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We use this protocol and it's working

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Preparation of 1L of Nematode Growth Medium (NGM)

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ABSTRACT

Original source of the protocol: WormBook Methods http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html.
This protocol is for making Nematode Growth Medium (NGM), a standard agar-based medium to grow *C. elegans* in petri dishes. It describes both medium preparation and pouring.

MATERIALS

Agar powder: Agar **Sigma Catalog #A1296-10KG**
Cholesterol: Cholesterol powder **Sigma Catalog #C3045-5G**
Absolute ethanol:
Ethanol absolute ≥99.8% AnalaR NORMAPUR® ACS Reag. Ph. Eur. Analytical Reagent **VWR Chemicals Catalog #20821.310-1L**
Sodium chloride (NaCl): Sodium chloride **Sigma Catalog #S5886-1KG**
Peptone: Peptone (Bacto) **Bd Catalog #211677-500G**
Tissue culture dishes (100x20mm):
Falcon® 100 mm TC-treated Cell Culture Dish **Corning Catalog #353003**

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

- 5mg/mL cholesterol in absolute ethanol (do not autoclave) (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)
- 1M magnesium sulfate (as prepared in: https://www.protocols.io/view/preparation-of-1m-magnesium-sulfate-solution-mgso4-ca4rsgv6)
- 1M calcium chloride (as prepared in: https://www.protocols.io/view/preparation-of-1m-calcium-chloride-solution-cacl2-b8parvie)

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

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

4 Add 2 g ±0.02 of peptone.

5 Add 780 mL of milliQ water.

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

7 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.

8 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:

9 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.

10 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.

11 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 (5mg/mL in ethanol, stored in freezer).

12 Add 0.8 mL of 1M calcium chloride (CaCl) 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.

13 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.
We typically use 100x20mm (diameter x height) petri dishes, pouring 18 mL in each.

14 We store our NGM plates at room temperature for 3-4 days. We then flip them to avoid condensation in the lid, and store them at 4°C with a cover on each stack to limit evaporation.