Acriflavine Centrifuge Stain for Labyrinthulomycetes

Modified by Sabrina Geraci-Yee FROM: RAGHUKUMAR S, SCHAUMANN K. 1993. AN EPIFLUORESCENCE MICROSCOPY METHOD FOR DIRECT DETECTION AND ENUMERATION OF THE, THE THRAUSTOCHYTRIDS. LIMNOL. OCEANOGR.

Abstract

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Protocol

Step 1.

Centrifuge 1 ml of culture for 5 minutes at 10,000 x g. This protocol works well for *Schizochytrium 28209* growing in 1/10 790 or *Artemia* soup.

NOTES

Laura Halligan 30 Mar 2017

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.

Pour off media and resuspend cells in 1 ml of 0.05% acriflavine in 0.1 M citrate buffer at pH 3.0. (A 0.5% stock solution of acriflavine in distilled water is used to make the 0.05% acrflavine working solution).

ANNOTATIONS

Laura Halligan 31 Mar 2017

Keep acriflavine in dark.

Step 3.

Let stain for 3 minutes.

Step 4.

Spin cells at 10,000 X g for 2 minutes.

Step 5.

Pipette out all the acriflavine.

Step 6.

To destain, resuspend cells in 1ml 75% isopropanol and spin at 10,000 x g for 2 minutes.

(This entire step can be skipped if destaining is not required; go straight to step 8).

Step 7.

Pour off 75% isopropanol and wash cells with 1ml sterile ASW and spin at 10,000 x g for 2 minutes.

Step 8.

Resuspend with 100 µl ASW.

Step 9.

Cells can be mounted directly onto slide or can be concentrated on a filter. Place a cover slip over cells or filter. If a filter is used, add a drop of water or immersion oil on top of filter before placing cover slip.

Step 10.

Under epiflourescence microscopy, labyrinthulomycete cells should have a red-fluorescent cell wall and green-fluorescent cytoplasm. In our cultures, the cell wall is often thin and hard to see.

Preferred excitation filters: 420-490 nm violet-to-blue or 450-490 nm blue.