

## 2. MATERIALS

### 2.1 Equipment

- Laminar flow or sterile working bench (BDK Luft- und Reinraumtechnik GmbH, Sonnenbühl-Genkingen, FRG)
- Gel Doc 2000, Mini-Trans-Blot Cell, Mini Protean 2 chamber, Model 1000/500 power supply, Sub Cell GT electrophoresis chamber (Bio-Rad GmbH, Munich, FRG)
- EL 800 Universal microplate reader (Bio-Tek Instruments GmbH, Bad Friedrichshall, FRG)
- Centrifuge 5417 R (Eppendorf, Wesseling-Berzdorf, FRG)
- Allegra™ 6R centrifuge (Beckman Coulter GmbH, Krefeld, FRG)
- Steril-Cult 200 incubator (Forma Scientific, Frankfurt, FRG)
- Ultra-Turrax TM homogenizer (Ika Werke GmbH, Staufen, FRG)
- Electrophoresis power supply ST 606 (Invitrogen Life Technologies, Karlsruhe, FRG)
- Fluoroskan Ascent FL Type 374 (Labsystems, Frankfurt, FRG)
- Gene Amp PCR System 970 (Perkin Elmer LAS GmbH, Rodgau-Jügesheim, FRG)
- Spectrophotometer UV-1202 (Shimadzu Europa, Duisburg, FRG)
- Light microscope (Leica Mikrosysteme Vertrieb GmbH, Bensheim, FRG)
- Axioplan II fluorescence microscope (Zeiss, Jena, FRG)
- LSM 510 META confocal microscope (Zeiss, Jena, FRG)
- EOS 500 digital camera (Canon USA Inc., Lake Success, USA)
- Curix 60 (AGFA, Berlin, FRG)
- Cryo 1°C freezing container (Nalgene Nunc International, Hamburg, FRG)
- 6-well plates (TPP, Traisadingen, Switzerland)
- 4-well plates (Nunc, Wiesbaden, FRG)
- 96-well plates (Perkin Elmer LAS GmbH, Rodgau-Jügesheim, FRG)
- 12-well plates (Costar, Corning, USA)
- Glas microchip, glas slide (Menzel Glasbearbeitungswerke GmbH & Co. KG, Braunschweig, FRG)

### 2.2 Plasmid vectors

- pcDNA.3.1 (Invitrogen Life Technologies, Karlsruhe, FRG)

- pGL3Basic (Promega GmbH, Mannheim, FRG)

### 2.3 Enzymes

- Restriction enzymes and corresponding buffers: AseI, KpnI, NcoI, Sall, XbaI (Amersham Biosciences, Freiburg, FRG)
- Taq polymerase and PCR reagents (InViTek, Berlin, FRG)
- T4 DNA ligase, T4 DNA polymerase and corresponding buffers (New England Biolabs, Frankfurt, FRG)

### 2.4 Bacteria

- TOP10 (Invitrogen Life Technologies, Karlsruhe, FRG)

### 2.5 Plasmid purification

- QIAprep Mini Prep kit (Qiagen GmbH, Hilden, FRG)
- Qiagen Plasmid Maxi kit (Qiagen GmbH, Hilden, FRG)

### 2.6 Oligonucleotides

- hALC-1 promoter primer sense : 5'-attggtaccTTGCCTGTAAAACAGCATG-3'  
 hALC-1 promoter primer antisense : 5'-ctcccatggTGTCTTGTTGGGATCTTTGGC-3'  
 (hALC-1 promoter sequence in capital letters)

- Luc sense : 5'-ACACCCGAGGGGGATGATAA-3'  
 Neo antisense : 5'- CGGCACTTCGCCCAATAGCA-3'

- Sequencing primer sense : 5'-CTAGCAAATAGGCTGTCCC-3'  
                                   sense : 5'-TGGCCAAGTTGTTTCATCCAG-3'  
                                   antisense : 5'-CTTTATGTTTTTGGCGTCTTCCA-3'

All oligonucleotides: BioTez, Berlin, FRG

## 2.7 Molecular weight markers (M)

- Ma:1 Kb DNA ladder (Invitrogen Life Technologies, Karlsruhe, FRG); band sizes (in bp): 75, 134, 154, 201, 220, 298, 344, 396, 506/517, 1018, 1636, 2036, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000, 12000
- Mb: 100bp DNA ladder (Invitrogen Life Technologies, Karlsruhe, FRG); band sizes (in bp): 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 2072
- Roti-Mark standard (Roth, Karlsruhe, FRG)

## 2.8 Cell lines

- H9c2 cell line (European Collection of Animal Cell Cultures (ECACC), Wiltshire, UK)

## 2.9 Cell culture reagents

- DMEM (1x) High Glucose, DMEM (1x) High Glucose lacking  $\text{Ca}^{2+}$  (PAA Laboratories, Cölbe, FRG)
- Fetal bovine serum (FBS), G418 sulfate (Geneticin), L-Glutamine, Phosphate buffered saline (PBS (1x); pH 7.4), Trypan blue stain 0.4%, Trypsin-EDTA (Invitrogen Life Technologies, Karlsruhe, FRG)
- Albumin bovine Fraction V (BSA; pH 7.0) (AppliChem GmbH, Darmstadt, FRG)

## 2.10 Chemicals and inhibitors used in cell culture experiments

- (Arg<sup>8</sup>) Vasopressin, Cyclosporin A (Sigma, Taufkirchen, FRG)
- Bisindolylmaleimide, Ionomycin, KN93, KN92 (Calbiochem, Schwalbach, FRG)
- 4',6-Diamidino-2-phenylindole dihydrochlorid (DAPI) 5mg/ml stock solution in methanol (Roche Diagnostics GmbH, Penzberg, FRG)

## 2.11 Kits

- Calcium Phosphate Transfection System (Invitrogen Life Technologies, Karlsruhe, FRG)

- GeneShuttle™ Transfection Reagent (Q Biogene, Heidelberg, FRG)
- GenePorter™ Transfection Reagent (Gene Therapy Systems Inc., San Diego, USA)
- Nucleofector Kit (Amaxa, Köln, FRG)
- Twin Prep DNA/RNA Kit (InViTek, Berlin, FRG)
- Luciferase Assay (Promega GmbH, Mannheim, FRG)
- DC Protein Assay (Bio-Rad GmbH, Munich, FRG)
- ECL System (Amersham Biosciences, Freiburg, FRG)

## **2.12 Membranes and films**

- Hybond ECL nitrocellulose mebrane (Amersham Biosciences, Freiburg, FRG)
- Gel blot chromatography paper, 0.4 mm (Schleicher & Schuell Bioscience GmbH, Dassel, FRG)
- Hyperfilm ECL autoradiography film (KODAK, New York, USA)

## **2.13 Antibodies**

- anti-ALC-1 rabbit polyclonal antibodies (cf. 3.10.1)
- Peroxidase (POD)-conjugated anti-rabbit IgG (BioGenes GmbH, Berlin, FRG)
- anti-NFAT3 goat polyclonal antibody (NFATc4; Santa Cruz Biotechnology, Santa Cruz, USA)
- blocking peptide for NFAT3 (Santa Cruz Biotechnology, Santa Cruz, USA)
- anti-CaMKII $\delta$  rabbit polyclonal antibody (cf. Hoch et al. 1998)
- anti-CaMKIV mouse monoclonal antibody (BD Biosciences Pharmingen, San Diego, USA)
- Alexa 594-conjugated anti-rabbit IgG, Cy3-conjugated anti-mouse and anti-goat IgG (Molecular Probes, Eugene, USA)

## **2.14 Animals**

- WKY rats were obtained from the animal facility of the Max-Delbrueck-Center

## 2.15 Chemicals

- DNA agarose (Biozym, Hess. Oldendorf, FRG)
- $\beta$ glycerophosphate (AppliChem GmbH, Darmstadt, FRG)
- Leupeptin, NaF,  $\text{NaN}_3$ ,  $\text{Na}_3\text{VO}_4$ , Ovalbumin, Protease inhibitor cocktail, Urea (Sigma-Aldrich, Munich, FRG)
- PCR reagents: 50 mM MgCl, 10x PCR buffer, dNTPs (InViTek, Berlin, FRG)
- 10 x Tris-borate EDTA (TBE) buffer (Invitrogen Life Technologies, Karlsruhe, FRG)
- Recombinant luciferase (Promega GmbH, Mannheim, FRG)
- Ammonium-peroxidsulfate (APS), Blotting grade blocker non-fat dry milk, BSA standard, N,N,N,N-Tetramethyl-ethylene diamine (TEMED) (Bio-Rad GmbH, Munich, FRG)
- Ethylenediamine-tetraaceticacid (EDTA), Ethyleneglycol-bis-( $\beta$ -aminoethylether)-N,N,N',N'-tetraaceticacid (EGTA), Ethanol, Ethidiumbromide, Glycine, Hydrochloric acid (HCl), Methanol, NaCl, Phenol, Rotiphorese Gel 30 (Acrylamide), Saccharose, Sodium acetate (Roth, Karlsruhe, FRG)
- Dimethylsulfoxide (DMSO), Dithiothreitol (DTT), Glycerol, Hepes, Ponceau S, Sodium dodecyl sulfate (SDS), 5-sulfosalicylic acid, Tris-(hydroxymethyl)-aminomethane (Tris), Tween-20 (Serva, Heidelberg, FRG)
- Bromophenol blue, Chloroform, PMSF, Trichloroacetic acid (Merck, Darmstadt, FRG)
- Aqua polymount (Polysciences Europe GmbH, Eppelheim, FRG)