Acriflavine Direct Detection Technique for Labyrinthulomycetes

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Abstract

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Protocol

Step 1.

Collect 100 µl of live cells on a 0.22 µm membrane filter.

This protocol works well for *Schizochytrium 28209* growing in 1/10 790 or 1/2 790; the volume filtered can be adjusted to accomodate the culture cell density.

The cells can also be fixed with formaldehyde; we have not observed a big difference in fresh vs fixed cells.

Step 2.

Rinse cells on filter with filter-sterilized artifical seawater.

We generally use half-strength artificial seawater.

Step 3.

Add 3-4 mL of 0.05% acriflavine in 0.1 M citrate buffer at pH 3.0 to the cells and let stand for 4 minutes.

A 0.5% stock solution of acriflavine in distilled water is used to make the 0.05% acrflavine working solution.

ANNOTATIONS

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Keep acriflavine in dark.

Step 4.

Vacuum drain the stain and once it is fully drained, add 2 ml of 75% isopropyl alcohol while still drawing the vacuum.

Step 5.

Once all isopropyl alcohol is drained, rinse filter with sterile distilled water.

Step 6.

Place filter on microscope slide, add a drop of water (or immersion oil) on it and place cover slip on top.

Step 7.

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.