Oct 07, 2024

Chemical fixation of *Solarion arianae* for transmission electron microscopy

DOI
dx.doi.org/10.17504/protocols.io.kxygxyd5zl8j/v1

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DOI: dx.doi.org/10.17504/protocols.io.kxygxyd5zl8j/v1

Protocol Citation: Marek Valt, Pavla Hrubá 2024. Chemical fixation of Solarion arianae for transmission electron microscopy . **protocols.io https://dx.doi.org/10.17504/protocols.io.kxygxyd5zl8j/v1**

Protocol status: Working
We use this protocol and it's working

Created: September 27, 2024

Last Modified: October 07, 2024

Protocol Integer ID: 108556

Abstract

This is an optimized version of the protocol for standard chemical fixation for transmission electron microscopy that was used for the fixation of the culture of *Solarion arianae*.

Cell harvest

1 Centrifuge a well-grown culture at 1250 g at 4 °C.

5m

1.1 Discard supernatant and collect pellet.

Chemical cell fixation

2d 6h 3m

2 Immerse pellet with 2,5% glutaraldehyde fixative in 0,1M cacodylate buffer solution for 1h. Work on ice.

1h

3 Wash with 0,1M cacodylate buffer, 3 times.

4 Post-fix with 2% OsO4 in 0,1 M cacodylate buffer for 1h, on ice

1h

5 Wash fixed samples in distilled water, 3 times.

6 Dehydrate samples in ethanol series 30%, 50%, 70%, 80%, 90%, 95%, (100% 3 times)

7 Impregnate the dehydrated sample with acetone 1:1 ethanol solution.

8 Impregnate with 100% acetone.

9 Impregnate acetone 1:1 resin (EMbed 812) mixture.

10 Transfer the sample to (EMbed 812) resin. Repeat 3 times every 4h. (can be left longer or overnight)

4h

11 Polymerize samples embedded in resin at 70 °C for 48h. 2d

Section preparation

12 Cut sections.
For *Solarion,* 80nm thick sections were cut with a diamond knife on an Ultracut E ultramicrotome (Reichert).

13 post-contrast with uranyl acetate and lead citrate.