

# Appendixes

## Appendix A.

### (1) 4 X Sample buffer

0.5 M/pH 6.8 Tris-base: 1 ml

Glycerol: 0.8 ml

10 % SDS: 1.6ml

2-mercaptoethanol (2-ME): 0.4 ml

0.1 % bromophenol blue: 0.4 ml

ddH<sub>2</sub>O: 3.8 ml

### (2) SDS-PAGE (15 %)

	Running gel	Stacking gel
ddH <sub>2</sub> O	0.88 ml	2.1 ml
1.5 M Tris pH 8.8	1.04 ml	-
1 M Tris pH 6.8	-	0.38 ml
10 % SDS	40 µl	40 µl
30 % Acrylamide mixture (Acrylamide:Bis- Acrylamide)=29:1	2 ml	0.5 ml
10 % ammonium persulfate (APS)	40 µl	30 µl
N,N,N,N,-tetramethylethylenediamine (TEMED)	1.6 µl	3 µl
Total volume	8 ml	3 ml

### (3) Running buffer

Glycine: 72 g

Tris-base: 15.15 g

SDS: 5g

ddH<sub>2</sub>O: 5 L

**(4) Transfer buffer, pH 8.3**

Glycine: 2.9 g

SDS: 0.37 g

Tris-base: 5.8g

Methanol: 200 ml

ddH<sub>2</sub>O: 800 ml

**(5) Blocking buffer**

Nonfat milk powder: 2.5 g

PBS: 50 ml

**(6) PBST**

Tween 20: 0.5 ml

PBS: 1L

**(7) Substrate solution**

DAB: 0.0149 g

35 % H<sub>2</sub>O<sub>2</sub>: 5µl

50 mM Tris-buffer pH 7.3: 50 ml



**Appendix B.**

**(1) Minimum Essential Medium Eagle (MEM), pH 7.4**

MEM: 10 g

NaHCO<sub>3</sub>: 1.5 g

Penicillin-Streptomycin-Amphotericin B solution: 10 ml

ddH<sub>2</sub>O: 1 L

**(2) Dulbecco's Modified Eagle's Medium (DMEM), pH 7.4**

DMEM: 10 g

NaHCO<sub>3</sub>: 1.5 g

Penicillin-Streptomycin-Amphotericin B solution: 10 ml

**Appendix C.**

**(1) Propidium Iodide (PI) staining solution**

10 X RNase A: 1 mg/ml

10 X PI: 400 µg/ml

1 X PBS: 10 X RNase A: 10 x PI = 8:1:1

**Appendix D.**

**(1) HEPES-Tyrode buffer, pH7.4**

10 mM HEPES: 1.1915 g

150 mM NaCl: 4.383 g

12 mM NaHCO<sub>3</sub>: 0.504 g

2.5 mM KCl: 0.0931 g

2 mM MgCl<sub>2</sub>: 0.095 g

0.4 mM NaH<sub>2</sub>PO<sub>4</sub>: 0.02759 g

0.1 % Glucose: 0.5 g

0.02 % BSA: 0.1 g

ddH<sub>2</sub>O: 500 ml

**(2) Substrate/Lysis solution, pH 5.0**

1 % Triton X-100

6 mg/ml *p*-Nitrophenyl Phosphate

50 mM sodium acetate buffer



## Appendix E.

### (1) 2 x Sample buffer

0.5 M/pH 6.8 Tris-HCl: 1 ml

Glycerol: 0.8 ml

10 % SDS: 3.2 ml

0.1 % bromophenol blue: 0.4 ml

ddH<sub>2</sub>O: 2.8 ml

### (2) Reaction buffer, pH 7.6

Tris-HCl: 6.06 g

CaCl<sub>2</sub>: 1.47 g

NaCl: 2.92g

Brij35: 0.5

ddH<sub>2</sub>O: 1 L



## Appendix F.

### (1) PCR

Components	Volume ( $\mu$ l )
cDNA	1
10 $\mu$ M Forward primer	1
10 $\mu$ M Reverse primer	1
10 mM dNTPs	1
10 $\times$ reaction buffer	2.5
ddH <sub>2</sub> O	18
Taq DNA polymerase	0.5
Total volume	25