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Permanent specimen preparation by protargol staining

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Marek Valt[1], Michael Kotyk[1]

[1]Charles University, Faculty of Science, Department of Zoology, Prague, Czechia

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Marek Valt
Charles University, Faculty of Science, Department of Zoolog...

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

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Abstract

Protargol staining according to the protocol by Bodian (Bodian 1936) and later modified by Nie (Nie 1950), further optimized for staining of small flagellates.

Protargol staining optimized for a small flagellates, used for staining of Solarion arienae.

2d 1h 15m

1 Mix 1 µm of culture sediment with a drop of sterile egg albumin, on a coverslip.
For *Solarion arienae*, egg albumin was not diluted to achieve better adherence to the cells to coverslip.

2 Fix in Bouin-Hollande's solution overnight. (0,175 mol/l picric acid, 0,138 mol/l Cu^{+2} acetate, 4% formaldehyde, and 5% acetic acid in water solution)

3 Next day, transfer coverslips throught a graded ethanol series (70%, 50%).

4 Wash in distilled water.

5 Bleach in 0,5% $KMnO_4$ solution for 5 minutes.

5m

6 Wash in distilled water (5x).

7 Treat coverslips with 5% oxalic acid for 5 minutes.

5m

8 Wash again in distilled water (5x).

9 Stain in 1% protargol solution, for 48 hours at 37°C in a beaker with copper wire pieces placed between the coverslips.

2d

For *Solarion*, protargol produced by Bayer, I. G. Farbenindustrie Actinengesellschaft (out of business since 1952) was used.

10 Wash in distilled water (2x).

11 Treat with freshly prepared reducing solution (1% hydroquinone and 5% Na_2SO_3), for 10 minutes.

10m

12 Wash in distilled water (5x).

13 Tone with 1% $AuCl_3$, for 5 minutes. 5m

14 Wash in distilled water (2x).

15 Treat with 2% oxalic acid, for 5 minutes. 5m

16 Wash in distilled water (5x).

17 Treat with 5% $Na_2S_2O_3$ for 10 minutes 10m

18 The final wash is done under a constant stream of tap water for 20 minutes. 20m

19 Dehydrate the coverslips in an ethanol series (50%, 70%, 80%, 96%, 100%) and 3 times in xylene (5 minutes each).

20 Dehydrate in xylene, 3 times (5 minutes each). 15m

21 Finally, the stained, dehydrated coverslips are mounted on glass slides with DPX mounting medium (Sigma–Aldrich).