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Refractive index adjusted imaging medium: High RI Iodixanol, Iohexol - 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 high 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

OptiPrep Density Gradient Medium

Sigma Life Science

Cat#: D1556-250ML

Lot#: 120M1221

Deionized Water

Equipment:

50 ml laboratory bottle with screw cap



50 ml centrifuge tube with cap

1ml pipette

25 ml pipette

stirring hot plate

magnetic stirring bar

micro scales

autoclave

oven

thermometer

refractometer

Before start

Have the following prepared:

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

Iodixanol crystals:

Preparation:

transfer 40 ml Iodixanol (Optiprep) solution into a 50 ml centrifugation tube

place the tube opened into an oven set to 40°C

through evaporation the refractive index of the Iodixanol solution rises:

	A	B
	time in days	RI
	0	1.4315
	2	~1.45
	4	~1.47
	6	~1.5
	7-10	crystalisation

Iodixanol solution - refractive index increase in 40°C oven over time



1 Compound medium for autoclave

STEP CASE

Iodixanol 11 steps

This protocol describes the preparation of high refractive index yeast imaging medium using Iodixanol (~OptiPrep).

1.1

Note

It is possible to use existing medium with Iodexanol adjusted refractive index as a base. In this case only steps 1.7 and 1.8 apply.

Fill a 50 ml flask with  25 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 synthesise 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 resolving the powders.

- 1.6 Add  1.4 mL ddH₂O.

- 1.7 Add and resolve Iodixanol (~Optiprep) crystals.

Note

For reasons of cost efficiency it possible to use a portion of the mixed solution with 1.4 and add the Iodixanol crystals to increase the RI.
! Be aware that the Glucose 20% volume added in step 3 must be adjusted to the correct ratio for the volume used!

- 1.8 Measure the refractive index with a refractometer. If the RI measured is below your target RI, repeat step 1.7  [go to step #1.7](#) until the targeted RI is reached.

- 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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