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We use this protocol and it's working

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Preparation of 1L of Foraging Buffer

Alfonso Pérez Escudero[1], Alid Al-Asmar[1], gabrielmadirolas[1]

[1]Research Centre on Animal Cognition (CRCA), Centre for Integrative Biology (CBI), Toulouse III University, CNRS, Toulouse, France

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ABSTRACT

Protocol is mainly inspired from: WormBook Methods http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html.
We use this protocol for our foraging experiments with the worm *Caenorhabditis elegans*, using different strains of bacteria.
This protocol describes how to produce the buffer we use to wash our bacteria after growing them in LB. The buffer has a similar composition as our Foraging Medium, but contains no cholesterol. It contains antibiotics at bacteriostatic concentrations (for our bacterial strains), in order to prevent bacteria from multiplying after they have been washed.

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:

- 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 2.32 g ±0.02 of sodium chloride (NaCl).

3 Add 780 mL of milliQ water.

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

5 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.

6 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:

7 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.

8 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.

9 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.

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

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

12 We store our buffer at room temperature, usually for 2-3 weeks without problem. After this time, it is very common that salts will start precipitating in the bottom of the bottle, but we sometimes use the buffer for a few more weeks if it shows no crystals.