

Aug 23, 2024

Pre-Imaging Liquid Growth Medium - Yeast

DOI

dx.doi.org/10.17504/protocols.io.81wgbzxe1gpk/v1



Mathias Hammer¹, Ammeret Rossouw¹, Azra Lari², Ben Montpetit³, David Grunwald¹

¹UMass Chan Medical School, RNA Therapeutics Institute, Worcester, MA, USA;

³University of California, Department of Viticulture and Enology, Davis, CA, USA



Mathias Hammer

UMASS Chan Medical School/RTI





DOI: dx.doi.org/10.17504/protocols.io.81wgbzxe1gpk/v1

Protocol Citation: Mathias Hammer, Ammeret Rossouw, Azra Lari, Ben Montpetit, David Grunwald 2024. Pre-Imaging Liquid Growth Medium - Yeast. protocols.io https://dx.doi.org/10.17504/protocols.io.81wgbzxe1gpk/v1

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working We use this protocol and it's working

Created: May 27, 2024

Last Modified: August 23, 2024

Protocol Integer ID: 100681

Keywords: yeast imaging, yeast medium, yeast mRNA labeling

Funders Acknowledgement:

NSF

Grant ID: 1917206

²University of Alberta, Department of Cell Biology, Edmonton, AB, Canada;



Disclaimer

DISCLAIMER - FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

This protocol describes the steps to prepare liquid culture medium for Saccharomyces cerevisiae. This liquid medium is used to optimize yeast cultures for fluorescence imaging by the reduction of auto-fluorescence through an abundance of Adenine [1].



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

Deionized Water

Equipment:

500 ml laboratory bottle with screw cap 1ml pipette 50 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

Optional:

SC-xx 10x 100ml solution:

Concentration: 19.2 g/l

 $mix 1.92 g into 100 ml ddH_2O$

YNB 20x 100ml solution:

Concentration: 134.4 g/l

mix 13.44 g into 100 ml ddH_2O



1 Compound medium for autoclave

CT	-ED	CA	ISE
υı		\cup_{Γ}	۱JL

Medium preparation with pre-resolved components 7 steps

This version of the protocol shows the preparation of the medium from SC-XX 10x and YNB 20x solutions.

- 1.1 Fill a 500 ml flask with 320 mL ddH₂O.Add a magnetic stirring bar and place the flask on a stirring hot plate.
- 1.2 Add 25 mL YNB 20x solution (Yeast Nitrogen Base with Ammonium Sulfate without Amino Acids).
- 1.3 Add 4 100 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.

1.4 Add 4 5 mL Adenine 100x solution.

Note

The additional Adenine is supposed to repress the Adenine synthesize to reduce a possible accumulation of red pigment [1].

2 Autoclave for (5) 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 \bot 50 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