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Dispensing agar into multiwell plates

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

Protocol for pouring agar into 96 well plates using Integra VIAFILL dispenser. Agar should be prepared in advance, and kept in 60°C waterbath until ready to dispense, and whilst dispensing. The X, Y, Z positions for dispensing can be adjusted according to the multiwell plates being used.

Materials

MATERIALS

 Whatman UNIPLATE 96-Well Clear Microplates **VWR International (Avantor) Catalog #WHAT7701-1651**

Troubleshooting

Configure Integra VIAFILL

- 1 Prepare a 250ml bottle of hot milliQ water in the microwave and keep in the waterbath along with the agar. The water is important to have on hand in case of tubing blockages.
- 2 Insert large cassette into the machine
- 3 Configure X, Y, and Z settings for the multiwell plate by clicking on tool symbol → stage alignment.

Note

For UNIPLATE96SQWLF 650U:

X = 95.6

Y = 4.2

Z = -22.5

For 48WP:

- 3.1 Put the plate into the stage and then press 'Move' so that the plate moves so that it is under the dispensing cassette.
 - 3.2 Use the up and down arrows to move the pipette tip so that they hover just over the plate and make note of the height (this will be entered into the dispensing program at a later step). Press 'Fast/Slow' button to switch between fast and slow movements.
 - 3.3 Use the X, Y arrows to move the plate so that the pipette tips are centered in the middle of column 5.
 - 3.4 Save all settings.
- 4 Exit settings by pressing the back button

Dispensing Agar



5 Press on the program you wish to use (see later for configuring your own program)

6 Make sure that the correct cassette is listed and change if necessary

7 Select the volume you wish to dispense

Note

For 96WP:
150 μ L

8 Select 'set height' and set the appropriate height for tip height (usually all the same)

9 Place the end of the tubing from the cassette into the agar that is being kept warm in the water bath

10 Press 'Prime' to prime the tubing and allow to finish so that agar flows from the pipette tips.

Note

IMPORTANT:

Once the agar is in the tubing it is important to act quickly to avoid agar solidifying and causing blockages. If you are particularly concerned about agar cooling in the tubing, wrap the tubing in aluminium foil to keep hot.

STEP CASE

Unblocking the tubing 9 steps

If the tubing does block, clear the blockage by 'reverse priming' as much of the agar as possible.

Then place tube ends in the hot water and prime continuously with hot water until the water runs all the way through.

If you are having trouble getting the water through, squeeze and massage parts of the tubing where you can see blockages to force the agar along and allow the water to pass.

Once all cleared, 'reverse prime', and reprime with the agar



- 11 Place a clean plate in the stage
- 12 Press run and then plate should fill with agar
- 13 Repeat steps 11-12 until all the plates have been filled.

Note

Little drops of agar can solidify on the tip ends. It is often good to remove these drops using a pipette tip every few runs so that blockages do not occur.

Cleaning the cassette

- 14 'Reverse prime' all the agar
- 15 Place the tubing ends into the hot water.
- 16 Prime so that the water runs through and clears all the agar
- 17 Reverse prime to remove the water
- 18 Release tension from the tubing and remove cassette
- 19 Double wrap the cassette in aluminium foil for autoclaving