Protocol for tube samples

Fluorescent beads in agar

Daniël Cox

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Given volumes are for a final solution of 500× diluted beads, with 4.5 g/L agar. Store in fridge.

1 Requirements

1.1 Ingredients

- Coverslip 0.17 mm × 76 mm × 26 mm
- Capillary glass tube
- Fluorescent beads: Droplets Polysciences Category #15700, 0.5 µm diameter YG beads
- Nail polish
- Demi water
- Agar powder (BMPI usually has stock)

1.2 Tools

- Mass scales
- Stirring magnet (next to scales)
- Heater
- Microwave (near entrance)
- Ultrasonic bath (in 2nd room)
- Beaker 100 mL (in 2nd room)
- Eppendorf tubes a.k.a. eppy
- Pipet

2 Making the sample

2.1 Agar solution

1. Take 100 mg agar from BMPI stock and put in 100 mL beaker.
2. Add 20 mL demi water to the beaker. Suggestion: use Accu-jet pro a.k.a. "Pipet Boy". → This gives a 5 g/L solution.
3. Stir with stirring magnet. Stir setting 5.

2.2 Beads dilution

1. Shake 'em up! Put the beads in their original vial in the Ultrasonic bath in the water. There should be a floater thing with holes in it to keep it upright.
2. Put 3 droplets of beads in an eppy.
3. Take 40 µL of those drops in a new eppy.
4. Add 1960 µL of demi water to get a 50× dilution. Resuspend in order to mix (suck it partially back in the pipet a few times).
5. Put the eppy in 'handwarm' water (about 50 °C) from the tap to keep it well above room temperature.

2.3 Agar-bead mixture in tube

1. Preheat the capillary tube. Set heater to 60 °C. Put tissue on heater to keep the tubes in place and to keep possible dirt off. Put capillary tube on tissue.
2. Prepare the pipets: 900 µL for the agar solution + 100 µL for beads dilution → gives 10× dilution → gives 500× dilution in total.
3. Take agar solution in beaker and heat to a boil in the microwave at 450 W. Make sure it doesn't boil over the edge. Restart a few times to make sure the liquid is fully boiled.
4. Stir agar solution in beaker with stirring magnet. Let it cool to about 60 °C, when it's cool enough to hold with your hands.
5. Add with pipet 100 µL beads dilution + 900 µL agar solution in a new eppy. Preferably while keeping it in the beaker with 'handwarm' tap water to prevent cooling. → This gives 500× beads dilution in total, and brings the agar solution down to 4.5 g/L.
6. Take capillary tube and suck in the mixture. Capillary action should do most of the work. If not, use the pipet bulb that came with the capillary tubes to suck in the liquid. For large tubes, keep the tube almost horizontal to help the capillary action.

2.4 Glue the tube

1. Put the filled capillary tubes on the coverslip. Put them as aligned as possible.
2. Seal the edges with nail polish. Be careful not to use to much, or the nail polish will spread underneath the entire tube. It helps to put a tiny droplet next to the tube end, and then drag it over the tube end.
3. Create a well around the tube using nail polish. This is for keeping the objective immersion fluid from spreading over the entire coverslip. Make sure this nail polish doesn't touch the tube or it will spread underneath.