Luria Broth Base, Miller's Modification

**Intended Use**
Recommended for the cultivation and maintenance of recombinant strains of *Escherichia coli* with or without addition of glucose.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0.500</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Direction**

Suspend 15.5 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired add 10 ml of 20% glucose solution. Mix thoroughly and pour into sterile test tubes or flasks.

**Principle And Interpretation**

This medium is based on original Luria broth formula described by Miller for the growth and maintenance of *E.coli* strains used in molecular microbiology (4). Luria broth Base, Miller is a nutritionally rich medium recommended for growth of pure cultures of recombinant strains. *E.coli* is grown in late log phase in LB medium. Some plasmid vectors may replicate to high copy numbers without selective amplification. Some vectors do not replicate so freely, and need to be selectively amplified. Chloramphenicol can be added to inhibit host synthesis and as a result prevent replication of the bacterial chromosome. (5) Luria Broth Base, Miller’s modification contains one tenth and one twentieth the sodium chloride level of the Lennox and Miller formulations of LB Agar respectively(3,4,5).This helps the user to select the optimal salt concentration for a specific strain. The medium may be aseptically supplemented with glucose, if desired.

Tryptone provides peptides and peptones while Vitamin B complex is provided by yeast extract. Sodium chloride provides sodium ions for membrane transport and also maintains the osmotic equilibrium of the medium.

**Type of specimen**
Recombinant strains of *E.coli*

**Specimen Collection and Handling**

ForRecombinant strain samples follow appropriate techniques for handling specimens as per established guidelines (3,4,5). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations**

1. Some strains may show poor growth due to nutritional variations.
2. Further biochemical and serological tests must be carried out for complete identification.

**Performance and Evaluation**

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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Please refer disclaimer Overleaf.
Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of Prepared Medium
Yellow to amber coloured clear to slightly opalescent solution

Reaction
Reaction of 1.55% w/v aqueous solution at 25°C, pH: 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Inoculum (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli ATCC 23724</td>
<td>luxuriant</td>
<td>50-100</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>luxuriant</td>
<td>50-100</td>
</tr>
<tr>
<td>Escherichia coli DH5 alpha MTCC 1652</td>
<td>luxuriant</td>
<td>50-100</td>
</tr>
</tbody>
</table>

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference
3. Lennox E.S. 1955, Transduction of Linked Genetic Characters of the host by bacteriophage P1., Virology, 1:190.

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