Long staining procedure of nuclei in Euplotes crassus using DAPI Version 4

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Abstract

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Protocol

Step 1.

Pellet Euplotes crassus cells at 400 rcf for 3 minutes, and remove as much supernatant as possible by pipetting.

NOTES
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Both algae and bacteria are autofluorescent. Better to have a completely starved Euplotes crassus culture.

Step 2.

Add 1 ml of 2% PFA in 1X PHEM or 4% PFA in 1X PBS to the cells, and incubate them for 10 minutes at room temperature.

Step 3.

Pellet Euplotes crassus cells by centrifugation at 400 rcf for 3 minutes, and remove as much supernatant as possible by pipetting.

Step 4.

Wash cells twice with 1X PBS (400 rcf for 3 minutes each time).

Step 5.

Add 1 ml of TBSTEM - 3% BSA and 0.5 µl of DAPI (0.1 mg/ml) to the cells, and stain for 10 minutes at room temperature.

Step 6.

Pellet Euplotes crassus cells by centrifugation at 400 rcf for 3 minutes.

Step 7.

Add 50 µl of Prolong medium.

Step 8.

Place an approx. 10 µl droplet of Euplotes crassus cells on a slide for observation by fluorescence microscopy.