

Preparation of Agar medium in petri dishes  
(Level 3 – mushroom expert)

The following formula makes 1 L of “MEA” – malt extract agar medium, convenient for most species of cultivatable mushrooms. Especially for working with spores, antibacterial agar media is recommended. Ready-to-use formulas are offered that only need to be mixed with water.

Recommended equipment:

24 g agar-agar  
20 g malt extract  
2 g dry yeast  
1 g peptone  
1 L drinking water

Erlenmeyer flask  
Sterile disposable petri dishes or glass petri dishes  
Measuring container  
Scale  
Parafilm  
Latex gloves  
Facemask and hairnet  
Disinfectant solutions for surfaces and hands  
Pressure cooker/Autoclave  
Sterile working area: Glove Bag/Glove Box or  
sterile air flow (HEPA-filter, laminar flow hood)

Mixing the substrate

Pour the ingredients into the Erlenmeyer flask along with hand-hot water, close the lid and shake well until mixed thoroughly. The maximum level of filling the flasks is reached at two-thirds of their volume. Remove the lid and wrap some aluminium around the flask's neck, forming a tube which you bend down at its free end. This attached duct will ban condensate to enter the unlocked flask during sterilisation.



#### Sterilisation of the agar medium

Agar medium should be sterilised for 45 minutes using a pressure cooker. Overlying a 2-3 cm water level at the bottom of the cooker, a separating insert keeps the flasks above the water line. Assure yourself that there is no contact between water and the upright standing flasks, decant some water if necessary (mind your pressure cooker's manufacturer informations!).

Get the lid locked and put the cooker on a hotplate. Sterilise for 45 minutes, counting from the moment as the pressure gauge reaches its highest stage (for household pressure cookers). If you've got a professional pressure steriliser at your disposal sterilise at 121°C/ 250°F/ 15 psi/ 1.05 bar.

Attention: Do not exceed temperatures specified above for a longer span of time. By overheating the nutrients, the culture medium can degrade and the solution's colour darkens from yellowish to brownish. If so, the medium is useless. Mind that other compositions than this mixture might have a different colour.

After 45 minutes of sterilisation let the cooker cool off in front of a HEPA-filter or in a clean place. During pressure equalization air is sucked in; a clean and wet cloth over the cooker's cover filters the aspired air, if you do not use a HEPA-filter.



#### Pouring the Petri dishes

Wear protective clothing, disinfect working area and hands. Do not open the cooker before the pressure gauge indicates zero. Continue processing from now on because the agar medium is only in its liquid stage above a certain temperature (If you work with glass petri dishes, do not forget to sterilise them too).

Pour about 25-30 ml of the warm agar medium per petri dish (90 mm in diameter), which gives 35-40 petri dishes per 1 L agar medium. For cooling down leave them in a place of low microbiological contamination, in front of a HEPA-filter or inside a Glove Box.

When the agar has hardened and cooled to room temperature, the petri dishes are ready for inoculation. If you do not use all casted petri dishes, you can seal up the remaining ones with Parafilm, wrap them in plastic foil or bag and keep them in a clean refrigerator for up to 4 weeks. (35.6-39.2°F / 2-4°C)

#### References:

„Mycelium running/ How mushrooms can help save the world“, Paul Stamets; Ten Speed Press, Berkeley/Toronto;

„The Mushroom Cultivator: A Practical Guide to Growing Mushrooms at Home“, Paul Stamets, Agarikon Press; First Edition (December 1983);

„Growing Gourmet and Medicinal Mushrooms“, Paul Stamets, Ten Speed Press, Berkeley/Toronto;