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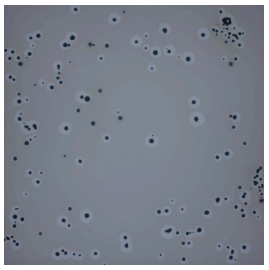
TAP 0.5%-0.75% impranil agarose plates



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We use this protocol and it's working

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Abstract

The protocol describes the preparation of TAP media plates containing 0.5% to 0.75% Impranil, with the inclusion of antifungal and ampicillin. Notably, agarose was employed instead of agar to achieve a lighter gel color. These specialized plates serve the purpose of identifying Chlamydomonas reinhardtii strains secreting the plastic-degrading enzyme PHL7, discerned by the halo formation around the colonies.

Guidelines

All steps described in this protocol are intended to be conducted in a research laboratory. Follow aseptic procedures

To keep plates from getting contaminated always use sterile technique

Shelf life 6 months

Materials

1 liter flask
Foil paper
Petri dishes
Pipette tips
Water bath
Stir bar
Stir plate
Autoclave
Flame or sterile area
Ampicillin
Antifungal
Infrared Thermometer
pH probe

For TAP media:
1M Tris Base
Phosphate Buffer II
Solution A
Trace Elements
Glacial acetic acid*
*adjust pH to 7.0 with Glacial acetic acid

Safety warnings

Wear appropriate PPE, including gloves, safety glasses or goggles, and protective clothing, as recommended by the SDS.

Work in a well-ventilated area or use local exhaust ventilation to minimize inhalation exposure.

Avoid direct skin contact. In case of skin contact, wash the affected area thoroughly with soap and water.

In case of eye contact, flush eyes with plenty of water for at least 15 minutes and seek medical attention if irritation persists.

Avoid breathing vapors or mists. If working in an area with potential inhalation exposure, use respiratory protection as recommended in the SDS.

Clean up spills immediately, following appropriate procedures outlined in the SDS. Use absorbent materials and avoid releasing the material into the environment.

Store the dispersion in accordance with the manufacturer's recommendations, considering temperature, humidity, and compatibility with other substances.

Follow safe handling procedures specified in the SDS. Dispose of waste in accordance with local regulations.

Some polyurethane materials may be flammable. Follow proper fire safety precautions, and avoid exposure to open flames or ignition sources.

Familiarize yourself with the first aid measures recommended in the SDS for exposure scenarios such as skin contact, eye contact, inhalation, or ingestion.

Anionic aliphatic polyester-polyurethane dispersion. Impranil DLN SD
Storage conditions:
- Storage in original sealed Covestro container.
- Recommended storage temperature: 5 - 30 °C.
- Protect from frost, heat and foreign material.

General information: The product cannot be used if it has been frozen. The containers must be well closed to prevent the evaporation of water which may result in the formation of a non-redispersible film. Brief heating to max. 50 °C has no adverse effect on product properties.

TAP media

1 Take a stir rod and place into a 1 L erlenmeyer flask then fill with 900 mL of Milli-Q water. Place flask on stir plate and stir gently.

2 Add 20 mL of 1M Tris Base
Add 1 mL of Phosphate Buffer II
Add 10 mL of Solution A
Add 1 mL of Trace Elements
Let it stir.

3 Place a pH probe into the TAP media solution and adjust to pH 7 with Glacial Acetic Acid. *Slowly add 1 mL - 1.5 mL .

4 Measure out 15g of agarose and add it to the TAP media solution to have [M] 1.5 % (v/v) per 1 L . Stir gently.

5 Top off TAP media with Milli-Q water until you get to 1 L , let it stir for a couple of minutes then remove it from the stir plate. Place a square foil paper on top of the flask to cover and label the flask. It is now ready to be autoclaved.

Autoclave

6 Securely place the flask inside a suitable autoclavable container. Add water to the container, ensuring sufficient coverage around the flask. Load the autoclave with the container and set the autoclave parameters according to the manufacturer's guidelines. Use a cycle time of 01:00:00 (1 hour) with settings appropriate for your specific autoclave model (e.g., gravity 30). Start the autoclave cycle, following standard operating procedures for your laboratory's equipment.

Note: Refer to the autoclave manufacturer's instructions for precise settings and safety guidelines. Adjust time and settings as needed for your specific autoclave.

Water bath cool down and sterile technique to pour plates 6h

7 Once the autoclave has completed, take out the flask, place on stir plate to give a quick stir then place in a water bath at a temperature of 55 °C for 02:00:00 - 04:00:00 before adding antibiotics. 6h

8 Place flask on stir plate and measure the temperature of the TAP media with an infrared thermometer. After the temperature is below 50 °C, using sterile technique, add the antibiotics and the rest of the ingredients. If temperature is still high, let it sit on the stir plate and cool down for a few more minutes. For 1 L of TAP media add 11 mL of [M] 1.0302 mg/mL antifungal, 1 mL of ampicillin [M] 150 mg/mL in [M] 70 % (v/v) ethanol and either 5.0 mL or 7.5 mL of impranil (Anionic aliphatic polyester-polyurethane dispersion. Impranil DLN SD) for [M] 0.5 % (v/v) or [M] 0.75 % (v/v) of impranil per 1 liter of media. Let it stir.

9 As the media is stirring, set up the petri dishes on a clean flat surface near a flame and you are ready to pour.

10 Label and leave petri dish to dry overnight.

Protocol references

Impranil website: https://solutions.covestro.com/en/products/impranil/impranil-dln-sd_57821411-17997446?SelectedCountry=AU