Staining Unfixed Labyrinthulomycetes with Nile Red

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Abstract

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Pandey A, Bhathena Z. 2014. Prevalence of PUFA Rich Thraustochytrids sps. along the Coast of Mumbai for Production of Bio Oil. Journal of Food and Nutrition Research 2(12): 993-999.

Citation: This protocol modified from: Pandey A, Bhathena Z. 2014. Prevalence of PUFA Rich Thraustochytrids sps. along the Coast of Mumbai for Produ Staining Unfixed Labyrinthulomycetes with Nile Red. **protocols.io**
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Protocol

Step 1.

Primary Nile Red (NR) stock: 1mg/mL in DMSO.

Working NR stock: 100 µg/mL in DMSO.

Keep NR in the dark.

NR is not very soluble in water

We have used this protocol successfully for *Schizochytrium 28209* grown in 1/2 790 and *Artemia* soup.

We've even seen stained zoospores still swimming!

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.

Add 10 µl of NR working stock to 100 µl of cell culture.

Final staining condition: 10 µg/mL in 10% DMSO.

Step 3.

Cover test tube (wrap in aluminum foil) to prevent NR light exposure and then vortex for 1 minute.

Step 4.

Let stained sample incubate in the dark at room temperature for 5 minutes.

Step 5.

After 5 minutes, mount cells on slide and place cover slip.

Step 6.

The photo shown was taken with a Lietz I3 filter cube (excitation BP 450-490 nm; dichroic 510 nm; emission LP 515).

Step 7.