

VII. APPENDIX

Name of Chemicals	Company
Acids	
Acetic acid	Merck
Hydrochloric acid, concentrated	Merck
Sulfuric acid, concentrated	Merck
Buffers	
HEPES (N-[2-hydroxyethyl]piperazine-N-[2-ethanesulfonic acid])	Sigma
Tris (Tris[Hydroxymethyl]aminomethane)	Sigma
Cell culture reagents	
Antimycotic (penicillin G and streptomycin)	Sigma
Disodium ethylenediaminetetra acetoacetic acid	Sigma
Fetal calf serum	Gibco-BRL
Ficoll Type 400	Sigma
Heparin	Sigma

Name of Chemicals	Company
D-glucose	Sigma
Ferrus sulfate (FeSO_4)	Sigma
Hydrogen peroxide	Witayasom
Magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$)	Sigma
Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	Sigma
Potassium chloride (KCl)	Sigma
Potassium dihydrogen phosphate (K_2HPO_4)	Sigma
Sodium bicarbonate (NaHCO_3)	Sigma
Sodium chloride (NaCl)	Sigma
Disodium hydrogen phosphate (Na_2HPO_4)	Sigma
Sodium hydroxide (NaOH)	Merck
Triton-X 100	Sigma
Trizma base	Sigma
Electrophoretic materials	
Agarose	Sigma
Low melting point agarose	Sigma
N-lauroylsarcosine	Sigma
Tris HCl	Sigma

Name of Chemicals

Company

Medium

RPMI-1640

Gibco-BRL

Organic solvent

Absolute ethanol

Merck

Acetic acid

Merck

DMSO

Sigma

Chloroform

Merck

Hexene

Merck

Protocol for Preparation of Reagent or Media

Incomplete RPMI-1640

RPMI-1640	9.8	g
HEPES	3.57	g
NaHCO ₃	2	g
penicillin G sodium	100,000	units
streptomycin	0.1	g

The solution was filtered with hydrophilic 0.2 μm filter and kept at 4 °C. The complete RPMI media was prepared by adding fetal calf serum to the final concentration of 10 %(v/v).

Modified Gey's buffer

NaCl	147	mM
KCl	5	mM
KH ₂ PO ₄	1.9	mM
Na ₂ HPO ₄	1.1	mM
Glucose	5.5	mM
CaCl ₂	1.5	mM
MgSO ₄ .7H ₂ O	0.3	mM
MgCl ₂ .6H ₂ O	1.0	mM
DW	1000	mL

The solution was adjusted to pH 7.4 and filtered with hydrophilic 0.2 μm filter.

The solution was kept at 4°C until used.

Stock Phosphate buffer (PBS) 3X

NaCl	24	g
KCl	0.6	g
Na ₂ HPO ₄	3.435	g
KH ₂ PO ₄	0.6	g
DW	1000	mL

The pH was adjusted to 7.2 and if the solution was to be use with the cell culture, the solution was to be filtered with 0.2 μ m filter or autoclaved at the condition of 120 °C with the pressure of 1.5 atmosphere for 15 minuted. The solution was kept at room temperature and diluted 1:3 before use.

Incomplete lysing solution for comet assay

NaCl	2.5 M	146.1 gm
EDTA-Na	100 mM	37.2 gm
Trizma base	10 mM	1.2 gm

The ingredients were added to about 700 mL of deionized water (dH₂O) with constant stirring of the mixture. Then NaOH 8 grams was added and allowed the mixture to dissolve (about 20 min). The pH was adjusted to 10.0 using concentrated HCl or NaOH then diluted to 890 mL with dH₂O stored at room temperature.

The working lysing solution was prepared by fresh 1 mL of Triton X-100 and 10 ml of DMSO were added in 89 mL of incomplete lysing solution and then

refrigerated for at least 60 minutes for protect DNA damage from high temperature of reagent.

Electrophoresis Buffer

NaOH	300 mM	12 g
EDTA-Na	1 mM	0.36 g

The electrophoresis buffer was stored at room temperature. The total volume depends on the gel box capacity. Prior to use, measured the pH of the buffer to ensure 13.

Neutralization Buffer

Tris	0.4 M	48.5 g
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Tris-aminomethan in approximately 800 mL dH₂O and adjusted to pH 7.5 with conc HCl then diluted to 1000 mL with dH₂O, and stored at room temperature.

The solution was stable for 1 month at 4 °C.

Stock staining solution (10X): Ethidium bromide

Ethidium bromide	10 mg
DW	50 mL

The staining solution was prepared by added 10 mg ethidium bromide in 50 mL dH₂O, stored at room temperature. The 20 µg/mL working staining solution was prepared by mixed 1 mL of stock solution with 9 mL dH₂O.

SYBRTM Green 1µL of provided stock solution was added to 10 mL of buffers (10 mM Tris-HCL, 1 mM EDTA, pH 7.5) to prepare a 1:10,000 dilution. Make fresh prior to use. Staining solution was stabled for several hours at room temperature and for 1-2 days when refrigerated. The pH was critical for stability.

3. Equipment

Name of equipment	Company
Automated cell counter (Hemacell)	Hycell
HPLC analyzer (CLC385)	Primus
Laminar flow (MSC12)	Juan
Microcentrifuge (MR1812)	Juan
Centrifuge (H-103RS)	KOKUSAN
Electrophoresis chamber (22x47 cm)	Biorad
Power supply (Power Pac 300, 283BR)	Biorad
Zeiss microscopoe model axioskop 2 (flurescent and light function linked to CCD camera)	Zeiss

VIII CURRICULUM VITAE

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