Yeast DAPI Staining

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Abstract

Stains the nucleus of budding or fission yeast with DAPI in order to see it with a fluorescence microscope.

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Protocol

Step 1.
Grow up yeast in liquid medium overnight.

NOTES
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The OD may not matter too much here, but something in the 0.8 - 2 range is probably ideal.

Step 2.
Add 333 µL (or 1 volume) yeast culture to a 1.5 mL microcentrifuge tube.

AMOUNT
333 µl Additional info:

Step 3.
Add 666 µL (or 2 volume) of 100% Ethanol to the 1.5 mL microcentrifuge tube.

AMOUNT
666 µl Additional info:

REAGENTS
Ethyl alcohol, Pure 200 proof, for molecular biology E7023 by Sigma Aldrich

Step 4.
Let the yeast ethanol mixture sit at room temperature for 30-60 minutes.

DURATION
00:30:00

Step 5.
Spin down yeast cells for 1 minute at 2500 RPM.

DURATION
00:01:00

Step 6.
Pour out the supernatant and resuspend the pellet in 1mL of 1 x PBS, then centrifuge for 1 minute at 2500 RPM.

AMOUNT
1 ml Additional info:

REAGENTS

✓ 1X PBS (Phosphate-buffered saline) by Contributed by users

Step 7.
Pour out the supernatant and resuspend the pellet in 200 µL of a 1 x PBS / 1:2000 Dilution DAPI mixture.

AMOUNT
200 µl Additional info:

PROTOCOL

. PBS / DAPI 1:2000 Dilution Mixture

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Step 7.1.
Add 1 mL 1 x PBS to a 1.5 mL microcentrifuge tube.

AMOUNT
1 ml Additional info:

REAGENTS
✓ 1X PBS (Phosphate-buffered saline) by Contributed by users

Step 7.2.
Add 0.5 µL of a 2.5 mg/mL (or a 1:2000 dilution) of DAPI to the 1 x PBS.

REAGENTS
✓ DAPI (2.5mg/mL) by Contributed by users

Step 8.
Add one drop of the yeast suspended in the PBS / DAPI mixture onto a microscope slide, add a coverslip on top, and go observe the stained yeast.

NOTES
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Make sure after you do this part to go look at it within a few hours of resuspending in PBS / DAPI. The sooner you can get to a microscope the better.

Warnings

DAPI is light sensitive.

You must centrifuge at low RPM to avoid displacement of the nucleus.