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Pre-Imaging Solid Growth Medium - Yeast

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We use this protocol and it's working

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Abstract

This protocol describes the steps to prepare solid culture medium for Saccharomyces cerevisiae.
This solid medium is used for cultures that are going to be investigated by fluorescence imaging. Of that reason the medium is prepared with an abundance of Adenine to reduce auto-fluorescence [1].

Materials

SC-Ura Powder
Sunrise Science Products
Cat#: 1306-030
Lot#: 23K3083
Exp: 10/2027

Yeast Nitrogen Base Without Amino Acids
Sigma Life Science
Cat#: Y0626-250G
Lot#: SLBG0555V

Glucose
Sunrise Science Products
Cat#: 1907-1kg
Lot#: 3A0036

Agar
Sunrise Science Products
Cat#: 1910-500
Lot#: 3B0104

Sodium Hydroxide
Fisher Scientific
Cat#: S318-500
Lot#: 130802

L-Adenine
Sigma Life Science
Cat#: A-9795
Lot#: 33H12895

Deionized Water

Equipment:

500 ml laboratory bottle with screw cap
1ml pipette
50 ml pipette
stirring hot plate
magnetic stirring bar
micro scales

autoclave
thermometer
10cm polystyrene Petri dishes (20 pieces)
4°C fridge

Before start

Have the following solutions premixed:

Glucose 20% 500 ml solution:
Concentration: 200 g/l
mix 100 g Glucose in 500 ml deionized water (ddH_2O)

Sodium Hydroxide 1M solution 50 ml:
Concentration: 39.997 g/l
mix 1.99985 g NaOH into 50 ml ddH_2O

Adenine 100x 100 ml solution:
Concentration 3 g/l
mix 0.3 g Adenine in 100 ml ddH_2O

Optional:
SC-xx 10x 100ml solution:
Concentration: 19.2 g/l
mix 1.92 g into 100 ml ddH_2O

YNB 20x 100ml solution:
Concentration: 134.4 g/l
mix 13.44 g into 100 ml ddH_2O

1 Compound medium for autoclave 2m

STEP CASE

Medium preparation with pre-resolved components 13 steps

This version of the protocol shows the preparation of the medium from SC-XX 10x and YNB 20x solutions.

1.1 Fill a 500 ml flask with 369 mL H_2O.
Add a magnetic stirring bar and place the flask on a stirring hot plate.

1.2 Add 25 mL YNB 20x solution (Yeast Nitrogen Base with Ammonium Sulfate without Amino Acids).

1.3 Add 50 mL SC-XX 10x solution.

Note

In regard to cover all optional dropout media the amino acid base holds the notification -xx, where xx stand for the amino acid(s) that is as selection factor, missing in the medium.

1.4 Add 10 g Agar

1.5 Add 5 mL Adenine 100x solution

Note

The additional Adenine is supposed to repress the Adenine synthesize to reduce a possible accumulation of red pigment [1].

1.6 Add 1 mL NaOH 1 molar solution.

Note

This is essential for the solidification of the medium!

2 Autoclave for 00:15:00 at 121 °C .

Note

Remove the stirring bar before going to autoclave.

3 Cooling and platting

3.1 Add a sterile stirring bar and thermometer. Let the medium stir to homogenize its temperature, while cooling.

3.2 When the medium cooled down to around 80 °C add 50 mL sterile Glucose 20%.

3.3 When the medium cooled down to 60 °C , plate 25 mL per 10 cm Petri dish (~20 dishes).

3.4 Let the medium solidify and cool down to Room temperature .

4 Seal the prepared dishes in a plastic bag to prevent condensation and store them in 4 °C fridge.

Note

The agar plates can be store in the 4°C fridge for 2 to 3 months.

Protocol references

[1] Kokina, Agnese et al. "Adenine auxotrophy–be aware: some effects of adenine auxotrophy in Saccharomyces cerevisiae strain W303-1A." *FEMS yeast research* 14.5 (2014): 697-707. doi:10.1111/1567-1364.12154