Protargol Method

Reagents:

Bleach:

6% NaOCl → diluted to 0.6% stock

Protargol:

1% in DIW; we have had success with "Strong silver proteinate " from Johnson and Matthey ; it seems stronger than what you get from Polysciences.

Developer:

95ml DIW

5g sodium sulfite (Na_2SO_3)

1g hydroquinone

[Foissner says may be dil to 2-5% for some ciliates; others, use full strength]

fixative:

1L DIW

25g sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)

1. Fix organisms in Bouin's fluid (regular Bouin's, 1:1 with culture; add 7.5 ml Bouin's to 15 ml Falcon tube, then add 7.5 ml of the culture) for 10-30 minutes.
2. Spin at 500 rpm 5 minutes 15 C; take off supernatant with pipette and resuspend ciliates in the last few ml and transfer to well insert for 12-well plate; wash organisms 3-4 times with distilled water, wicking away the fluid on a Kimwipe until the color of Bouin's disappears.
3. Put sodium hypochlorite (NaClO) on the cells and bleach them until they become a little transparent (a few seconds): the concentration of NaClO depends on the cells that will be stained. Normally, we use 6% NaClO diluted to 0.6% stock, then 2 drops in 18 drops DIW, then pipette on top of ciliates, which are in a very small amount of DIW. If bleaching is too strong, cells either dissolve or cannot be stained well. Watch under dissecting 'scope until cells are slightly transparent (only 10-20 seconds).
4. Dilute with DIW, then wick away the fluid several times to wash out the bleach.
5. Add 1% warm protargol solution; set on hot plate at 50 C and impregnate for 1 hour, or until cells become a little dark.
6. Remove from hot plate. Wick away most of the protargol solution and add some drops of developer to the remainder containing the organisms: usually fresh developer, which should be stored in fridge (4 ℃) and can last for several weeks, is used. If the developer becomes a little yellow, it should be discarded. Control development in dissecting microscope. As soon as the

infraciliature becomes faintly visible, development must be stopped by adding a few drops of sodium thiosulfate.

7. Wick away the fluid and stabilize the impregnation by adding sodium thiosulfate for approximately 5 minute.

8. Wash thoroughly in distilled water (5-8 times) to remove sodium thiosulfate. Pipette the ciliates in about 1 ml of DIW and place in microcentrifuge tube. Spin for 5 minutes, then remove most of the supernatant.

9. Put 1 drop each of albumen-glycerol and concentrated organisms in the center of an alcohol-cleaned cover slip. Mix drops with a mounted needle and spread over the slide. Remove extra water and albumen-glycerol until you get a moderately thick layer. Dry preparation for at least 2 hours.

10. Transfer cover slip(s) through 70%-80%-90%-100%-100% alcohol-1:1 alcohol/xylene for about 5 minutes.

11. Clear with two 5 minute transfers through 100% xylene.

12. Mount in synthetic neutral mounting medium (Permount): keep slide(s) from drying between steps 11 and 12. Dry preparation overnight before viewing with oil immersion.