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APPENDICES

APPENDIX A

REAGENTS, MATERIALS AND INSTRUMENTS

A. Media and Reagents

Absolute ethanol	(Merck, Germany)
AccuGel™ 29:1 (40% Acrylamide)	(Kimberly research, U.S.A.)
Acetic acid	(Merck, Germany)
Agarose	(Research Organic, Inc., U.S.A.)
Ammonium persulfate	(Bio Basic, U.S.A.)
Ampicilin	(Bio Basic, U.S.A.)
BCIP/NBT	(KBL, U.S.A.)
Bromphenol blue	(USB, U.S.A.)
100 bp DNA ladder	(Fermentas, EU)
DNA extraction kit	(Qiagen, U.S.A.)
dNTPs	(Promega, U.S.A.)
DTT	(Bio Basic, U.S.A.)
Ethidium bromide	(Bio-Rad, U.S.A.)
Fetal Bovine Serum	(PAA, Austria)
Goat anti-mouse IgG	(Santa Cruz, U.S.A.)
Goat normal IgG	(Santa Cruz, U.S.A.)
Gold colloidal solution	(Sigma-Aldrich, U.S.A.)
Guanidine hydrochloride	(Bio Basic, U.S.A.)
HPV16E6 goat polyclonal antibody	(Santa Cruz, U.S.A.)
HPV16E6 mouse monoclonal antibody	(Santa Cruz, U.S.A.)
HPV16L1 goat polyclonal antibody	(Santa Cruz, U.S.A.)
HPV16L1 mouse monoclonal antibody	(Santa Cruz, U.S.A.)
IPTG	(Bio Basic, U.S.A.)
LB broth powder	(Bio Basic, U.S.A.)
MEM medium	(GIBCO™ Invitrogen, UK)

Methanol	(Merck, Germany)
Penicilin G	(Bio Basic, U.S.A.)
Plasmid extraction	(Qiagen, U.S.A.)
Polyethyleneglycon	(Sigma-Aldrich, U.S.A.)
Potassium chloride	(Merck, Germany)
Potassium phosphate	(Bio Basic, U.S.A.)
Prestained protein ladder	(Fermentas, EU)
Proteases inhibitor cocktail tablets	(Roche, Germany)
SDS	(Bio Basic, U.S.A.)
Sodium bicarbonate	(Bio Basic, U.S.A.)
Sodium chloride	(Merck, Germany)
Sodium phosphate	(Bio Basic, U.S.A.)
Sodium tetraborate decahydrate	(Sigma-Aldrich, U.S.A.)
Streptomycin	(Bio Basic, U.S.A.)
Taq DNA polymerase	(Fermentas, U.S.A.)
TEMED	(Bio Basic, U.S.A.)
Transformation kit	(Zymo Research, U.S.A.)
Triton X-100	(Sigma-Aldrich, U.S.A.)
Tris Base	(Research Organic, U.S.A.)
Trypsin 1:250	(Bio Basic, U.S.A.)
Quant-IT™ protein assay kit	(Invitrogen™, UK)

B. Materials

Affinity column GSTrap FF	(GE healthcare, U.S.A.)
Centrifuge tube	(Labcon, Germany)
Cervix brush	(RMD, Netherlands)
Disposable cuvettes	(Bio-Rad, U.S.A.)
Disposable serological pipette	(Labcon, Germany)
Filter Tip	(Labcon, Germany)
Microcentrifuge tube	(Sorenson, U.S.A.)

Tissue culture flask (Nunc, Denmark)

C. Instruments

Autoclave (model-SX-700)	(Tomy, Japan)
CO ₂ Incubator	(Thermo Forma, U.S.A.)
Culturing shanking incubator	(Ztictene, China)
DNA thermocycle system	(Takara, Japan)
Electrophoresis chamber	(CBS, U.S.A.)
Incubator	(Mettler, Germany)
Microcentrifuge	(Fotodyne, U.S.A.)
pH meter	(Accumet Basic, Singapore)
Power supply (Model 1000/500)	(Bio-Rad, U.S.A.)
Qubit fluorometer	(Invitrogen™, UK)
Refrigerator	(Sanyo, Japan)
Spectrophotometer (SmartSpect™ 3000)	(Bio-Rad, U.S.A.)
Sonicator	(SONIC, U.S.A.)
Vertical electrophoresis chamber	(Cleaver scientific Ltd., UK)
Water bath	(Julabo, Germany)
Western blot chamber	(Cleaver scientific Ltd., UK)

APPENDIX B

REAGENTS PREPARATION

Reagent for preparation of HPV-16 antigens

1. Luria-Bertani broth

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Distilled water to	1 L
Sterilized by autoclaving 121°C 15 minutes	

2. Luria-Bertani agar plate

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Agar	10 g
Distilled water to	1 L

Sterilized by autoclaving 121°C 15 minutes

To pour plates, agar was allowed to cool about 50 °C and then ampicillin 100 mg/ml was added. After drying, plates were stored at 4 °C until used.

3. Binding buffer

NaCl	8 g
KCL	20 mg
NaHPO ₄	1.1 g
KH ₂ HPO ₄	20 mg
Deionized distilled water to	90 ml

Adjusted to pH 7.3 and adjusted volume to

100 ml with additional deionized distilled water

Sterilized by autoclaving and stored at room temperature

4. Elution buffer

Tris-HCl	1.21 g
Glutathione reduced	0.61 g
Deionized distilled water to	190 ml
Adjusted to pH 8.0 and adjusted volume to	
200 ml with additional deionized distilled water	
Sterilized by filtration and stored at room temperature	

Reagents for SDS-PAGE

1. Running gel solution

Deionized distilled water	3.03 ml
1.5 M Tris-HCl pH 8.8	1.90 ml
10% (w/v) SDS	75 μ l
40% Acrylamide	2.47 ml
10%(w/v) APS	37.5 μ l
TEMED	4.95 μ l

2. Stacking gel solution

Deionized distilled water	1.80 ml
0.5 M Tris-HCl pH 6.8	750 μ l
10% (w/v) SDS	30 μ l
40% Acrylamide	402 μ l
10%(w/v) APS	15 μ l
TEMED	3 μ l

3. 10X Tris-borate buffer (TBE)

Tris-base	60.50 g
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Boric acid	30.85 g
Na ₂ EDTA·2H ₂ O	3.72 g
Distilled water to	1 L
Sterilized by autoclaving and stored at room temperature	

Reagents for Western blot

1. TBS buffer

1 M Tris pH 7.5	20 ml
NaCl	29.22 g
Distilled water to	1 L
Sterilized by autoclaving and stored at room temperature	

2. TTBS buffer

TBS	100 ml
Tween20	100 µl

3. Blocking buffer

TBS	10 ml
Bovine serum albumin	0.3 g

4. Alkaline phosphate buffer

1 M Tris pH 9.5	50 ml
NaCl	2.92 g
2 M MgCl ₂	625 µl
Distilled water to	1 L
Sterilized by autoclaving and stored at room temperature	

5. 10X Blotting buffer

Tris base	30.3 g
Glycine	144 g
Distilled water to	1 L

Sterilized by autoclaving and stored at room temperature

Reagents for cells cultivation

1. Penicillin 100,000 Units/ml

1,000,000 Unit Penicillin G 1 ampoule

Deionized Distilled water 10 ml

Sterilized by filtration through membrane pore size
of 0.2 μm and stored at -20°C

2. Streptomycin 100,000 $\mu\text{g}/\text{ml}$

1 gm Streptomycin 1 ampoule

Distilled water 10 ml

Sterilized by filtration through membrane pore size
of 0.2 μm and stored at -20°C

3. 1 M HEPES

HEPES 23.83 g

Deionized distilled water to 100 ml

Sterilized by autoclaving and stored at 4°C

4. 10 % NaHCO_3

NaHCO_3 10 g

Deionized distilled water to 100 ml

Sterilized by autoclaving and stored at 4°C

5. Growth medium

MEM medium 90 ml

100,000 unit/ml Penicillin G 1 ml

100,000 $\mu\text{g}/\text{ml}$ Streptomycin 1 ml

Fetal Bovine Serum (FBS) 100 ml

1M HEPES 1 ml

10 % NaHCO_3 2 ml
Stored at 4 °C

6. 5% Trypsin

Trypsin 5 g
Deionized distilled water to 100 ml
Sterilized by filtration and stored at -20 °C

7. 1% EDTA

EDTA 1 g
Deionized distilled water to 100 ml
Sterilized by filtration and stored at 4 °C

8. Trypsin/EDTA (0.025% trypsin, 0.01% EDTA)

5% Trypsin 0.5 ml
1% EDTA 0.2 ml
1X PBS up to 100 ml
Do not autoclave
Sterilized by filtration and stored at 4 °C

9. 10 X PBS

NaCl 80 g
KCL 2 g
NaHPO₄ 11.5 g
KH₂HPO₄ 2 g
Deionized distilled water to 900 ml
Adjusted to pH 7.4 and adjusted volume to
1000 ml with additional deionized distilled water
Sterilized by autoclaving and stored at room temperature

10. 1X PBS

10 X PBS	100 ml
Deionized distilled water	900 ml

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