1. Chemicals & Strains

All the chemicals used in this work were of ultra pure grade and purchased from *Sigma-Aldrich, St. Louis, MO-63103, USA.* Growth media contents were from *HiMedia laboratories, Mumbai, India.* Ortho-phosphoric acid for Bradford reagent was purchased from *Qualigens India Ltd., Mumbai, India.*

Plasmid Isolation kits and PCR gel extraction kits were from *Macherey-Nagel.*

Protein purification materials, columns, column materials and molecular biology kits were obtained from *Amersham Biosciences* (Part of *GE* healthcare), *UK.*

All the restriction enzymes were from *New England biolabs (UK) Ltd., Herts, SG4 0TY, UK.* T4 polynucleotide kinase was purchased from *Amersham Biosciences* (Part of *GE* healthcare), *UK.*

γ-^{32}P ATP was purchased routinely from *BRIT* (*Board of radiation and isotope technology*), *BARC, Mumbai, India.*

T7 based expression vectors pET21d, pET28a, bacterial strain BL21 (DE3) and other strains to aid cloning and expression were from *Novagen, EMD Biosciences Inc., Madison, WI-53719, USA,* pRSET B expression vector was from *Invitrogen Life Technologies,* while T5 promoter based expression vector pQE60 was from *Qiagen GmbH, Hilden, Germany.*

Centricons were purchased from *Amicon, Beverly, MA-01915, USA.* Hyperfilm-XP for autoradiograms was purchased from *Amersham Biosciences* (Part of *GE* healthcare), *UK.*

Primers were synthesized from *Sigma-Genosys, The woodlands, TX-77380, USA.* Lyophilized samples were dissolved in TE to make a 100 nM stock.

2. Reagents and Composition

All purification buffers, as mentioned in section 4, were properly filtered with 0.22 μm filters and degassed as per the guidelines of the columns manufacturers.

A. Luria-Bertani (LB) media

Tryptone 10 g, Yeast extract 5 g, NaCl 10 g in 950 ml of deionized water. The contents are dissolved, made-up and sterilized by autoclaving for 20 min at 15 lb/in^2.

For solid LB, 1.5% agar is added before autoclaving.

B. Bradford Reagent

For 100 ml

Brilliant blue G 250	10 mg
85% Ortho phosphoric acid	10 ml
Absolute ethanol	5 ml

10 mg G-250 was dissolved in 5 ml ethanol and then thoroughly mixed with 10 ml ortho-phosphoric acid. Volume was made up to 100 ml with TDW and solution was filtered through whatman filter no.1 and kept in brown bottles.

C. Tris Borate EDTA (10X)

For 1000 ml

Tris	108 g
Boric acid	55 g
0.5 M EDTA	40 ml

D. SDS PAGE

(i) Resolving gel (5 ml)	10%	12%
H_2O	1.9 ml	1.6 ml
30% Acrylamide	1.7 ml	2.0 ml
1.5 M Tris, pH 8.8	1.3 ml	1.3 ml
10% SDS	0.05 ml	0.05 ml
10% APS	0.05 ml	0.05 ml
TEMED	0.002 ml	0.002 ml

(ii) Stacking gel	2 ml
H_2O	1.4 ml
30% Acrylamide	0.33 ml
1.0 M Tris, pH 6.8	0.25 ml
10% SDS	0.02 ml
10 APS	0.02 ml
TEMED	0.002 ml

E. PAGE running buffer (1X)

Tris	3.02 g
Glycine	18.8 g
10% SDS	10.0 ml

F. Native PAGE (5%)

Gel volume	50 ml
30 % Acrylamide	8.33 ml
10 X TBE	2.5 ml
10% APS	1.0 ml
TEMED	0.05 ml

Volume was made up with TDW.

0.25 X TBE was used as running buffer. Gels were run at 150 volts.

G. Native gel loading dye

Sucrose	40% (w/v)
Bromophenol blue	0.25% (w/v)

H. 5X SDS Loading dye **10ml**

50% Glycerol	5.25 ml (60%)
250 mM Tris pH 6.8	2.5 ml
500 mM β-Me	0.340 ml
10% SDS	1 g
0.5% Bromophenol Blue	0.05 g
MQ	1.91 ml

I. Staining Solution

Brilliant blue R 250	0.25%
Methanol	45%
Acetic acid	10%
Water	45%

J. Destaining Solution

Methanol	45%
Acetic acid	10%
Water	45%

K. Antibiotics Stock

100 mg/ml and 50 mg/ml stock solutions for ampicillin and kanamycin were prepared in TDW and filtered with 0.22 μM syringe filters (*Millipore*, Massachusetts, USA).

L. IPTG & PMSF

1 M stock for isopropyl-beta-D-thiogalactopyranoside (IPTG) was prepared each time in TDW and filtered with 0.22 μM syringe filters and stored at -20°C. 200 mM stock of Phenyl methyl sulphonyl fluoride (PMSF) was made in Isopropanol and stored at -20°C.

3. Gel drying and development of autoradiogram

All the native PAGE gels were dried on a model 583 Gel drier (*Bio-Rad*, CA, USA) at 65°C for 2 hrs. Dried gels were exposed to hyperfilm XP for an appropriate time period and developed as per standard protocol (Sambrook *et al.*, 1989).