Jun 30, 2023

LB + Mg + Glucose for better Gram-N growth (agar + antibiotics possible) 1L

DOI
dx.doi.org/10.17504/protocols.io.6qpvr3o9bvmk/v1

Dylan brettingham[1,2]

[1]University of Guelph; [2]iGEM Guelph 2023

D Dylan brettingham

DOI: https://dx.doi.org/10.17504/protocols.io.6qpvr3o9bvmk/v1

Protocol Citation: Dylan brettingham 2023. LB + Mg + Glucose for better Gram-N growth (agar + antibiotics possible) 1L. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr3o9bvmk/v1

Protocol status: Working
We use this protocol and it's working

Created: June 30, 2023

Last Modified: June 30, 2023

Protocol Integer ID: 84310

Keywords: glucose for better gram, 1l lb media with additive, glucose in the original formulation, glucose, concentration the gram, cell growth, thegrowth of gram, 1l lb media, lps synthesis, negative microbe, gram, growth, antibiotic, additive, better gram, bacteria, original formulation

Abstract

LB media with additives to improve thegrowth of gram-negative microbes. LB is Mg defficeint and some of the proteins invovled in LPS synthesis require Mg as a co-factor, so by increasing the concentration the gram-N bacteria will be able to grow faster. LB also used to have glucose in the original formulation and this was removed, but the media is also pretty carbon starved and the glucose will simply aid in cell growth.

Blog Small Things Considered has a good post about this:
https://schaechter.asmblog.org/schaechter/2009/11/the-limitations-of-lb-medium.html

Guidelines

- Media out of the autoclave is very hot! Be careful
- The ingredients are fine powders for the most part and should not be inhaled

Troubleshooting

LB-2X media

1 Weigh out ingredients and add to clean bottle
- 10g Tryptone
- 5g Yeast Extract
- 5g NaCl (this is for Lennox formulation, 10g can be used for Miller Formulation)
- 2.46g MgSO4 * 7H20 (10mM)
- 10g D-glucose (1%)
+ 15g Agar if making plates (1.5%)

2 - Add stir bar to bottle and 1000mL of ddH20
- Stir until any chunks are dissolved
- Autoclave at temp (121C) for 40 minutes
  - If adjusting recipe to 500mL or below 30min autoclave is enough

3 Let cool (putting on a stir plate speeds this up) before pouring plates using aseptic technique
  - Can be poured in a laminar flow hood or biosafety cabinet
  - Heating the lip of the bottle well before pouring will help in preventing bubbles (they get popped as they pass over the hot lip)
  - Shaking the bottle will cause bubbles, try and not do this, use the stir plate
  - The thickness of the plates will affect how long they can be used for growth until they begin to dry, the thicker the longer they will last but will use more material
- If adding antibiotics, add them just prior to pouring not to the hot media out of the autoclave
  eg: if wanting 25ug/mL chloramphenicol in final media, add 1mL of 25mg/mL stock solution

4 Plates should be left out overnight and checked for growth the next day to be sure they are sterile
  - Labelling plates with media type before putting themaway will make life easier in the future!

Lightly spraying and wiping down outsdie of plates with 70% ethanol will help keep them sterile while being stored

Put plates away in the fridge (4C)
  - They will keep well for a month, and ive successfully used plates much older than that but its best to only make as much as you will use in a month

- If antibiotics were added the 1 month limit is more strict as the antibiotic will loose efficacy over time