Acridine Orange Staining

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Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

Modified from the Handbook of Methods in Aquatic Microbial Ecology (p. 220)

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Protocol

Step 1.

Form a meniscus with Milli-Q H_2O (used with freshwater samples) or ESAW (saltwater samples) on the vacuum round

Step 2.

Place 0.45 µm HAWP filter on meniscus and turn on vacuum to 5 mmHg

Step 3.

Close knob

Step 4.

Make another bubble with Milli-Q H_2O or ESAW on the filter

Step 5.

Place a 0.2 µm GTBP filter onto the bubble

Step 6.

Vacuum and close knob

Step 7.

Place the filter tower over the filter

Step 8.

Pour 2 mL of a preserved water sample (or a sample which has been diluted to 2 mL with Milli-Q or ESAW) onto the filter

Step 9.

Add 10 µL of 1% w/v filtered Acridine Orange (AO) and mix

REAGENTS

Acridine orange AC423340010 by Fisher Scientific

Step 10.

Keep the AO in a dark place when not using it, and use it under low light conditions

Step 11.

Stain for 3 min

DURATION

00:03:00

Step 12.

Vacuum and rinse with 2 mL Milli-Q H_2O or ESAW when the meniscus of the sample reaches the filter surface

Step 13.

Rinse again

Step 14.

When the entire sample has been filtered, remove the filter tower

Step 15.

Remove the membrane filter while still under a vacuum and place onto a prepared slide

NOTES

Alyssa Alsante 06 Jun 2017

To prepare a slide, place 5 small drops of immersion oil on a cover slip and one drop on the slide. Place the cover slip on the slide. Before laying the filter on the slide, lift off the cover slip.

Step 16.

Replace the cover slip and remove any air bubbles

Step 17.

View the slides with the Leica DM4000-6000 epifluorescent microscope using the L5 (blue) fillter at excitation = 482 nm and emission = 525 nm