PREPARATION OF MEDIA AND REAGENTS

LB Agar (per Litre)
- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- 20 g of agar
- Add deionized H₂O to a final volume of 1 liter
- Adjust pH to 7.0 with 5 N NaOH
- Autoclave
- Pour into petri dishes (~25 ml/100-mm plate)

LB–Kanamycin Agar (per Litre)
- Prepare 1 litre of LB agar
- Autoclave
- Cool to 55°C
- Add 5 ml of 10-mg/ml, filter-sterilized kanamycin
- Pour into Petri dishes (~25 ml/100-mm plate)

LB–Ampicillin Agar (per Litre)
- 1 litre of LB agar, autoclaved
- Cool to 55°C
- Add 10 ml of 10-mg/ml filter-sterilized ampicillin
- Pour into Petri dishes (~25 ml/100-mm plate)

LB–Kanamycin Broth (per Liter)
- Prepare 1 litre of LB broth
- Autoclave
- Cool to 55°C
- Add 50 mg of filter-sterilized kanamycin

NZY Broth (per Litre)
- 5 g of NaCl
- 2 g of MgSO₄·7H₂O
- 5 g of yeast extract
- 10 g of NZ amine (casein hydrolysate)
- Add deionised H₂O to a final volume of 1 liter
- Adjust the pH to 7.5 with NaOH
- Autoclave
NZY Top Agar (per Liter)

- Prepare 1 liter of NZY broth
- Add 0.7% (w/v) agarose
- Autoclave
- All purification buffers were properly filtered with 0.22 \( \mu \)m filters and degassed as per the guidelines of the column manufacturers.

Bradford Reagent

For 100 ml
Coomassie 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.

Tris Acetic acid EDTA (50X)

For 1000 ml
Tris 242 g
Acetic acid glacial 57.1 ml
0.5 M EDTA 100 ml

SDS PAGE (10%)

**Resolving gel** 5 ml

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
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<tbody>
<tr>
<td>H₂O</td>
<td>1.9 ml</td>
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<tr>
<td>30% Acrylamide</td>
<td>1.7 ml</td>
</tr>
<tr>
<td>1.5 M Tris, pH 8.8</td>
<td>1.3 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>0.05 ml</td>
</tr>
<tr>
<td>10% APS</td>
<td>0.05 ml</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.002 ml</td>
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</table>

**Stacking gel** 2 ml

<table>
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<th>Component</th>
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<tbody>
<tr>
<td>H₂O</td>
<td>1.4 ml</td>
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<tr>
<td>30% Acrylamide</td>
<td>0.33 ml</td>
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<tr>
<td>1.0 M Tris, pH 6.8</td>
<td>0.25 ml</td>
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<tr>
<td>10% SDS</td>
<td>0.02 ml</td>
</tr>
<tr>
<td>10% APS</td>
<td>0.02 ml</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.002 ml</td>
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</table>

**PAGE running buffer (1X)**

<table>
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<th>Volume</th>
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<tbody>
<tr>
<td>Tris</td>
<td>3.02 g</td>
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<tr>
<td>Glycine</td>
<td>18.8 g</td>
</tr>
</tbody>
</table>
10 % SDS  
10.0 ml

Native gel loading dye

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

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 absolute ethanol and stored at -20°C.
Research Publications


Abstracts Published

