## MEDIA AND REAGENTS

### ASN III Medium

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>25.0g</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>2.0g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.5g</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>0.75g</td>
</tr>
<tr>
<td>K₂HPO₄.3H₂O</td>
<td>0.02g</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>3.5g</td>
</tr>
<tr>
<td>CaCl₂.2H₂O</td>
<td>0.5g</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.003g</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.003g</td>
</tr>
<tr>
<td>EDTA (disodium salt)</td>
<td>0.0005g</td>
</tr>
<tr>
<td>Na₂CO₃.H₂O</td>
<td>1.0ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

#### Trace metal mix

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₃PO₃</td>
<td>2.86g</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>1.81g</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>0.222g</td>
</tr>
<tr>
<td>Na₂MoO₄.2H₂O</td>
<td>0.390g</td>
</tr>
<tr>
<td>CuSO₄.5H₂O</td>
<td>0.079g</td>
</tr>
<tr>
<td>Co (NO₃)₂.6H₂O</td>
<td>0.0494g</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
</tr>
</tbody>
</table>

### PEPTONE BROTH

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>4.0g</td>
</tr>
</tbody>
</table>
pH 7.2

**MANNITOL SALT AGAR**

- Beef extract 1.0g
- Peptone or polypeptide 10.0g
- Sodium chloride 75.0g
- Mannitol 10.0g
- Phenol red 0.025g
- pH 7.4
- Agar 15.0g

**BLOOD AGAR**

- Nutrient Agar 1000ml

After sterilization add 50ml of defibrinated sheep blood

**BLOOD AGAR BASE**

- Beef heart muscle infusion 375.0g
- Tryptose or peptic digest of animal tissue 10.0g
- Agar 15.0g

Sterilize, cool to 50° C and add 5% of defibrinated sheep blood

**MUELLER HINTON AGAR**

- Beef infusion 300ml
- Casein hydrolysate 17.5g
- Starch 15g
- Agar 15.0g
- pH 7.4
LURIA BERTANI BROTH

Bacto tryptone 17.0g
Yeast extract 5.0g
Sodium chloride 10.0g
Distilled water 1000ml

Add the ingredients to 950ml of deionized water stir for complete solubility. Adjust pH to 7.0 with 5N NaOH. Adjust the volume to 1000ml with deionized water sterilize by autoclaving for 20mins at 15lbs.

REAGENTS

Reagent for DNA isolation

SDS
Phenol
Chloroform
95% ethanol
Sodium acetate
TE Buffer
Agarose
TAE Buffer
Ethidium bromide

Reagent for SDS PAGE

Stock acrylamide solution

Acrylamide 30.0%
Bisacrylamide 0.8%

Make up to 100ml with double distilled water and filter (it is light sensitive, store it in an amber coloured bottle)

Separating gel 4X (pH 8.8)

Tris HCL 1.5M
Adjust the pH with 2N HCL (Take 4.16ml and make to 25ml)
Stacking gel buffer 4X (pH 6.8)

Tris HCL 0.5M
Dissolve 3.02g, adjust pH with HCL and make it up to 50ml and store at 4° C

Polymerizing agent

Ammonium persulphate 10%
TEMED (it is added for activation of polymerization)
SDS 10%
Dissolve 5g in 50ml and keep it at 40° C for half a day. Store at room temperature.

Electrode buffer 5X (pH 8.4-8.8)

Tris HCL 0.25M
Glycine 1.92M
SDS 10%
Water 500ml

Sample buffer 4X

Tris HCL 0.5M
SDS 10%
Glycerol 40%
2-mercaptoethanol 20%
Bromophenol blue 0.5%
Store frozen in small aliquots and diluted to 1X concentration just before use.

Destaining solution

Methanol (40%) 40ml
Acetic acid (10%) 10ml
Distilled water 50ml

Staining solution

Coomassie brilliant blue R 250 (1%) 100mg dissolved in destaining solution.
(First the dye was dissolved in methanol and proceeded use fresh preparation every time)
Note: Acrylamide, bisacrylamide, buffer and water can be prepared in large batches and
either stored at 4° C for one month or frozen in aliquots and used indefinitely. Remove the required amounts, warm to room temperature, and add ammonium per sulphate and TEMED before use only.

**McFarland’s standard**

The turbidity of the standard is equivalent to overnight broth culture.

1. Prepare 1% sulphuric acid solution.
2. Prepare 1.175% solution of barium chloride by dissolving 2.35g of dehydrates barium chloride (BaCl₂.2H₂O) in 200ml of distilled water.
3. To make the turbidity standard, add 0.5ml of sulphuric acid solution and mix. The standard can be stored in dark at room temperature for up to 6 months.