

# Protocol for Staining of Transparent Exopolysaccharide Particles (TEP)

Written 1995 WKoz

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Alcain Blue stain is kept in refrigerator. If no working stock is made up, dilute the concentrated stock v/v 1:50 with DI water. The working stain needs to be prefiltered through a 0.2 $\mu$  syringe tip filter before each use.

Use a dedicated TEP filtration rig – it should include a Gelman 250ml 2.5cm filtration cup with a 250 ml sidearm flask. This rig should be rinsed with distilled water after every use.

After water collection:

1. Set up TEP filtration rig with a 2.5cm, 0.4 $\mu$  nucleopore filter shiny side up (**do not** use the 0.4 $\mu$  **black** nucleopore filters), and a connection to the vacuum pump. Check that pump is pulling at 100 mm (4 in) Hg before filtering samples.

2. Make you Blank: using a P-1000 Pipetman, place 500 $\mu$ l of the filtered working stain onto the filter.

Rinse funnel with DI water - Turn on pump only enough to start the water through the filter. Shut off vacuum BEFORE filter is dry.

3. Place damp filter into a labeled ("TEP", date, station, depth, "Blank") cryo-vial.

Rinse filtration rig with DI water; replace 0.4 $\mu$  filter.

4. Filter the sample: filter 100ml water from TEP container through filter, turning off vacuum just before filter is dry.

Add 500 $\mu$ l Alcain Blue stain at this point, allowing the residual vacuum to pull the stain through the filter.

Rinse funnel with DI water - Turn on pump just long enough to start the water through the filter. Shut off vacuum BEFORE filter is dry.

5. Place damp filter in a labeled ("TEP", date, station, depth, vol. filtered) cryo-vial.

6. Repeat steps four and five two more times, so for each station you have three sample filters and one blank.

7. Put all four vials into a ziplock bag, labeled with the date, into the large TEP ziplock in the -60 $^{\circ}$  freezer.

8. Be sure to note on Station Log Form the actual volumes filtered (ie. 3x100ml + blank).

If a sample is especially concentrated, less volume may be filtered (as little as 50 ml).