

Feb 05, 2022

Soft agar for yeast imaging

leonhard.bandilla ¹¹(independent)

protocol .

LBandilla



leonhard.bandilla

This is an unverified and experimental protocol. Use this protocol at your own risk, use this for informational purposes only.

The here described medium can be used to image yeast without immobilization or compression while allowing for growth in all directions. The medium consists of YPD with agar added at a very low concentration to form a soft gel that allows for free growth in all directions. The media can be poured into any multiwell plate or dish to be imaged with an inverted microscope. This protocol is written for 1l of final medium but smaller quantities can be used.

leonhard.bandilla 2022. Soft agar for yeast imaging . **protocols.io**
<https://protocols.io/view/soft-agar-for-yeast-imaging-b4q8qvzw>



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When transferring the warm media to the final container, allow for enough height to image and for inoculation.

The final product is fragile and should not be tilted, shaken or inverted.

Wear standard PPE for the biosafety level of the laboratory where this protocol is performed.

The contents of the flask can remain dangerously hot for a long time after autoclaving.




Always follow the instructions of your autoclave, tightening bottles can be dangerous due to the pressure differences involved.


When autoclaving or heating, only fill bottles to half their volume to avoid foaming, splashing and spills.

Read the SDS of all materials involved before following the protocol.

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- 1 To an autoclavable bottle add per 1l: 5m
 -  **10 g** Yeast extract
 -  **20 g** Peptone
 -  **1 g** Agar

- 2 Add  **950 mL** of water of appropriate quality per 1l of final media 1m

- 3 Invert the bottle to dissolve everything apart from the agar 2m

- 4 Autoclave or heat up sufficiently to dissolve the agar and insure sterility 20m

- 5 Per 1l add  **50 mL** of sterile 40% glucose and mix, avoid introducing air bubbles 1m

- 6 Let the solution cool down so that it can be safely handled and pour or pipette into the final 10m
container in which the imaging will take place. Avoid air bubbles as they can interfere with
imaging. Make sure to avoid contamination by working near a bunsen burner or in a laminar
flow hood

- 7 Wait for the agar solidify over night before innoculating the media. This media takes 12h
significantly longer to set, allow for multiple hours before any change in consistancy is

noticable, be aware that the final product is very soft and can easily be mistaken for liquid media