguanosine triphosphate)	No. R-0161, MBI Fermentas
dTTP (desoxythymidine triphosphate)	No. R-0171, MBI Fermentas
Ethidium bromide	No. 7870, Roth
Formaldehyde 37%, Rotipuran	No. 979.1, Roth
Formamide	No. 6749.1, Roth
GeneRuler 100bp Plus DNA marker	No. SM-0322, MBI Fermentas
Glycoblue co-precipitant	No. 9515, Ambion
Mixed bed ionexchange resin	No. AG-501-X8, BioRad
Smartladder DNA marker	No. MW-1700-04, Eurogentec

### 5.3 Antibodies

Anti-human $\lambda$ Pure	No.345130, BD
Anti-human B cell CD19	No. M-0740, Dako
Anti-human bssc 17.1AxCD3 (bsscEpCAMxCD3)	non-commercial, Micromet AG
Anti-human bssc CD19xCD3 (MT103)	non-commercial, Micromet AG
Anti-human bssc Wue-1xCD3 (MT105)	non-commercial, Micromet AG
Anti-human CD29	No. 0790, Immunotech
Anti-human CD48	No. 0847, Immunotech
Anti-human CD54	No. 7970, BD
Anti-human HC-10	Gift from Dr. A. Rehm, MDC Berlin, Germany
Anti-human IgG Fc specific	No. 109-006-098, Dianova
Anti-human Mue-1 hybridoma supernatants (IgM)	Gift from Dr. A. Greiner, University of Würzburg, Germany
Anti-human Wue-1 ascites (IgG <sub>1</sub> )	custom-made, Eurogentec
Anti-mouse IgG	No. M-1397, Sigma-Aldrich
Anti-mouse IgG (Fab') <sub>2</sub> -Biotin,	No. E-0413, Dako
Anti-mouse IgG HRP conjugate	No. W-402B, Promega
Donkey anti-goat IgG HRP conjugate,	No. sc-2020, Santa Cruz Biotechnology
Goat anti-mouse IgG F(ab') <sub>2</sub> -fragment PE conjugated	No. 115-116-146, Dianova
Goat anti-mouse IgM F(ab') <sub>2</sub> -fragment PE conjugated	No. 115-116-075, Dianova
Goat anti-mouse IgM HRP conjugated	No. 115-036-075, Dianova
IgG <sub>1</sub> isotype control	No. X-0931, Dako
IgM isotype control	No. X-094201, Dako

### 5.4 Enzymes

DNase I, RNase-free	No. 79254, Qiagen
Klenow fragment	No. 27-9250D, Amersham
Lysozyme	No. L-7651, Sigma-Aldrich
<i>Pfu</i> Polymerase	No. M-774A, Promega
Phospholipase C, phosphatidylinositol specific	No. P-8804, Sigma-Aldrich
Pronase	No. 165921, Roche
Restriction enzymes	various, MBI Fermentas

Shrimp alkaline phosphatase (SAP)	No. M-820A, Promega
T4 DNA Ligase	No. EL 0014, MBI Fermentas
<i>Taq</i> Polymerase	No. 30203022, Invitex

## 5.5 Plasmids

pBS-SK-FSS-LSP-scWue-1	AG Bommert, MDC Berlin, Germany
pBullet-607	Gift from Prof. H. Abken, University of Cologne, Germany
pCDNA3.1	Invitrogen
pCDNA6/V5-HisA	Invitrogen
pEGFP-N3	Clontech
pGEM®T Easy	Promega

## 5.6 Oligonucleotides and radionucleotides

Custom made, gene specific oligonucleotides were ordered from Biotex, Berlin, Germany.

### 5.6.1 General Oligonucleotides

SP6	5' -ATT TAG GTG ACA CTA TAG AA-3'
T3	5' -AAT TAA CCC TCA CTA AAG GG-3'
T7	5' -GTA ATA CGA CTC ACT ATA GGG C-3'

### 5.6.2 Cloning Oligonucleotides

<i>Bam</i> HI-wue <sub>rev</sub>	5' -GCG GAT CCG CGG AGA CGG TGA CCG TGG-3'
<i>Xba</i> I-HA-wue <sub>fw</sub>	5' -GCT CTA GAT ATC CAT ATG ATG TTC CAG ATT ATG CTC AGC TGA CCC AGT CTC C-3'
<i>Xba</i> I-wue <sub>fw</sub>	5' -GCT CTA GAC AGC TGA CCC AGT CTC C-3'

### 5.6.3 Sequencing Oligonucleotides

pBullet-kappa <sub>fw</sub>	5' -GCT CTA GAC TGC CAT GGA TTT TCA GGT GC-3'
pBullet-wue seq F1	5' -CCA AGC TCG AGA TCA AAG GTG-3'
pBullet-wue seq F2	5' -ACC GTC AGT CTT CTT CTT CC-3'
pBullet-wue seq F3	5' -GCA CAA CCA CTA CAC GCA GAA-3'
pBullet-wue seq R1	5' -GAA TGA GTA ACC AGA ACC CTT-3'

## 5.6.4 Radionucleotides and radiochemicals

$[\alpha\text{-}^{32}\text{P}]$ dATP (3000 Ci/mmol, 10mCi/ml) Easytides	No. BLU/NEG-512H, NEN Perkin Elmer
$[\alpha\text{-}^{32}\text{P}]$ dCTP (3000 Ci/mmol, 10mCi/ml) Easytides	No. BLU/NEG-513H, NEN Perkin Elmer
$[\alpha\text{-}^{32}\text{P}]$ rUTP (800 Ci/mmol, 10mCi/ml) Easytides	No. 007 X 03203, NEN Perkin Elmer
L- $[\text{}^{32}\text{S}]$ -methionine (1175Ci/mmol, 7.9mCi/ml)	No. NEG-072, NEN Perkin Elmer

## 5.7 Buffers and solutions

### 5.7.1 Western blotting and immunoprecipitation

Buffer	Composition
0.1M sodium phosphate buffer (pH8)	93.2ml of 1M $\text{Na}_2\text{HPO}_4$ , 6.8ml of 1M $\text{NaH}_2\text{PO}_4$
10x PBS	8mM $\text{Na}_2\text{HPO}_4$ , 2mM $\text{NaH}_2\text{PO}_4$ , 140mM NaCl
4x Laemmli buffer (pH6.8)	200mM Tris-base, 40% glycerol, 16% SDS, 20% $\beta$ -mercaptoethanol, 0.01% bromphenolblue
Colloidal Coomassie destaining buffer I	10% glacial acetic acid, 25% MeOH, in $\text{H}_2\text{O}$
Colloidal Coomassie destaining buffer II	25% MeOH in $\text{H}_2\text{O}$
Colloidal Coomassie fixing buffer	7% glacial acetic acid, 40% MeOH in $\text{H}_2\text{O}$
Coomassie destaining buffer	7.5% glacial acetic acid, 25% ethanol
Coomassie staining solution	0.25% coomassie brilliant blue R-250, 10% glacial acetic acid, 45% ethanol
Glycine buffer (pH2)	0.5M glycine, 0.15M NaCl, 0.02% sodium azide
High salt buffer	0.5M NaCl, 1% NP40, 50mM Tris-HCl (pH8)
PBS-T	1xPBS, 0.05% Tween® 20
PVDF transfer buffer (pH8.3)	25mM Tris-base, 192mM glycine, 20% MeOH,
Storing buffer (sepharose columns)	1x PBS, 0.02% sodium azide
TBS-T	0.5M NaCl, 20mM Tris-HCl (pH7.5), 0.1% Tween-20

### 5.7.2 Lysis buffers

Protease inhibitors were added to the buffers as required just prior to use.

Buffer	Contents
1x NP40 lysis buffer	500 $\mu$ l 2x NP40 basis buffer, 1% NP40, 374 $\mu$ l $\text{H}_2\text{O}$
2x NP40 basis buffer	40mM HEPES (pH7.9), 700mM NaCl, 2mM $\text{MgCl}_2$ , 1mM EDTA (pH8), 0.2mM EGTA in $\text{H}_2\text{O}$
2x SDS lysis buffer	2% SDS, 20mM Tris-HCl (pH7.5), 4mM EDTA (pH8) in $\text{H}_2\text{O}$
2x Triton X-100 lysis buffer	150mM NaCl, 1% Triton X-100, 10% glycerine, 30mM Tris-HCl (pH7.5)
Buffer H	250mM sucrose, 10mM HEPES (pH7.2), 2mM $\text{MgCl}_2$ , 10mM NaF, 1mM sodium orthovanadate in $\text{H}_2\text{O}$

Hypotonic lysis buffer	10mM Tris-HCl (pH8), 0.1mM DTT in H <sub>2</sub> O
Urea/CHAPS buffer	8M Urea, 2% CHAPS, in H <sub>2</sub> O

### 5.7.3 Protease inhibitors

Stock solution	Final concentration	Supplier
100μM Aprotinin	100nM	No.236624, Roche
100μM Pepstatin A	1μM	No. P-5318, Roth
100mM DTT	0.5mM	No. 6908.2, Roth
100mM EDTA-Na <sub>2</sub>	1mM	No. ED-2SS, Sigma-Aldrich
100mM Na-orthovanadate	1mM	No. S-6508, Sigma-Aldrich
100mM Pefabloc®	1mM	No. A-154.2, Roth
100mM PMSF	0.1mM	No. P-7626, Sigma-Aldrich
10x Complete Mini	1x	No. 1836170, Roche

### 5.7.4 RNA, DNA and Northern blotting

Buffer	Contents
10x MOPS (pH7)	200mM MOPS, 50mM sodium acetate, 10mM DEPC-EDTA (pH8) NOTE: Do not autoclave. Adjust with 10M NaOH. Storage at 4°C in the dark
20 x SSC (pH7)	3M NaCl, 0.3M tri-sodium citrate, adjust pH with HCl
4M guanidinium solution	4M guanidinium isothiocyanate, 20mM sodium acetate (pH5.2), 0.1mM DTT, 0.5% N-lauryl-sacrosine (sacrosyl) in DEPC-H <sub>2</sub> O
Deionized formamide	5g mixed bed ionexchange resin in 100ml formamide
Denaturing RNA-gel	2.34g agarose in 169.5g DEPC-H <sub>2</sub> O, 19.5ml 10x MOPS, 5.85ml formaldehyde
RNA loading buffer	20μl 0.5M DEPC-EDTA, 25μl 0.05% bromphenol blue, 25μl xylene cyanol, 6.25ml 80% glycerol, 3.68ml DEPC-H <sub>2</sub> O
RNA premix	2.5ml 10x MOPS, 4.4ml 37% formaldehyde, 12.5ml deionized formamide
RNA running buffer	1x MOPS
TBE	0.1M Tris-base, 0.1M boric acid, 2.5mM EDTA
TE buffer	10mM Tris-HCl (pH7.5), 1mM EDTA
Wash buffer I	2x SSC, 0.05% SDS
Wash buffer II	0.1x SSC, 0.1% SDS
0.1M DEPC-EDTA (pH8)	0.1M Na <sub>2</sub> -EDTA, 0.1% DEPC, adjust to pH8, autoclave twice
DEPC-H <sub>2</sub> O (0.1%)	1ml DEPC ad 1000ml H <sub>2</sub> O bi-dest, stir 30min. at RT; autoclave twice
3M sodium acetate (pH5.2)	3M sodium acetate, 0.1% DEPC, adjust to pH5.2 with 96% acetic acid, autoclave twice
10mM TE <sub>10/5</sub> (pH7.4)	0.5ml sterile 1M DEPC-Tris-HCl (pH7.5), 2.5ml sterile 0.1M DEPC-EDTA (pH8), ad 50ml sterile DEPC-H <sub>2</sub> O

## Materials

---

5.7M Caesiumchloride solution (pH5.5;  $\rho=1.693\text{g/ml}$ )      5.7M CsCl<sub>2</sub>, 100mM EDTA (pH8), 0.1% DEPC, adjust to pH5.5 with HCl), autoclave twice

### 5.7.5 FACS

Buffer	Contents
AKV buffer	1x PBS, 1% Venimmun, 0.1% sodium azide
1x PI (propidium iodide) working solution	30 $\mu\text{g/ml}$ in 1xPBS

### 5.7.6 ELISA

Buffer	Contents
10% Neutral buffered formalin (pH7)	10ml Formalin (37-40% Formaldehyde solution), 0.65g Na <sub>2</sub> HPO <sub>4</sub> , 0.4g NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O, H <sub>2</sub> O ad 100ml
Blocking buffer	3-5% BSA in PBS
Carbonate Buffer (pH9.6)	34mM Na <sub>2</sub> CO <sub>3</sub> , 100mM NaHCO <sub>3</sub> , adjust to pH9.6
IFN- $\gamma$ ELISA blocking buffer	1x PBS, 5% BSA
IFN- $\gamma$ ELISA dilution buffer	1xPBS, 0.05% Tween® 20, 1% BSA
IFN- $\gamma$ ELISA wash buffer	1x PBS, 0.01% Tween® 20
OPD substrate buffer (pH5)	0.05M citric acid, 0.05M sodium phosphate; prior to use add 1 $\mu\text{l}$ 30% H <sub>2</sub> O <sub>2</sub> per ml substrate buffer
PBS-B	1x PBS, 1% BSA
PBS-B+	1x PBS, 1% BSA, 0.1% sodium azide
PBS-T	1x PBS, 0,05% Tween® 20
Sodium acetate buffer (pH4.5)	50mM sodium acetate in H <sub>2</sub> O
Sodium borohydride solution	50 mM Na-borohydride in PBS
Sodium-m-periodate solution	20mM Na-periodate in 50mM acetate buffer (pH4.5)
Stop solution (2.25M H <sub>2</sub> SO <sub>4</sub> )	12.5 ml concentrated sulfuric acid ad 100ml H <sub>2</sub> O
Washing buffer	1xPBS, 0.01% Tween-20

### 5.7.7 Immunoselection

10x Trypsin/EDTA (0.5%/0.25% (w/v))	No. L-2153, Biochrom
Chloroquine	No. C-6628, Sigma-Aldrich
DEAE dextran	No. D-9885, Sigma-Aldrich
DMSO	No. A-994.1, Roth
Ficoll solution (specific density 1.077g/ml)	No. L6115, Biochrom
PEG (polyethyleneglycol) 1450	No. 25322-68-3, ATCC
PI (propidium iodide) stock solution (10mg/ml)	No.P-4170, Roth



<b>Buffer</b>	<b>Contents</b>
50% PEG 1450 solution	50%(w/w) PEG 1450 in DMEM w/o serum, pH7 adjusted with 7.5% Na <sub>2</sub> CO <sub>3</sub>
Blocking buffer	1x PBS, 1mg/ml BSA
Coating buffer	50mM Tris-HCl (pH9.6)
EA	0.5mM EDTA, 0.02% sodium azide
EAF	0.5mM EDTA, 0.02% sodium azide, 2% Ficoll
EAV	0.5mM EDTA, 0.02% sodium azide, 0.1% Venimmun
Hirt lysis buffer	0.6% SDS, 10mM EDTA
SM buffer	5.8g NaCl, 2g MgSO <sub>4</sub> ·7H <sub>2</sub> O, 50ml 1M Tris-HCl (pH7.5), 5ml of 2% gelatine, ad 1l H <sub>2</sub> O
Sucrose-MgCl <sub>2</sub> buffer	10%(w/v) sucrose, 10mM MgCl <sub>2</sub> in DMEM
Sucrose-Tris-HCl buffer	20% (w/v) sucrose, 50mM Tris-HCl (pH8)
Transfection mix	10mg/ml DEAE dextran, 2.5mM chloroquine in PBS + DNA
Wash buffer	0.15M NaCl

### 5.7.8 Ready made solutions and buffers

8M guanidine-HCl solution (pH1.5)	No. 24115, Pierce
ExpressHyb™ solution	No. 636831, Clontech
ImmnoPure (A) IgG1 Binding buffer (pH8)	No. 21001, Pierce
ImmnoPure Mouse IgG Elution buffer (pH2.8)	No. 21004, Pierce
ImmnoPure Mouse IgG <sub>1</sub> Mild Elution buffer (pH6)	No. 21034, Pierce
LDS 4x sample buffer	No.NP 0007, Invitrogen
Lithium Chloride Precipitation Solution (7.5M LiCl <sub>2</sub> ),	No. 9480, Ambion
MES running buffer	No. NP-0002, Invitrogen
Poly-L Lysine solution	No. P-8920, Sigma-Aldrich

## 5.8 Cells

### 5.8.1 Mammalian cells

<b>name</b>	<b>origin</b>	<b>supplier</b>
ARH-77	human plasma cell leukaemia	No. ACC 512, DSMZ
BL60-2	Burkitt lymphoma	obtained from Dr. B. Schories, MDC, Berlin
Buffy coat	Leucocytes and thrombocytes	obtained from the University Clinic, Berlin Steglitz
COS-7	African green monkey kidney	No. ACC 60, DSMZ
DOHH-2	human B-cell lymphoma	No. ACC 47, DSMZ
Ina-6	human multiple myeloma	obtained from Dr. M. Chatterjee, MDC, Berlin
Jurkat	human T cell leukaemia	No. ACC 282, DSMZ
L-363	human plasma cell leukaemia	No. ACC 49, DSMZ

## Materials

---

MM.1S	human multiple myeloma	obtained from Dr. M. Chatterjee, MDC, Berlin
Mue-1 hybridoma	RPMI/NSO-D mouse hybridoma	obtained from Dr. A. Greiner, University of Würzburg
Nalm-6	human B-cell precursor leukaemia	No. ACC 128, DSMZ
NCI-H929	human multiple myeloma	No. ACC 163, DSMZ
OPM-2	human multiple myeloma	No. ACC 50, DSMZ
RPMI-8226	human multiple myeloma	No. ACC 402, DSMZ
U-266	human multiple myeloma	No. ACC 9, DSMZ
Wue-1 hybridoma	Balb-c/NSO-D mouse hybridoma	obtained from Dr. A. Greiner, University of Würzburg

### 5.8.2 Bacteria

name	selection	medium	supplier
<i>E.coli</i> DH5- $\alpha$	/	LB	No. 53868, ATCC
<i>E.coli</i> VCS257	/	LB, 0.2% (w/v) maltose, 10mM MgSO <sub>4</sub>	Stratagene
<i>E.coli</i> XL1-Blue MRF'	Tet <sup>R</sup>	LB, 0.2% (w/v) maltose, 10mM MgSO <sub>4</sub>	Stratagene
<i>E.coli</i> XL0LR	Tet <sup>R</sup>	NZY w/o supplements	Stratagene

### 5.9 Media

$\beta$ -Mercaptoethanol	No. 4227.1, Roth
$\alpha$ -thioglycerol	No. M-6145, Sigma
1% EDTA	No. L-2113, Biochrom
100mM Sodium Pyruvat	No. L-0473, Biochrom
10x Trypsin/EDTA, 0.5%/0.25% (w/v)	No. L-2153, Biochrom
200mM Glutamax (L-glutamine)	No. 35050-038, Gibco
Bacto Agar	No. 214010, Bacto Laboratories
Bacto Tryptone	No. 211705, Bacto Laboratories
Bacto Yeast extract	No. 288620, Bacto Laboratories
BCS (bathocuproine disulfonic acid disodium salt)	No. B-1125, Sigma
Dimethylsulfoxid (DMSO)	No. A-992.1, Roth
DMEM	No. FG-0415, Biochrom
DMEM High Glucose	No. E-15009, PAA Laboratories
Fetal Calf Serum (FCS)	No. S-0115, Biochrom
NZ amine (casein hydrolysate)	No. N-4517, Roth
RPMI	No. E-15-039, PAA Laboratories
RPMI 1640 Special	No. T-15-055, PAA Laboratories

### 5.9.1 Basic cell culture medium

Medium	Contents
DMEM complete	
DMEM High Glucose complete	10% FCS, 2mM L-glutamine, 1mM sodium pyruvate 25µg/ml gentamycin (or 100U penicillin/0.1mg/ml streptomycin)
RPMI complete	

### 5.9.2 Special cell culture medium

Medium	Contents
BL-60 Medium	RPMI complete, 10mM HEPES, 0.5ml BCS/α-thioglycerol
Freezing Medium	70% DMEM or RPMI complete, 20% FCS, 10% DMSO
Labelling Medium	90% RPMI 1640 Special (w/o methionine), 10% FCS
NCI-H929 Medium	RPMI complete, 2µM β-mercaptoethanol
RPMI 1640 Special (methionine-free)	10% FCS, 2mM L-glutamine, 25µg/ml gentamycin, 1mM sodium pyruvate, 2.5mM CaCl <sub>2</sub>

### 5.9.3 Bacterial medium

Medium	Contents
LB	1% bacto-tryptone, 0.5% yeast extract, 0.5%g NaCl, (1.5% agar agar)
NZY	0.5% NaCl, 8mM MgSO <sub>4</sub> , 0.5% yeast extract, 1% NZ amine
SOC	2% tryptone, 0.5% yeast extract, 10mM NaCl, 2.5mM KCl, 10mM MgCl <sub>2</sub> , 10mM MgSO <sub>4</sub> , 20mM glucose

### 5.9.4 Antibiotics

Stock solution	Final concentration	Supplier
Ampicillin (100mg/ml)	100µg/ml	No. K-029.2, Roth
Gentamycin (50mg/ml)	25µg/ml	No. A-271-26, Biochrom
Kanamycin (50mg/ml)	50µg/ml	No. K-1377, Sigma-Aldrich
Penicillin (10.000units/ml)	100U/ml	No. P-0608100P, PAN Biotech
Spectinomycin (100mg/ml)	0.1mg/ml	No. S-2647, Sigma-Aldrich
Streptomycin (100mg/ml)	1mg/ml	No. P-0611100P, PAN Biotech
Tetracyclin (12.5mg/ml)	12.5µg/ml	No. T-7660, Sigma-Aldrich

### **5.10 Appliances**

Agfa Curix 60 tabletop image processor	Agfa
Benchmark Plate Reader	Biorad
Electroporator Gene Pulser X Cell	BioRad
FACS-Calibur flow cytometer	BD Biosciences
Floor Centrifuge Varifuge 3.0R	Heraeus
Glass homogenizer 5ml	Sartorius
Liquid Scintillation Analyzer TriCarb 1900 TR	Packard
NuPAGE "X-Cell II Blot Module"	Invitrogen
NuPAGE "X-Cell Sure Lock Mini Cell"	Invitrogen
Peltier Thermal Cycler PTC 200	MJ Research
Semidry Blotter Trans Blot SD	Biorad
Spectrophotometer DU640	Beckman
Speed Vac SC 110A	Savant
Tabletop Centrifuge Biofuge 15	Heraeus
Tank Blot Trans Blot Cell 5365/1488	BioRad
Ultracentrifuge Optima L60, rotor Ti-70.1	Beckman
UV Stratalinker 2400	Stratagene

### **5.11 Software**

4Peaks	<a href="http://www.mekentosj.com">www.mekentosj.com</a> (non-commercial)
CellQuest Pro	BD Biosciences
Lasergene,	DNA Star Inc.