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Refractive index adjusted imaging medium: Iodixanol (RI ~ 1.4) - Yeast

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

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Disclaimer

Abstract

This protocol describes the steps to prepare imaging medium for Saccharomyces cerevisiae with adjusted refractive index. This medium is optimized for fluorescence imaging by the reduction of auto-fluorescence through an abundance of Adenine [1] and the repression of of the Met-promoted pp7- CP expression [2].

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

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

L-Methionine
Sigma Life Science
Cat#: M-5308
Lot#: 129H0322

OptiPrep Density Gradient Medium
Sigma Life Science
Cat#: D1556-250ML
Lot#: 120M1221

Deionized Water

Equipment:

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

autoclave
thermometer

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)

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

Methionine 200x 50 ml:
Concentration: 17.12 g/l
mix 856 mg into 50 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

STEP CASE

Medium preparation with pre-resolved components 9 steps

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

1.1 Fill a 50 ml flask with 21 mL OptiPrep.
Add a magnetic stirring bar and place the flask on a stirring hot plate.

1.2 Add 0.3 mL Adenine 100x solution.

Note

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

1.3 Add 0.3 mL Methionine 200x solution.

Note

The additional Methionine represses the Met promoter, which drives PP7 syntheses [2].

1.4 Add 3 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.

1.5 Add 1.5 mL YNB 20x solution (Yeast Nitrogen Base with Ammonium Sulfate without Amino Acids).

1.6 Add 0.9 mL ddH_2O.

2 Autoclave for 00:15:00 at 121 °C .

Note

Remove the stirring bar before going to autoclave.

3 When the medium cooled down to around 80 °C add 3 mL sterile Glucose 20%.

4

Note

The medium can be store at the bench 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
[2] Lari, Azra, et al. "Live-Cell Imaging of mRNP–NPC Interactions in Budding Yeast." *Imaging Gene Expression: Methods and Protocols* (2019): 131-150. doi.org/10.1007/978-1-4939-9674-2_9