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Refractive index adjusted imaging medium: lodixanol (RI ~ 1.4) -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 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 (ddH₂0)

Adenine 100x 100 ml solution:

Concentration 3 g/l

mix 0.3 g Adenine in 100 ml ddH₂O

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

 $\rm mix~1.92~g~into~100~ml~ddH_2O$

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 4 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 A 0.3 mL Methionine 200x solution.

Note

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

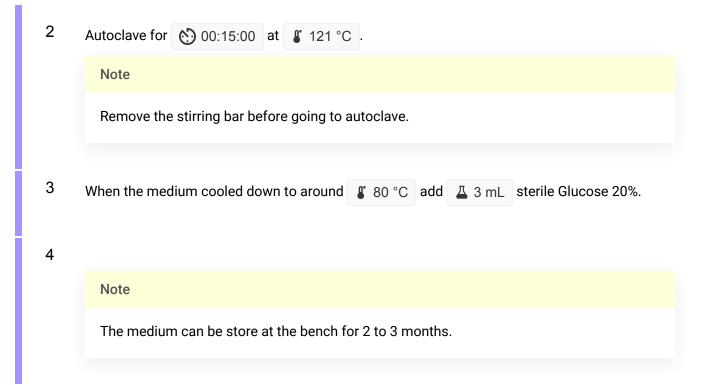
1.4 Add 🗸 3 mL SC-XX 10x solution.

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 <u>Add</u> 1.5 mL YNB 20x solution (Yeast Nitrogen Base with Ammonium Sulfate without Amino Acids).
- 1.6 Add \perp 0.9 mL ddH₂0.





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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