Staining Labyrinthulomycetes with Propidium Iodide

This protocol was modified by Sabrina Geraci-Yee and is from: Pozarowski P, Darzynkiewicz Z. 2004. Analysis of cell cycle by flow cytometry. Methods Mol Bio. 281:301-311.

Abstract

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Protocol

Step 1.

NOTES
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ATCC Medium: 790 By+ Medium

Yeast Extract.....1.0 g

Peptone.....1.0 g

D+-Glucose.....5.0 g

Seawater.....1000 ml

https://www.atcc.org/~/media/920FDAC93FF84B79851C29FBB8049862.ashx

'Artemia Soup' medium

1.25 g ground, freeze-dried brine shrimp

autoclaved in 1 liter artifical seawater

Step 2.

Re-suspend cell pellet in 1.5 ml 70% EtOH to fix the cells. Store at 4°C for at least 1 hour (and up to several weeks).

Step 3.

Prepare PI staining solution (Keep propidium iodide stain in dark) :

- 0.1% (v/v) Triton X-100 → 1 ul per 1 ml stain
- 10 ug/mL Propidium iodide (PI) → 1 ul per 1 ml stain
- 100 ug/mL DNase-free RNase A → 10 ul per 1 ml stain
- Bring up to 1 ml by adding 1X PBS → 988 ul per 1 ml stain

Step 4.

Centrifuge ethanol-suspended cells at 10,000 x g for 5 minutes, remove ethanol thoroughly. Pour off and blot the top of the tube. If necessary, pipette out remaining ethanol without disturbing pellet.

Step 5.

Re-suspend the cell pellet in 1 ml of PI staining solution; keep in dark at room temp for 30 min or at 37°C for 10 min.

Step 6.

View cells under fluorescence (you may also want to concentrate the cells on a filter). Excitation maximum is 535 nm and emission maximum is 617 nm.