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YPS+CRAD Media preparation

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We use this protocol and it's working

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Disclaimer

This protocol is provided for educational and research purposes only. The procedures described herein should be conducted by trained personnel in a properly equipped laboratory, adhering to all relevant safety and ethical guidelines. The authors and publishers of this protocol are not responsible for any injuries, damages, or legal consequences that may result from the improper use of this protocol. The use of chemicals, reagents, and equipment should be done with caution, and users must ensure they are compliant with all local, national, and institutional regulations.
Users are responsible for ensuring that their work complies with applicable biosafety and bioethics standards, particularly when handling genetically modified organisms (GMOs), pathogens, or other hazardous materials. This protocol does not constitute medical, legal, or professional advice, and should not be used as a substitute for consultation with qualified professionals in those fields.

Abstract

YPS (Yeast Extract, Peptone, Sucrose) is a culture medium specifically designed for the general isolation of fungi. This medium is composed of yeast extract, peptone (or tryptone), and a carbohydrate source such as glucose or sucrose, providing a nutrient-rich environment that supports the growth of various fungi. The addition of CRAD (Chloramphenicol, Rifampicin, Ampicillin, Danitol) to the medium after sterilization creates a selective environment, inhibiting the growth of bacteria and other unwanted microorganisms, while allowing fungi to proliferate under controlled conditions.

CRAD in the context of YPS medium includes the following antibiotics and chemicals:

- **Chloramphenicol**: A broad-spectrum antibiotic that prevents the growth of Gram-positive and Gram-negative bacteria.
- **Rifampicin**: An antibiotic effective against Gram-positive bacteria, dissolved in DMSO to ensure complete incorporation into the medium.
- **Ampicillin**: A broad-spectrum beta-lactam antibiotic that inhibits bacterial cell wall synthesis.
- **Danitol**: An acaricide and insecticide used to control specific organisms, such as insects or mites.

Guidelines

- **Aseptic Technique**: Strict aseptic techniques must be followed throughout the preparation process to avoid contamination. Ensure that all materials and equipment are sterile.
- **Component Quality**: Use high-purity reagents and freshly prepared solutions to maintain the integrity and effectiveness of the medium.
- **Homogeneous Mixing**: Continuous slow stirring during the addition of CRAD components is essential to ensure that the antibiotics and other chemicals are evenly distributed throughout the medium.
- **Timing**: Prepare and use the medium as soon as possible after preparation to ensure the viability of the antibiotics.

Materials

- 1 g of **yeast extract**
- 1 g of **peptone** or **tryptone**
- 1 g of **glucose** or **sucrose**
- 0.1 g of **chloramphenicol**
- 18 g of **agar**
- 0.025 g of Rifampicin dissolved in 500 µl of DMSO
- 100 µl of Danitol stock solution (500 µl Danitol 2.4 EC in 100 ml acetone)
- 0.1 g of Avid 0.15 EC
- Distilled water (1 liter)
- Sterile Petri dishes

Equipment

Adventurer™ Analytical Balances	NAME
Analytical balance	TYPE
Ohaus	BRAND
30100600	SKU
https://www.fishersci.com/shop/products/ohaus-adventurer-analytical-balances-7/p-4918285	LINK

Equipment

8-Liter Autoclave

NAME

Portable Stainless Steel Pressure Steam Sterilizer

TYPE

China

BRAND

XFS-D-8L

SKU

https://www.dentalplaza.co.uk/Dentist-8L-Portable-Steam-Autoclave-Sterilizer-168696-dental.html

LINK

SPECIFICATIONS

Voltage: 220 V (AC)
Power: 1.2 kW
Working Medium: Steam
Design Pressure: 0.17 MPa
Working Temperature: 129 °C
Frequency: 50 Hz
Useful Life: 5 Years
Delivery Date: 3. Oct, 2019

JPG

Equipment

Laminar Flow Hood

NAME

Benchtop workstation

TYPE

Envirco

BRAND

TT4830

SKU

Safety warnings

- **Chemical Handling**: DMSO and antibiotics like rifampicin should be handled with care. DMSO can penetrate the skin and carry other substances with it, so wear appropriate protective gear, including gloves and lab coats.
- **Sterilization Safety**: Follow proper autoclaving procedures to prevent accidents. Never open the autoclave until it has fully depressurized and cooled.
- **Antibiotic Sensitivity**: Rifampicin and other antibiotics are sensitive to light and temperature. Store them in a dark, cool place, and only expose them to light and ambient conditions when necessary.

Ethics statement

This protocol is designed for use in laboratory research settings. All experimental procedures involving microorganisms should be conducted in accordance with institutional biosafety guidelines and regulations. Researchers are responsible for ensuring that their work complies with ethical standards, particularly when dealing with genetically modified organisms (GMOs) or pathogens.

Before start

Before starting the preparation of YPS+CRAD medium, the following steps must be completed:

Prepare Rifampicin Solution in DMSO:

- Dissolve 0.025 g of rifampicin in 500 µl of DMSO. Ensure the solution is completely homogeneous.

Prepare Danitol Solution:

- Prepare a stock solution by diluting 500 µl of Danitol 2.4 EC in 100 ml of acetone. From this stock solution, take 100 µl to add to the medium.

Ensure that all necessary materials and equipment are available and sterile before beginning the procedure.

Dissolve YPS Components:

1 **(For 1 liter of medium)**
In a 2-liter Erlenmeyer flask with a stir bar, dissolve the following components in 1 liter of distilled water (dH2O):

1 g of yeast extract.

1 g of peptone or tryptone.

1 g of glucose or sucrose.

0.1 g of chloramphenicol.

18 g of agar.

Stir and heat the mixture until all components are fully dissolved.

Sterilize:

2
- Autoclave the solution at 121 °C for 20 minutes to sterilize the medium.
- Allow the medium to cool to approximately 50 °C in a room temperature water bath, with slow stirring to facilitate the dissolution and mixing of ingredients.

Add CRAD:

3 Add the following components to the cooled medium:

Rifampicin: Incorporate the solution prepared in DMSO (0.025 g dissolved in 500 µL of DMSO).

Ampicillin: Add the required amount as per protocol.

Danitol: Add of the pre-prepared stock solution.

Pour into Petri Dishes:

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- Work aseptically and pour the medium into sterile Petri dishes.
- Allow the medium to solidify at room temperature.