

## A. Medium Preparation

1	Preparation of Liquid LB Medium	<ol style="list-style-type: none"> <li>① calculate the required volume of liquid culture medium and the quality of the added substances;</li> <li>② take a conical flask;</li> <li>③ add 1% NaCl, 1% peptone, 0.5% yeast extract, and the required volume of deionized water;</li> <li>④ remove the sub-instrument and sub-tubes marked with LB;</li> <li>⑤ dispense liquid medium, small tubes carry 5mL, large tubes carry 7mL.</li> </ol>
2	Preparation of Liquid M9 Medium	<ol style="list-style-type: none"> <li>① calculate the required volume of liquid culture medium and the quality of the added substances;</li> <li>② 0.6% Na<sub>2</sub>HPO<sub>4</sub>, 0.3% KH<sub>2</sub>PO<sub>4</sub>, 0.05% NaCl, 0.1% NH<sub>4</sub>Cl, 0.05% MgSO<sub>4</sub>, 0.2% glucose, 0.0015% CaCl<sub>2</sub>.</li> </ol>
3	Preparation of Solid LB Medium	<ol style="list-style-type: none"> <li>① calculate the volume of solid culture medium required and the quality of the added substance;</li> <li>② take a conical flask;</li> <li>③ add 1% NaCl, 1% peptone, 0.5% yeast extract, 1.5% agar and the required volume of deionized water;</li> <li>④ seal the conical flask with a sealing film.</li> </ol>
4	High Temperature Sterilization	<ol style="list-style-type: none"> <li>① open the high pressure steam pot;</li> <li>② make sure the water is over the hole;</li> <li>③ put a iron basket in the pot, then place the medium in the iron basket, and covered with iron cover;</li> <li>④ close the steam pot, close the exhaust valve;</li> <li>⑤ adjustment mode for the "sterilization", press the start button 4s;</li> </ol>

		⑥ sterilization at least 2h.
5	Cool Down	<p>① close the high pressure steam pot;</p> <p>② check the air pressure is reduced to 0, if not, then continue to wait;</p> <p>③ if so, then open the exhaust valve, open the steam pot when the gas is released;</p> <p>④ place the liquid medium in the beaker, stored at room temperature;</p> <p>⑤ put solid medium on the table to cool;</p> <p>⑥ Wait for the solid medium to cool to 60 ° C.</p>
6	Add Antibiotics	<p>① calculate the amount of chlorogenic or ampicillin, add chloramphenicol 2 ‰, or ampicillin 1 ‰;</p> <p>② remove and ignite the alcohol lights;</p> <p>③ use sterile tips to add antibiotics in the medium beside the alcohol lights.</p>
7	Preparation of the Solid Culture Dishes	<p>① next to the alcohol lamp, open the culture dishes;</p> <p>② gently pour the solid medium liquid to one half of the volume of a solid culture dish;</p> <p>③ shock culture dishes lightly to make the liquid evenly distributed;</p> <p>④ repeat steps to complete all solid medium.</p>
8	Refrigeration	<p>① at room temperature, standing for about 20min;</p> <p>② after the solid medium is solidified, wrap these dishes up</p> <p>③ invert in the 617 room 4 °C freezer storage.</p>