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Make serotonin and naloxone drug plates

Forked from a deleted protocol

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Behavioural Genomics

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We use this protocol and it's working

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Abstract

Protocol for making a set of three serotonin drug plates and a set of three naloxone drug plates. Serotonin (20 mM) is added to molten agar, whereas naloxone (10 mM) is made into a 10X stock solution in water first before being spread over agar plates to achieve the final concentration. Each 35 mm drug plate contains 3 mL low peptone NGM agar and the drug, and is always made up fresh the day before imaging experiments.

For naloxone methods, refer to https://www.ncbi.nlm.nih.gov/pubmed/25898004.

Materials

Serotonin creatinine sulfate monohydrate (Sigma H7752)
Naloxone (Sigma PHR1802)

fume hood
analytical balance

Troubleshooting

Safety warnings

Check toxicity of drugs

1 First identify the drugs (serotonin and naloxone) to be used in the study and ensure that they are correctly labelled and handled.

2 Get six 35 mm petri dishes and label three as serotonin (20 mM) plates and three as naloxone (10 mM) plates.

3 Since these are powered compounds, calculate the desired weight required for 10 mL of serotonin agar (at 20 mM final concentration), and for 1 mL of 10X naloxone stock solution (10X is 100 mM, for 10 mM final concentration). These volumes will give 3 drug plates each.

4 Make up 500 mL low peptone NGM agar and put it on autoclave.

Note

Do not attempt to use a smaller volume even though not much agar is required for drug plate preparation, as chemical weights will be too small to be measured accurately.

5 Inside a fume hood, with an analytical balance weigh out the desired amount of serotonin and leave aside. Weigh out the desired amount of naloxone and transfer the powder into a 1.5 mL Eppendorf tube labelled with the compound and the final concentration. To the naloxone tube, add 1 mL sterile water to make up the 10X stock solution, and close the lid of the Eppendorf tube.

6 When the autoclave cycle is done, add salts as normally required.

7 Remove 10 mL of molten low peptone NGM agar, take it to the fume hood and add the pre-weighed serotonin to achieve 20 mM final concentration. Mix well. Dispense 3 mL into each of three pre-labelled plates using a 10 mL serological pipette. Let serotonin plates set.

8 Take the naloxone stock solution tube out of the fume hood. To ensure the compound is dissolved fully, vortex the Eppendorf tubes (ensuring the lid is firmly in place) at setting 10 for 30 seconds.

9 Using a different 10 mL serological pipette, draw up 10 mL of molten agar (without serotonin) and dispense 3 mL each into a different set of three pre-labelled plates. Let the plates set (these will become the naloxone plates).

10 Add 300 µL of 10X naloxone stock solution on top of each plate from the last step. Let these plates dry in a laminar flow hood for 3 hours.

11 Wrap drug plates in foil and leave them overnight at 4°C to let the drugs diffuse. Use for experiments immediately the next day.