**LB Agar Plates**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Water** | **100 ml** | **250 ml** | **300 ml** | **500 ml** | **1000 ml** |
| **Yeast Extract** | 0.5 g | 1.25 g | 1.5 g | 2.5 g | 5.0 g |
| **Tryptone** | 1.0 g | 2.5 g | 3.0 g | 5.0 g | 10.0 g |
| **NaCl** | 0.5 g | 1.25 g | 1.5 g | 2.5 g | 5.0 g |
|  |  |  |  |  |  |
| **Bacto Agar** | 1.5 g | 3.75 g | 4.5 g | 7.5 g | 15 g |

 *Note: For convenience, all media ingredients are kept in the same*

*location in the chemical cabinet, regardless of name. They are in the*

*right-hand cabinet, about half way down the right side of shelves.*

*Note: 300 ml of media will make about 15 plates when poured*

*conservatively -- more if you pour them very thinly.*

Making media

Add designated amount of water to appropriately sized beaker.

Add Yeast Extract, Tryptone, and NaCl.

Stir until dissolved.

Adjust pH to 7.0 with 1M NaOH (made fresh monthly).

Transfer to orange capped bottle (put stir bar into bottle as well)

Add Bacto Agar and stir for 1-2 minutes to mix.

Turn on 50o C water bath while stirring (and check water level in bath).

Autoclave for 20 min (30 min for 1L media) on liquid cycle.

Pouring plates (we want the media at 50o C when pouring; too hot will kill the antibiotic)

After autoclaving, place bottle into 50o C water bath for 20 minutes (minimum) to cool.

While media is cooling, prepare plates in the hood

 (mark with date, media type, and antibiotic designation).

Thaw out antibiotic stock and warm to room temp while media is cooling.

After cooling, remove bottle and place on stir plate with moderate stirring.

Add antibiotic and allow to mix for about 1 minute.

Pour media into plates in hood.

Arrange plates and remove lids about half way.

Allow plates to dry in hood for 20-30 min.

Replace lids and store plates in plastic wrap or bag at 4o C.