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**Preparing Phytagel Nutrientless Media**

**Rating:**

**Red (requires the presence of at least one supervising adult/advisor)**

The autoclave potion of this lab requires special training. See [here](https://docs.google.com/document/d/1wdfuowrRGsAwny2qLxFeqAjW1A5wEcmRXNA-Eoa_ayI/edit?usp=sharing) for more information.

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### **Introduction:**

This protocol will guide you through the process of preparing nutrient media (phytagel or agar). Media solidifies quickly when cooled so it is a good idea to use the prepared media as soon as possible, preferably right after the end of this protocol. The media prepared in this protocol does not contain sugar and thus makes the oat the only supply of food within the plate.

Estimate time: 1 hour

### **Safety Information:**

*This lab utilizes the autoclave. You cannot use the autoclave unless you have the necessary safety training.* If you are authorized to operate the autoclave, remember to refrain from standing next to the steam vent and use personal protective equipment such as gloves and paper towels. For more information on how to run the autoclave, please reference the [autoclave operation protocol](https://docs.google.com/document/d/1wPfdM4vD_3b_5CwyjAYJELYgKJxf-hC1Y6hnzeNOJ60/edit#).

If there is an emergency call 911 or reference our [list of emergency contacts](https://docs.google.com/document/d/1yHtU0-1RkkxgRz55dj0mR-y-fYJJy3vjRIxNxnydBKg/edit#) [link pending]

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### **Materials:**

* Deionized Water
* Phytagel
* Autoclave bottle
* Autoclave tape
* Autoclave
* Magnetic stir plate and bar
* Graduated cylinder
* Weight scale

**Procedure:**

1. Add phytagel or agar so as to reach a concentration of 10g/L for each bottle.
2. Using a graduated cylinder, measure and pour desired amount of Deionized (DI) water into autoclave bottles: one if making 750mL or less, and two if making more than 750mL (Rule of thumb: 25 ml makes 1 round petri dish and 35 ml makes one square petri dish).
3. Stir with magnetic stir bar and magnetic stirrer until homogeneous.
4. After putting autoclave tape on bottle lids and ensuring the lids are screwed slightly off (to allow for steam to escape). Autoclave bottles for 30 min. Let cool for 15 min before opening autoclave.
5. Let stand until cool enough to handle.
6. Media is now ready to pour to make plates.

**Storage:**

Phytagel solutions solidify when cooled. If the media cannot be used immediately to pour plates, but needs to be used within the day, store it in a warm place such as the heat oven to prevent solidification. If the prepared media needs to be stored for long term, allow it to solidify as it can be heated up at a later date for use.

**Clean up:**

Please be courteous and wash the dishes. Simply rinse all used glassware seven times with distilled water and let dry. For further information look at the instructions above the sink.