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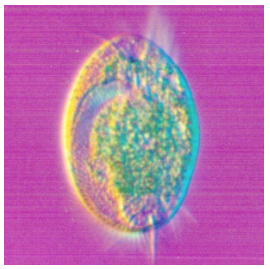
Increased sensitivity of Euplotes crassus to selective agents using 0.3 M glucose-based culture conditions.



Forked from Increased sensitivity of Euplotes crassus to selective agents using 0.3 M glucose-based culture conditions.

In 1 collection

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We use this protocol and it's working

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Abstract

Numerous unpublished studies indicate that *Euplotes crassus* is resistant to extremely high concentrations of commonly used selective agents when grow in artificial seawater. We have found that when *E. crassus* is grown in 1 part artificial seawater + 9 parts 0.3 M glucose, they are much more sensitive to a number of selective agents. For example, 1080 ug/ml paromomycin in artificial seawater only inhibited growth, as little as 120 ug/ml was effective in killing cells when grown with 0.3 M glucose. Similarly, G418 at 400 ug/ml had little effect in seawater, but as little as 100 ug/ml blocked growth in 0.3 M glucose. This may not prove true for all selective agents, as we saw no effect with paclitaxel up to 100 uM under either growth condition.

1 Distribute a dense culture of *Dunaliella salina* to two 50 ml screw-cap centrifuge tubes, and pellet the algae by centrifugation at 1,000 RPM (~200 x g) for 2 min. in a clinical centrifuge.

2 Remove all but the last 2.5 ml of supernatant from each tube, resuspend the algae pellets, and combine.

3 To the above tube, add 2.5 ml of a log phase culture of *Euplotes crassus* (grown with ***Dunaliella* in artificial seawater as the food source**), and 45 ml of 0.3 M glucose (freshly prepared using deionized water).

4 Add selective agent to desired final concentration. 200 ug/ul G418, 200 ug/ml paromomycin, or 40 ug/ml puromycin.

5 Cells can be distributed microtiter plates. For example, 2 ml per well for a 24-well microtiter plate.

6 Incubate at room temperature in a humidified chamber (e.g., plastic box with wet paper towels or beaker of water). Cell death occurs within 2-3 days.

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