JUL 31, 2023

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Protocol Citation: rebecca.bennett 2023. P5ARP/P10ARP Media Preparation. **protocols.io** https://protocols.io/view/p5arp-p10arp-media-preparation-cxv7xn9n

Protocol status: Working
We use this protocol and it's working

Created: Jul 28, 2023

Last Modified: Jul 31, 2023

PROTOCOL integer ID: 85663

P5ARP/P10ARP Media Preparation

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ABSTRACT

How to make P5ARP and P10ARP media plates, simplified from https://wiki.bugwood.org/PARP_or_PARP

Contains: pimarcin, ampicillin, rifampicin, pentachloronitrobenzens.
Pimaricin is a broad-spectrum antifungal antibiotic that inhibits the true fungi but not most members of the Pythiaceae. Once in solution, it is inactivated by light so that culture plates must be stored and incubated in the dark. Growth of true fungi indicates the inactivation of pimaricin. Concentrations above 10 mg a.i./liter inhibit oospore germination of Pythium and Phytophthora

Plates will last for 1-1.5 weeks before antibiotics are degraded.

Keywords: PARP, P5ARP, P10ARP, selective media, Pythium media

MATERIALS

Difco cornmeal agar
RO
rifampicin
DMSO
microcentrifuge tubes
ampicillin
Delvocid (50% natamycin)
Terraclor 75 WP (wettable powder - see safety notes)
Petri dishes (90 or 60 mm)

Equipment:
1.5 or 2L Erlenmeyer flask
Stir plate and bars
Fine weigh scale
Autoclave and materials
Hot water bath
Biosafety Hood
Ethanol (80%)
1 L media bottles

SAFETY WARNINGS

Terraclor - May cause sensitization by skin contact. Experimental carcinogen and neoplastigen
Personal Protective Measures: Nitrile Gloves × Adequate Ventilation × Safety Glasses× Dust Mask

BEFORE START INSTRUCTIONS

See Safety Warnings.

Preparation

1 Sign up for Autoclave and turn on.

2 Add 17 g of Difco cornmeal agar to each 2 L Erlenmeyer flask

2.1 Add RO water to 1000 mL line (pre-measure line and mark with sharpie or use a graduated cylinder).
Cover top with foil so that at least 2" of foil is below flask lip.

2.2 Add stir bar and mix well over stir plate.

3 Autoclave media in flask alongside 1 L media bottle (loosely capped), for 20 minutes on liquid cycle.

4 Turn on hot water bath and set to 50 °C.

5 Prepare hood by wiping down with 80% EtOH and labeling Petri dishes.

6 Prepare rifampicin solution: 0.01 g rifampicin dissolved in 1 mL DMSO in a microcentrifuge tube.
Cover tube with foil and keep away from light.

After Sterilization

7 Check temperature of hot water bath (between 50 °C to 55 °C).

Place sterilized basal media in hot water bath and wait for temperature to decrease target range. Higher temperatures will inactivate antibiotics!

8 **Fume hood:**

Add to each 1 L media bottle (wear gloves, eye protection, and dust mask!) on a stir plate:

0.25 g ampicillin sodium salt

5 mg = 0.005 g Delvocid (50% natamycin)

> **Note**
>
> Use 10 mg (0.01 g) for P_{10}ARP

10 mg = 0.01 g rifampicin dissolved in 1 mL DMSO

1 g Terraclor WP (wettable powder)

9 Mix completely.

Plating

10 Pour **half** into the sterile 1L media bottle.

10.1 Immediately pour into Petri dishes.

10.2 Refill with remaining media and dispense into Petri dishes.

10.3 Immediately fill empty bottle with tap water when finished so that agar does not dry on glass.

11 Mark plates with a single red bar using a Sharpie. Attach lab tape with date and your initials to

stack of plates.

11.1 When agar has solidified (8-24h), place back into sleeves and label bottom outside of bag with date made and your initials.

11.2 Store in fridge as soon as possible to preserve the antibiotics.

12 Wash bottles before media dries.