ANNEXURE - 1

A1. General Media for Cell culture

1. DMEM (For adherent cells)

DMEM/F-12, GlutaMAX™ supplement (Thermo Fisher Scientific # 10565-018)	890 ml
Fetal Bovine Serum (Thermo Fisher Scientific # 10438018)	100 ml
Penicillin-Streptomycin (10,000 U ml^{-1}) (Thermo Fisher Scientific # 15140148)	10 ml

2. RPMI (for floating cells)

RPMI 1640 Medium (Thermo Fisher Scientific # 11875085)	880ml
Fetal Bovine Serum (Thermo Fisher Scientific # 10438018)	100 ml
Penicillin-Streptomycin (10,000 U ml^{-1}) (Thermo Fisher Scientific # 15140148)	10 ml
HEPES (1 M) (pH 7.4)	10ml

A2. General Reagents and Salt Solutions

1. Phosphate buffer saline (pH 8.0)

a. Sodium dihydrogen orthophosphate ($NaH_2PO_4 . 2H_2O$)	0.78 g
b. Disodium hydrogen phosphate (Na_2HPO_4)	13.48 g
c. Distilled water up to	1000 ml

pH is adjusted with 1N NaOH/HCl to 8

2. Phosphate buffered saline-Glucose (PBS-G), pH 8.0

a. PBS (pH 8.0)	60 ml
b. Distilled water	40 ml
c. Glucose (1% W/V)	1 g

3. HBSS (pH 7.4)

Calcium Chloride ($CaCl_2$) (anhyd.)	0.140 g
Magnesium Chloride ($MgCl_2 \cdot 6H_2O$)	0.100 g
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	0.100 g

Potassium Chloride (KCl)	0.400 g
Potassium Phosphate monobasic (KH2PO4)	0.060 g
Sodium Bicarbonate (NaHCO3)	0.350 g
Sodium Chloride (NaCl)	8 g
Sodium Phosphate dibasic (Na2HPO4) anhydrous	0.048 g
HEPES (pH 7.4)	2.38 g
D-Glucose (Dextrose)	1 g
Distilled water up to	1000 ml

A3. Solution for Plasmid Isolation

1. **Solution I**

Glucose	0.9 g
Tris-Cl	0.303 g
EDTA	0.372 g
Distilled water up to	100 ml

pH was adjusted to 8.0 using 1N NaOH/ HCl. Autoclaved the solution and store at 4 ^{o}C.

2. **Alkaline lysis solution II**

Sodium hydroxide (NaOH)	0.8 g
SDS	1.0 g
Distilled water up to	100 ml

Autoclaved and store at room temperature.

3. **Potassium acetate solution (5M)**

Potassium acetate	29.44g
Distilled water up to	60 ml

4. **Solution III (pH 4.8-5)**

Potassium acetate solution (5M)	60 ml
Glacial acetic acid	11.5 ml
Distilled water	28.5 ml
Autoclaved and store at	4^{0}C

A4. Solutions for Agarose Gel Electrophoresis

1. **0.5 M Ethylene diamine tetra acetate (EDTA), pH 8.0**

EDTA disodium salt	18.61 g
Distilled water	80 ml

NaOH pellets added while stirring the solution to adjust pH 8.0. Final volume made to 100 ml, sterilized by autoclaving and store at 4°C

2. **Tris-Acetate-EDTA (TAE) stock solution (50X)**

Tris base	242g
Glacial acetic acid	57.1ml
0.5 M EDTA, pH 8.0	100ml
Distilled water	1000ml

Sterilized by autoclaving and stored at RT. For working solution (1X), stock TAE was diluted in water.

3. **Ethidium bromide solution (10mg ml^{-1})**

Ethidium bromide	10mg
Distilled water	1ml

A5. Bacteriological Solution

1. **Luria-Bertani (LB) broth**

LB broth powder	25g
(Casein hydrolysate 10g, Yeast extract 5g, NaCl 10g)	
Distilled water	1000ml

Autoclaved and stored at 37°C.

2. **LB agar**

LB agar powder	40g
(Casein hydrolysate 10g, Yeast extract 5g, NaCl 10g, Agar 15g)	
Distilled water	1000ml

Heated to melt agar in the water and then autoclaved.

3. **1M Isopropyl thio-β-D-galactoside (IPTG)**

IPTG	0.238g
Distilled water	1 ml

Filter sterilized and stored at -20°C. For working 1M IPTG was diluted 10 times.

A6. Solution for Protein Purification

1. **Lysis buffer (5ml)**

Tris-HCl-pH 7.5	100 mM
NaCl	150 mM
ß-Mercaptoethanol	5 mM

Glycerol	20 %
PMSF	1mM
Protease inhibitor cocktail	1 %
Benzonase	25 U ml^{-1}
Triton X -100	1 %
Imidazole	10 mM
Lysozyme	0.5 mg ml^{-1}

2. Equilibrium buffer

Tris -HCl - pH 7.5	100 mM
NaCl	150 mM
ß - Mercaptoethanol	5 mM
Glycerol	20 %
Imidazole	10 mM

3. Wash buffer

Tris -HCl - pH 7.5	100 mM
NaCl	150 mM
ß - Mercaptoethanol	5 mM
Glycerol	20 %
Imidazole	40 mM

4. Elution buffer

Tris -HCl - pH 7.5	100 mM
NaCl	150 mM
ß - Merc aptoethanol	5 mM
Glycerol	20 %
Imidazole	500 mM

5. Dialysis buffer

Tris -HCl - pH 7.5	50 mM
NaCl	150 mM

Dialysis was performed overnight at 4 °C with thrice buffer change

A7. Solutions for SDS PAGE

1. 30 % acrylamide stock solution

Acrylamide	29.2 g
N'- N'- bis - methylene acrylamide	0.82 g
Distilled water up to	100 ml

Filter the solution through Whatman No 1 filter paper and stores at 4ºC in a dark bottle.

2. 10% ammonium per sulphate

Ammonium per sulphate	100 mg
Distilled water	1 ml

The solution was di scarded after 7 days and prepared freshly.

3. SDS running buffer

SDS	1 g
Glycine	14. 4 g
Tris buffer	3.03 g
Distilled water up to	1000 ml

4. 4 x Sample buffer

Glycerol	40 ml
SDS	8 g
Tris buffer (pH 6.8)	2.9 g
Bromophenol blue	0.040 g
Distilled water up to	100 ml
ß Mercaptoethanol (Freshly added)	2.5%

pH is adjusted with 1N NaOH/HCl to 6.8 and stores at RT

Working solution will be prepared as 1X by diluting the 4X sample buffer in water

5. Staining solution

Coomassie brilliant blue R -250	250 mg
Methanol	40 ml
Acetic acid	10 ml
Distilled water upto	50 ml

First the dye was dissolved in methanol and then acetic acid and water were added.

The entire mixture was stirred. Finally the staining solution was passed through Whatman No 1 filter paper.

6. Destaining solu tion

Methanol	40 ml
Glacial acetic acid	10 ml
Distilled water upto	50 ml

7. Resolving gel (12%) 10 ml

Distilled water	3.3 ml
30% acrylamide	4 ml
1.5M Tris (pH - 8.8)	2.5 ml
10% SDS	0.1 ml
10% APS	0.1 ml
TEMED	0.004 ml

8. Stacking gel (5%) 4 ml

Distilled water	2.7 ml
30% acrylamide	0.67 ml
1 M Tris (pH - 6 .8)	0 .5 ml
10% SDS	0. 04 ml
10% APS	0. 04 ml
TEMED	0.004 ml

ANNEXURE - 2 (LIST OF PUBLICAT IONS)

Poster presented:

- Gupta B, Chakraborty S, Chaudhury A. Therapeutic potential of shikonin in pain via sodium channel modulation. 14 th - 17 th Nov 2013. 1st w orld symposium of the world institute of the pain foundation and 3rd ICIPM of world institute of Indian section. Kolkata, West Bengal, India.

Research articles:

- Gupta B, Chakraborty S, Saha S, Chandel SG, Baranwal AK, Banerjee M, Chatterjee M, Chaudhury A (2016). Antinociceptive properties of shikonin: in vitro and in vivo studies. Can. J. Physiol. Pharmacol 94: 788 - 96. [doi: 10.1139/cjpp - 2015 - 0465]. Epub 2016 Mar 6. pISSN : 0008 - 4212 (Print), eISSN 1205 - 7541 (Online).

- Gupta B, Chakraborty S, Chaudhury A (2016). Identification of novel targets for shikonin in inflammation and cancer. Pharmacologia 7: 350 - 360. [DOI: 10.5567/pharmacologia.2016.350.360]. pISSN: 2044 - 4648 (Print), eISSN: 2044 - 4656 (Online).