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

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Protocol status: Working

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 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

Histodenz

Sigma Life Science

Cat#: D2158-100G

Lot#: WXBC3389V

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 (ddH₂O)

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 ddH₂O

Optional:

SC-xx 10x 100ml solution:

Concentration: 19.2 g/l

mix 1.92 g into 100 ml ddH₂O

YNB 20x 100ml solution:

Concentration: 134.4 g/l

mix 13.44 g into 100 ml ddH₂O



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  21.9 mL ddH₂O

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  36.519 g Histodenz (Iohexol) powder.

Note

Warming the medium on the stirring plate helps resolving the powders.

2 Autoclave for  00:15:00 at  121 °C .

15m

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