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Dispensing agar into multiwell plates July 2019 updates

Forked from Dispensing agar into multiwell plates

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1 Works for me dx.doi.org/10.17504/protocols.io.46fgzbn

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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^{O}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

NAME	CATALOG #	VENDOR
Whatman UNIPLATE 96-Well Clear Microplates	WHAT7701-1651	VWR international Ltd

Configure Integra VIAFILL

1 Prepare a 250ml bottle of hot milliQ water in the microwave and keep in the 60^{O}C waterbath along with the agar. The water is important to have on hand in case of tubing blockages.

2 Insert large cassette (suitable for dispensing volumes from 5-9999µL) into the machine:

2.1 The pipette tip end slots into the dispenser holder over plates

2.2 The first plastic tube holder is placed on the metal plate on right hand side of the peristaltic arm

2.3 The second plastic tube holder is fitted onto the metal plate on the other side of hte peristaltic arm. Make sure the metal pins on either side slot into the holder

2.4 Flip the metal plate arm up so that it is in place

2.5 Secure everything in place by flipping up the cover

3 Configure X, Y, and Z settings for the multiwell plate by clicking on tool symbol -> stage alignment -> 96 well 8Ch.

For UNIPLATE96SQWLF 650U:

X = 95.6
Y = 4.2
Z = -22.5

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. The pipette tip closest to you should be over well 7H

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 (8 channel large) is listed and change if necessary

7 Select the volume you wish to dispense

For 96WP:
150 µL

8 Select 'set height' and set the appropriate height for tip height when dispensing (usually all the same as the Z height determined earlier)

9 Place the end of the tubing from the casettee into the agar that is being kept warm in the water bath set to 60^{O}C

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

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

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.

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 'Recover' to remove all the agar from inside the tubing.

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/place in tip box for autoclaving