APPENDIX
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Haemocytometer for cell counts/spore counts

Each square represents a total volume of 0.1m³ and 1cm³ is equal to 1ml. The subsequent cell concentration will be determined using the following calculations.

\[
\text{Cells/ml} \rightarrow \text{Average count/sq} \times \text{Dilution Factor} \times 10^4 (\text{count 1 sq})
\]

Total cells \rightarrow \text{cells/ml x original volume}

(* Do not count cells touching the middle line at bottom and right)

Precautions to have RNAse free

Bake glassware @ 180°C for 8hrs. Rinse with chloroform, immerse in DEPC (0.1%) which is a strong but not absolute inhibition of RNAse. Allow DEPC filled glassware or plastic ware to stand for 2hrs @ 37°C and then rinse several times with sterile water and then heat to 100°C for 15 min or autoclave for 15 min @15lb/sq in on liquid cycle. Which removes traces of DEPC that might otherwise modify residue in RNA carboxymethylation. The efficiency of translation is very low in cell free synthesis of carboxymethylated RNA.

Electrophoresis tanks

Clean with detergent solution, rinse in water, dried with ethanol and fill with a solution of 3% H₂O₂ and the tanks should be rinsed with water that has been treated with 0.1% DEPC.

Antibiotic Solutions

- Sterilize antibiotic solutions by filtration through a 0.2 um millipore membrane.
- Store solutions in light-tight containers.
- Do not add tetracycline to media with magnesium salts, since magnesium ions are antagonists of tetracycline.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>1,000 X Concentration</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>50 mg/ml in H₂O</td>
<td>-20°C</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>50 mg/ml in H₂O</td>
<td>-20°C</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 mg/ml in H₂O</td>
<td>-20°C</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>10 mg/ml in H₂O</td>
<td>-20°C</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10 mg/ml in H₂O</td>
<td>-20°C</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>5 mg/ml in H₂O</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
Cefotaxime Preparation

Cefotaxime is a white to off-white powder which is freely soluble in water. Variations in color of the freshly prepared solutions do not necessarily indicate changes in potency. Store this product in an airtight container protected from light. Aqueous solutions of cefotaxime at a pH of 4.3-6.2 are stable for 14-21 days when stored at 0-5°C. Cefotaxime is most effective against gram-negative bacteria.

Codon Table

http://psyche.uthct.edu/shaun/SBlack/geneticd.html