APPENDIX

BUFFERS AND SOLUTION FOR DNA EXTRACTION FROM FROZEN CERVICAL TISSUE, PAP SMEARS AND CELL CULTURE

*Phosphate buffered saline (PBS), pH 7.2*

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium phosphate (anhydrous)</td>
<td>1.52 g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.58 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>8.50 g</td>
</tr>
<tr>
<td>dH₂O</td>
<td>made up to 1 liter</td>
</tr>
</tbody>
</table>

*20X SSC, pH 7.0*

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>175.3 g</td>
</tr>
<tr>
<td>Sodium Citrate trihydrate</td>
<td>88.2 g</td>
</tr>
<tr>
<td>dH₂O</td>
<td>made up to 1 liter</td>
</tr>
</tbody>
</table>

*1M Tris - HCL, pH 8.0*

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trizma base</td>
<td>121.1 g</td>
</tr>
<tr>
<td>dH₂O</td>
<td>made up to 1 liter</td>
</tr>
</tbody>
</table>

The pH was adjusted with concentrated HCL

*SSC-buffered phenol*

Phenol was melted at 68°C. An antioxidant, 8-hydroxyquinoline was added to a final concentration of 0.1%. The melted phenol was then extracted with with equal volume of 1 X SSC, pH 8 by stirring at 4°C, overnight, until the pH of the aqueous phase is more than 7.6. The SSC buffered phenol was stored in a dark bottle at 4°C for up to 1 month.

CELL CULTURE

All media were filter sterilized and stored at 4°C.

*RPMI 1640 base medium*

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI 1640 base media</td>
<td>1 sachet</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Hydroxyethylpiperazine ethanesulfonic Acid (HEPES)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>dH₂O</td>
<td>made up to 1 liter</td>
</tr>
</tbody>
</table>
Reviving media

RPMI base media 77 ml  
Fetal calf serum (FCS) 20 ml  
Penicillin 2 ml  
Kanamycin 1 ml  
L-glutamine 0.012 g  

Media was filtered sterilized with 0.22 μm milipore filter

Culture Media

Preparation of culture media was the same as reviving media with the addition of 10% FCS.

Freezing solution

FCS 10 ml  
Dimethyl sulfoxide (DMSD) 4 ml  
RPMI 1640 base medium 6 ml  

The solution was prepared fresh and sterilized with 0.22 μm millipore filter.

GEL ELECTROPHORESIS

Tris-borate EDTA (TBE) 5X

Trizma base 54g  
Boric acid 27.5 g  
EDTA 0.05M pH 8.0 20 g  
dH₂O made up to 1 liter

Loading Buffer 6X

Bromophenol blue 0.25%  
Sucrose 40% (w/r)  
dH₂O made up to 100 ml  

20x SSC pH 7.0

SOUTHERN BLOT HYBRIDIZATION

Solutions are prepared as described by Boehringer Mannheim (1989). Reagents used were from the Non-radioactive DNA labelling and Detection kit.
Hybridization solution

5X SSC 22.5 ml  
N-lauroylsarcosine, Na-salt (Sigma) 0.1%(w/v)  
SDS 0.02% (w/v)  
Blocking reagent (vial 11) 1% (w/v)

ISOLATION OF PLASMID

LB broth

Bacto-Tryptone 10 g  
Bacto-yeast extract 5 g  
NaCl 10 g  
ddH₂O up to 1 litre

IN SITU HYBRIDIZATION

Denhardt's solution (50x)

Ficoll 400 5 g  
Polyvinylpyrrolidone-360 5 g  
BSA 5 g  
dH₂O added to 500 ml

The solution was sterilized using the disposable 0.22 µm Nalgene filter, dispensed into 25 ml aliquots and stored at -20°C.

4% paraformaldehyde

Paraformaldehyde 4 g  
PBS 100 g

Paraformaldehyde was dissolved by heating the solution 60°C-70°C and by adding drops of 2 N NaOH while adjusting the pH to 7.2.

Phosphate Buffered Saline (PBS) pH 7.6

Sodium chloride 7.75 g  
Potassium phosphate dibasic 1.5 g  
Potassium phosphate, monobasic 0.2 g  
dH₂O to 1 litre of the solution

The pH of the solution was adjusted to 7.6 with 3 M NaOH or 8.5% phosphoric acid.
**Prehybridization mixture**

- 50x Denhardt’s solution: 0.2 ml
- 50% dextran sulphate: 1.0 ml
- Sonicated salmon sperm DNA (10 mg/ml): 0.2 ml
- 20X SSC: 2.0 ml
- Formamide: 5.0 ml
- Sterile dH₂O: added to 10 ml

**Hybridization solution (probe-cocktail)**

- 50x Denhardt’s solution: 0.2 ml
- 50% dextran sulphate: 1.0 ml
- Sonicated salmon sperm DNA (10mg/ml): 0.2 ml
- 20X SSC: 2.0 ml
- 100% deionized formamide: 5.0 ml
- Digoxigenin labelled probe: 500 ng/slide
- Sterile dH₂O: to 10 ml

**100% deionized formamide**

- Formamide: 100 ml
- Resin: 10 g

The mixture was stirred vigorously for 30 minutes, filtered twice through Whatman filter paper and stored at -20°C.

**IMMUNOSTAINING**

The LSAB kit (Dako) contained the following reagents:

1. Biotinylated anti-mouse and anti-rabbit immunoglobulin in 0.05 M Tris-HCL buffer, pH 7.6.
2. Streptavidin-HRP conjugate in 0.05 M Tris-HCL buffer pH 7.6.

AEC substrate system (K697,Dako) contains the following reagents:

1. 0.1 M Acetate buffer, pH 5.2
2. 3% Hydrogen peroxide in water
3. 3% 3-amino-9-ethylcarbazole (AEC) in N,N dimethylformamide.

**ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)**

**0.1 M Carbonate buffer, pH 9.6**

- Na₂CO₃: 1.65 g
- NaHCO₃: 3.14 g
- dH₂O: up to 500 ml
Phosphate buffered Saline (PBS), pH 7.3

Sodium chloride 8.0
Potassium phosphate monobasic 0.2
Sodium phosphate dibasic 1.15
Potassium chloride 0.2
dH₂O up to 1 litre

PBS-0.05% Tween 20 (PBS-T) was prepared by adding 0.05% of Tween 20 to the above solution.

Lamb serum

Lamp serum (Gibco) was removed from the freezer and thawed at 37°C after which it was heat inactivated at 56°C for 30 minutes. The serum was then filtered through Whatman filter paper and 45 μm membrane filter. It was dispensed into aliquots and stored at -20°C.

ABTS (50x) (20mg/ml)

(2,2-azinodi-(3-ethylbenz thiazolin sulfonate(Boeriner) 0.2 g
dH₂O 10 ml

0.1 M Citrate Buffer, pH 4.0

Citric acid monohydrate 6.62 g
Sodium citrate dihydrate 5.44 g
dH₂O to 1 litre

Peroxidase substrate

50X ABTS 150 μl
30% Hydrogen peroxidase 1.8 μl
0.1M Citrate buffer up to 6 ml

The solution was prepared fresh.

Diethanolamine buffer - pH 9.8

Diethanolamine 97 ml
Magnesium chloride 0.1 g
dH₂O up to 1 litre

The solution was filtered through Whatman paper and stored in a dark bottle at room temperature.

Phosphatase substrate

Phosphatase substrate 104 (Sigma) 0.1 g
Diethanolamine buffer 100 ml
20% glucose

Glucose 10 g
Sterile dH₂O to 50 ml

The solution was transferred to a 50 ml tube and rotated end-over-end overnight at room temperature and stored at 4°C.

0.2 M NaH₂PO₄

NaH₂PO₄ 27.8 g
dH₂O to 1 litre

0.2 M Na₂HPO₄

Na₂HPO₄ 53.65 g
dH₂O to 1 litre

0.1 M Phosphate buffer, pH 6

0.2 M NaH₂PO₄ 87.7
0.2 M Na₂HPO₄ 12.3
dH₂O to 100 ml