**ABSTRACT**

*Vibrio natriegens* grows well in media containing v2 salts e.g. BHI + v2 and LBv2 ([link](#)). Furthermore, such media is routinely used for culturing this organism (Weinstock et al., 2016). However, care must be taken during preparation not to autoclave v2 salts and media together. This protocol first generates separate solutions before sterilising and combining them.


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Version created by Matthew Haines

**KEYWORDS**

Vibrio natriegens, Model prokaryotes, Synthetic biology

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MATERIALS TEXT

MATERIALS

- Sodium chloride Contributed by users
- Potassium Chloride Contributed by users
- Brain Heart Infusion Broth Dry

Medium Teknova Catalog #B9500 or
- LB-Broth Miller (= LB mix) Formedium Catalog #LMM0104
- Magnesium chloride hexahydrate Contributed by users

For certain reagents alternative suppliers are available and no supplier is endorse.

Prepare stock salt solutions

1. Prepare the following salt solutions at the given concentrations:
   - 5 Molarity (M) NaCl
   - 1 Molarity (M) KCl
   - 1 Molarity (M) MgCl2.6H2O

Prepare media

2. Dissolve 18.5 g BHI dry medium or 12.5 g LB Broth (Miller) in 400 mL ddH2O in a 1 L graduated bottle.

Sterilise and combine

3. Sterilise all solutions by autoclaving.

4. Under sterile conditions, transfer the following volumes of stock salt solutions to the BHI media:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Stock solution (M)</th>
<th>Volume (mL)</th>
<th>Final concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>5</td>
<td>20.4</td>
<td>204</td>
</tr>
<tr>
<td>MgCl2.6H2O</td>
<td>1</td>
<td>11.6</td>
<td>23.2</td>
</tr>
<tr>
<td>KCl</td>
<td>1</td>
<td>2.1</td>
<td>4.2</td>
</tr>
</tbody>
</table>

5. Adjust the volume to 500 mL using sterile ddH2O.

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