Soil extract for cocultures host-parasites media

Forked from Soil extract for algal media

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We use this protocol and it's working

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ABSTRACT

How to prepare soil extract

MATERIALS

Equipments

Autoclave

Laminar flow cabinet (or biosafety cabinet)

Vacuum pump

Magnetic hot plate stirrers

Materials

Glass fibre prefilters (Millipore, AP1507500)

Filtration unit Stericup GP Merck Millipore, pore size 0.22 µm (Millipore, S2GPU02RE)

BEFORE START INSTRUCTIONS

Please refer to our general recommendations to grow cultures :

https://www.protocols.io/private/A48906DC1374AD6281495CB86A8F092F

Soil sampling

1 Soil is sampled in a pine wood, located in Brittany, France.

To find a correct sampling area in your country, try to find a location devoid of pesticides and pollutants. It should be also a place where you know that you can collect the soil again and later in order to always use the same soil, especially if this one worked.

Collect the soil in plastic boxes.
Back to the laboratory, plastic boxes are placed at 37°C for 12 to 24 hours to let the soil dry. The soil must be dry before use, otherwise the boiling step should be longer.

Preparation of the soil extract solution (25g/l)

2 Weigh 30g of dried soil and place it in a 4L glass erlenmeyer.

3 Add 1200ml of MilliQ water to the soil.
Close the erlenmeyer with a aluminium foil.

4 Heat at 100°C with pemanent mixing during 1 hour to obtain an efficient boiling.
Adjust this time depending on the volume (more volume, more time).
Let cool.

5 Filtrate on 0.2 µm, using a glass prefilter and a pump vacuum.
As the prefilter will be rapidly clogged, better to add the boiled soil 100mL per 100mL.
If this happens, replace the glass prefilter, then the filter.
Transfer the solution in one or several glass bottles.

6 Autoclave at 121°C for 20 minutes.
Place the bottle of the filtered-boiled soil under a safety cabinet, and let it cool.

7 The day after, when the medium is at room temperature, place it at 37°C for 24 to 48 hours.
Then autoclave again at 121°C for 20 minutes.

This step allows to reactivate spores (fungi etc) contained in soil which have not been destroyed by the first sterilization, and to kill them during the second one.

8 Add 50ml of soil extract per 1 L of medium.