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Refractive index adjusted imaging medium: Iodixanol (RI ~ 1.41 and 1.42) - Yeast

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Mathias Hammer[1], Ammeret Rossouw[1], Azra Lari[2], Ben Montpetit[3], David Grunwald[1]

[1]UMass Chan Medical School, RNA Therapeutics Institute, Worcester, MA, USA;
[2]University of Alberta, Department of Cell Biology, Edmonton, AB, Canada;
[3]University of California, Department of Viticulture and Enology, Davis, CA, USA

Mathias Hammer
UMASS Chan Medical School/RTI

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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
5 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

1 Compound medium for autoclave

STEP CASE

Refractive Index (RI) ~1.41

9 steps

The case of the protocol describes the preparation of a yeast imaging medium adjusted to an refractive index of 1.41.

1.1 Fill a 50 ml flask with 22.5 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 57.6 mg SC-XX powder.

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 201.6 mg Yeast Nitrogen Base with Ammonium Sulfate without Amino Acids (YNB).

Note

Warming the medium on the stirring plate helps to resolve the powders.

1.6 Add 3.9 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 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.
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