MAR 08, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bp2l69n3rlqe/v1

Protocol Citation: Alfonso Pérez Escudero, Alid Al-Asmar, gabrielmadirolas 2023. Preparation of 1L of Foraging Medium. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l69n3rlqe/v1

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

Created: Sep 27, 2022

Last Modified: Mar 08, 2023

PROTOCOL integer ID:
70548

Preparation of 1L of Foraging Medium

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ABSTRACT

Protocol is mainly inspired from: WormBook Methods http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html.
This protocol is for making medium for foraging experiments with the worm *Caenorhabditis elegans*. The medium has the same basis as the standard Nematode Growth Medium (NGM), but contains no peptone (as no long-term worm growth is required for our experiments), and contains antibiotics that prevent our bacterial strains from growing (bacteriostatic concentrations).
It describes both medium preparation and pouring.

MATERIALS

Agar powder:

Agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1296-10KG**

Cholesterol:

Cholesterol powder **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C3045-5G**

Absolute ethanol:

Ethanol absolute ≥99.8% AnalaR NORMAPUR® ACS Reag. Ph. Eur. Analytical Reagent **VWR International Catalog #20821.310-1L**

Sodium chloride (NaCl):

Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S5886-1KG**

Peptone: Peptone (Bacto) **Becton Dickinson (BD) Catalog #211677-500G**

Chloramphenicol:

Chloramphenicol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #CO378 25G**

Novobiocin:

Novobiocin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #N1628-5G**

Tissue Culture Dishes:

100x20mm:

Falcon® 100 mm TC-treated Cell Culture Dish **Corning Catalog #353003**

60x15mm:

Petri Dishes 60x15mm with vent **greiner bio-one Catalog #628102**

35x10mm: Plastic petri dish 35 x 10 mm style **Falcon Catalog #353001**

1 In order to perform this protocol, you will need the following solutions:

- 5 mg/mL cholesterol in absolute ethanol (stored in freezer)
- pH=6 phosphate buffer (as prepared in: https://www.protocols.io/view/preparation-of-0-5l-of-phosphate-buffer-ph-6-0-n2bvj8r7bgk5/v1)
- 1 M magnesium sulfate (as prepared in: https://www.protocols.io/view/preparation-of-1m-magnesium-sulfate-solution-mgso4-ca4rsgv6)
- 1 M calcium chloride (as prepared in: https://www.protocols.io/view/preparation-of-1m-calcium-chloride-solution-cacl2-b8parvie)
- 10 mg/mL chloramphenicol in absolute ethanol (stored in freezer)
- 50 mg/mL novobiocin in milliQ water (filter-sterilized and then stored in freezer, in 1.8 mL aliquots to avoid defreezing-refreezing the whole stock)

2 Add 16 g ±0.2 of agar powder to a 1L clean bottle.

3 Add 2.32 g ±0.02 of sodium chloride (NaCl).

4 Add 780 mL of milliQ water.

5 Add a clean magnet (for stirring) and autoclave.

6 Put the bottle on a stirrer, and wait until it cools down to around 60°C. At this temperature, the bottle should feel very hot to the touch, but one can hold it for a while without feeling too uncomfortable. The agar should still be liquid.

7 While the mix is being stirred, do the following steps in that order, dispensing the reagents right above the surface (to avoid bubbles) with a sterile pipette:

8 Add 20 mL of phosphate buffer as prepared in https://www.protocols.io/view/preparation-of-0-5l-of-phosphate-buffer-ph-6-0-n2bvj8r7bgk5/v1. This should be added first to prevent any unnecessary precipitation of the next reagents. Wait for ~20 seconds after adding the buffer to give it time to mix well and stabilize the pH.

9 Add 0.8 mL of 1M magnesium sulfate ($MgSO_4$) solution as prepared in https://www.protocols.io/view/preparation-of-1m-magnesium-sulfate-solution-mgso4-ca4rsgv6.

10 For this step only, you can put the pipette right below the surface of the agar, otherwise cholesterol tends to stick to the surface.
Add 0.8 mL of cholesterol (5 mg/mL in absolute ethanol, stored in freezer).

11 Add 0.8 mL of 1M calcium chloride ($CaCl_2$) solution as prepared in https://www.protocols.io/view/preparation-of-1m-calcium-chloride-solution-cacl2-b8parvie. This step should make the medium a bit cloudier, but remain translucent.

12 Add 0.8 mL of chloramphenicol (10 mg/mL in absolute ethanol, stored in freezer).

13 Add 1.6 mL of novobiocin (50 mg/mL in milliQ water, stored in freezer).

14 Pour in petri dishes before it solidifies, using a pipette and trying to have the same volume in all dishes. We usually pour the plates on the bench, but to minimize the chance of contamination one can pour them in a microbiology hood.
These are our usual volumes:
For 100x20mm (diameter x height) petri dishes, we pour 18 mL.
For 60x15mm petri dishes, we pour 8 mL.
For 35x10mm petri dishes, we pour 2 mL.

15 We store our experimental plates at room temperature for 2 to 8 days. For longer storage and better plate quality, the plates are sometimes stacked in boxes that are sealed with a plastic bag, in order to reduce evaporation.