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

DOI

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# OPEN ACCESS



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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

#### 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 (ddH<sub>2</sub>0)

## Adenine 100x 100 ml solution:

Concentration 3 g/l

mix 0.3 g Adenine in 100 ml ddH<sub>2</sub>O

## 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

SI	ΓEΡ	CA	<b>\SE</b>

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 4 21.9 mL ddH<sub>2</sub>O 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 4 0.3 mL Methionine 200x solution.

Note

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

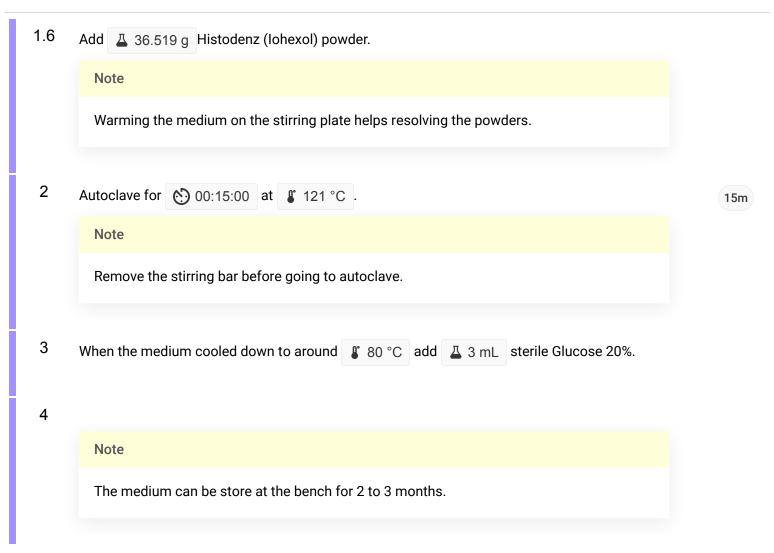
1.4 Add 🗸 3 mL SC-XX 10x solution.

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





## 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