

## 2. Materials

The materials were purchased from the following companies:

Amersham	Uppsala, Sweden
Amicon/Millipore Corporation	Bedford, MA, USA
BION ENTERPRISES	Des Plaines, IL, USA
BIO-RAD Laboratories	Hercules, CA, USA
Biosource International	Camarillo, CA, USA
BMA	Rockland, ME, USA
Boehringer	Ingelheim, Germany
Calbiochem	San Diego, CA, USA
Caltech Laboratories	Burlingame, CA, USA
Clontech	Palo Alto, CA, USA
Costar, Corning	Acton, MA, USA
Elkin-Sinn	Cherry Hill, NJ, USA
Eppendorf	Westbury, NY, USA
Fisher Chemicals	Pittsburgh, PA, USA
Fitzgerald	Concord, MA, USA
Immunotech	Marseille, France
Integrated DNA Technologies (IDT)	Coralville, IA, USA
Jackson ImmunoResearch	West Grove, PA, USA
Life Technologies (GibcoBRL)	Rockville, MD, USA
Miltenyi Biotec/MACS	Auburn, CA, USA
Molecular Devices Corporation	Sunnyvale, CA, USA
Molecular Probes	Eugene, OR, USA
New Brunswick Scientific	Edison, NJ, USA
New England Biolabs	Beverly, MA, USA
Operon Technologies	Alameda, CA, USA
Oxoid	Ontario, Canada

Perkin Elmer Life Sciences	Boston, MA, USA
Perkin Elmer Applied Biosystems	Foster City, CA, USA
Pharmacia	Peapack, NJ, USA
Pharmingen/BD Bioscience/Becton Dickinson	San Diego, CA, USA
Pierce PERBIO	Rockford, IL, USA
Promega	Madison, WI, USA
Quiagen	Valencia, CA, USA
Roche Molecular Biochemicals	Indianapolis, IN, USA
Serotec	Raleigh, NC, USA
SIGMA	St. Louis, MO, USA
Southern Biotechnology Ass.	Birmingham, AL, USA
Stratagene	La Jolla, CA, USA
The Jackson Laboratory	Bar Harbor, ME, USA
US BIO logical	Swampscott, MA, USA

## 2.1. Antibodies

### FACS analysis:

The antibodies listed are monoclonal mouse anti-human antibodies coupled with the specified fluorochromes: fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC) and biotin.

Anti-B220	BD Pharmingen
Anti-CD21	BD Pharmingen
Anti-CD23	BD Pharmingen
Anti-CD43	BD Pharmingen
Anti-human CD19	BD Pharmingen
Anti-human IgM	BD Pharmingen

Anti-human VpreB (PE)	Kind gift of Dr. C. Schiff, Centre d'Immunologie de Mar-seille- Luminy, 13288 Marseille Cedex 09, France
Anti-human $\kappa$	BD Pharmingen
Anti-human $\lambda$	BD Pharmingen

ELISA secondary antibodies:

Anti-human IgG, Fc $\gamma$ (HRP), whole antibody	Jackson ImmunoReseach
Polyclonal goat F (ab') <sub>2</sub> anti-human IgG (HRP)	Biosource International
Polyclonal goat F (ab') <sub>2</sub> anti-human IgM (HRP)	Biosource International
Polyclonal goat F (ab') <sub>2</sub> anti-human $\kappa$ (HRP)	Biosource International
Polyclonal goat F (ab') <sub>2</sub> anti-human $\lambda$ (HRP)	Biosource International

ELISA coating:

Anti-human IgG Fc $\gamma$ , goat	Jackson ImmunoReseach
Chimeric human IgG2 $\lambda$ , anti NP	Serotec
Human IgG1 $\kappa$	Sigma
Human IgG1 $\lambda$	Sigma
Human IgM	Sigma

Standard antibodies:

Human IgG1 $\kappa$	Sigma
M55, human polyreactive antibody	Kind gift of Dr. P. Casali, Weill Cornell Medical School, 10021 New York, USA

MACS microbeads:

Anti-CD19	Miltenyi Biotech
Anti-PE	Miltenyi Biotech

## 2.2. Enzymes and proteins

Bovine Serum Albumin (BSA)	New England Biolabs
Protein G Sepharose	Amersham
T4-DNA Ligase	New England Biolabs

### Restriction Enzymes:

AgeI Restriction Enzyme	New England Biolabs
BsiWI Restriction Enzyme	New England Biolabs
DdeI Restriction Enzyme	New England Biolabs
PstI Restriction Enzyme	New England Biolabs
SalI Restriction Enzyme	New England Biolabs
SacI Restriction Enzyme	New England Biolabs

### Polyreactivity assay:

Chitin	Sigma
Citrullinated Peptides	Sigma
Collagen Type II, from bovine tracheal cartilage	Sigma
Glucose-6-phosphate isomerase (GPI) Type XI, rabbit muscle	Sigma
Histone from calf thymus	Roche
IL-1 $\beta$ recombinant protein	Biosource International
Insulin, human recombinant	Fitzgerald
Lipopolysaccharid (LPS), E. coli 0111:B4	Sigma
Myoglobin, human	Sigma
Phosphorholine-hapten +BSA	Biosearch Technologie
Tetanus toxoid	Calbiochem
Thyroglobulin, human	US BIO logical

### PCR and single cell amplification:

HotStarTaq DNA Polymerase	Qiagen
Prime RNase Inhibitor	Promega

Proteinase K	Roche Molecular Biochemicals
RNAsin (40U/ $\mu$ l)	Promega
Superscript II reverse transcriptase	GibcoBRL
Taq DNA Polymerase	Roche Molecular Biochemicals

### 2.3. Nucleotides and nucleic acids

1 Kb DNA marker (ladder)	Promega
1 Kb Plus DNA ladder	Promega
Deoxynucleoside triphosphates (dNTPs)	Roche Molecular Biochemicals
Deoxyribonucleic acid	SIGMA
Oligonucleotides (The sequences of the oligonucleotides used in this work are listed in Table I)	Integrated DNA Technologies, GibcoBRL, Operon Technologies
Random hexamer for rtPCR reaction	GibcoBRL

### 2.4. Buffers, chemicals, and solutions

Blocking buffer for ELISA	1 mM EDTA 0.05 % Tween 20 (BIO-RAD Laboratories)
Cresol Red 5x Loading buffer	1x PBS 1 mM cresol red (Sigma) 60 % sucrose
Ethyl Alcohol – 200 PROOF	Aaper Alcohol and Chemical Co.
Glycine buffer 0.1M, pH3	
Isopropanol	Fisher Chemicals
RNase free PBS	Gibco
RNase free water	QIAGEN
Running buffer (SDS gel), pH 8.3	15 g/l Tris base 72 g/l Glycine

SDS reducing buffer 4x	5 g/l SDS to 600 ml with H <sub>2</sub> O 4 ml distilled water 1 ml 0.5 Tris-HCl, pH 6.8 0.8 ml Glycerol 1.6 ml 10 % (w/v) SDS 0.4 ml 2-β-Mercaptoethanol 0.05 % 9W/v) bromophenol blue
TE pH 8.0	10 mM Tris-Cl (pH 8.0) 1 mM EDTA (pH 8.0)
Wash buffer (SDS gel)	800 ml H <sub>2</sub> O 100 ml 100 % Ethanol 100 ml Acetic acid
<u>Transfection of A293 cells:</u>	
2,5 M CaCl <sub>2</sub>	
HBS buffer, pH 7.05	50 mM HEPES 10 mM KCl 12 mM Dextrose 280 mM NaCl 1.5 mM Na <sub>2</sub> HPO <sub>4</sub> -7H <sub>2</sub> O

## 2.5. Commercial kits and special chemicals

10x phosphate buffered saline (PBS)	GibcoBRL
Anti-nuclear antibody screen, ELISA	Sigma
Anti-Nuclear Test System, slides	BION Enterprises
Buffers for restriction enzyme digests	New England Biolabs
Centriplus, centrifugal filter, different size	Amicon/Millipore
CNBR-activated Sepharose <sup>TM</sup> 4 Fast Flow beads	Pharmacia

Cytofix/Cytoperm TM kit	Becton Dickinson
Ethidium bromide	SIGMA
Horseradish Peroxidase Substrate Kit	BIO-RAD Laboratories
QIAprep Spin Maxiprep Kit	Qiagen
QIAprep Spin Miniprep Kit	Qiagen
QIAquick Gel Extraction Kit	Qiagen
QIAquick PCR Purification Kit	Qiagen
SDS gel 4-20 %	BMA
TRIZOL LS Reagent	GibcoBRL
Tween 20	SIGMA

Reverse transcriptase reaction of single cells:

0.1M DTT	GibcoBRL
5x First Strand Buffer for rtPCR	GibcoBRL
NP-40	Sigma

**2.6. Culture media and additives**

Ampicillin	SIGMA
Dulbecco's Modified Eagle Medium (DMEM)	GibcoBRL
HEPES Buffer Solution (1M)	GibcoBRL
L-Glutamine (200mM, 100x)	GibcoBRL
Low IgG Fetal Calf Serum (FCS), heat inactivated (for one hour at 56°C)	GibcoBRL
Luria Agar	Oxoid
Luria Broth Media (LB)	Oxoid
MEM Sodium Pyruvate 100mM	GibcoBRL
MEM Non-essential amino acids solution 10 mM (100x)	GibcoBRL
Nutridoma	Roche
Penicillin/Streptomycin	GibcoBRL

Sodium Pyruvate (100mM)	SIGMA
Trypsin	GibcoBRL

## 2.7. Bacteria and vectors

DH5 $\alpha$ (for competent cells protocol)	CLONTECH
Original vector (used for heavy-, $\kappa$ - and $\lambda$ -chain)	Kind gift of Dr. J. Ravetch Rockefeller University, 1230 York Avenue, 10021 New York, USA
TA Cloning kit	CLONTECH

## 2.8. Cell lines

A 293 cells	Kind gift of D. Hawiger Rockefeller University, 1230 York Avenue, 10021 New York, USA
-------------	---

## 2.9. Special instruments and software

Autoclave	Amsco
Centrifuges:	
-GS6 KR	Beckmann
-6M	Beckmann
-GPR	Beckmann
-Biofuge pico	Heraeus
Easywash polystyrene plates for ELISA	Costar, Corning
Freezer	Revco
Flow Cytometers and Cell Sorters:	BD Bioscience
- FACS Calibur	
- LSR	



- FACS Vantage SE	
Heater	VWR Scientific
Incubator	VWR Scientific
Incubator shaker:	New Brunswick Scientific
- innova™ 4000	
- innova™ 4300	
Microscope	Nikon
Mikrowave	Panasonic
PH-meter	Corning
Photometer:	
- BioPhotometers	Eppendorf
Reader for ELISA assays:	
- Versa-max tunable microplate reader	Molecular Devices
PCR Machines	Perkin Elmer Applied Biosystems
Scale	Mettler
Steril Guard Hood	The Baker Company
Vortex	Fisher
Waterbath	Precision
<u>Software:</u>	
-CellQuest	Becton Dickinson
-IgBLAST	NCBI
-DNASTar Software	NCBI
-SoftMax (ELISA)	
-Adobe Illustrator	Adobe
-Adobe Photoshop	Adobe
-Microsoft Excel	Microsoft
-Microsoft Word	Microsoft