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Version 2

Chemical fixation of *Solarion arienae* for transmission electron microscopy V.2

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Protocol status: Working

We use this protocol and it's working

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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.