

Protocol for the Analysis of Ballast Water to determine the Concentration of viable Bacteria using the Adenosin-Triphosphate Method

1 CALIBRATION

1. Pour 100µl *Luminase* into a luminometer tube
2. Add two drops (=100µl) of *UltraCheck 1*
3. Insert luminometer tube into Luminometer, record RLU \Rightarrow RLU_{UC1}
4. If RLU_{UC1}<5000 with an LB 9509, use new bottle of *Luminase*

2 SAMPLE PREPARATION

The ballast water sample to be analyzed by the ATP method is generated through the sampling points on board the ships and additional, adequate sampling systems and procedures (cf. SOP xx)

1. Remove plunger from 60 ml syringe and attach 2 µm glass fiber filter to the front of syringe.
2. Pour filtrate into syringe, insert plunger.
3. Push sample through 2 µm filter, collect filtrate in small beaker.

3 SAMPLE ANALYSIS

1. Remove plunger from 60 ml syringe and fix *QuenchGone* filter to syringe.
2. Pour 60 ml of sample (from Sample Preparation step 4) into the syringe with attached filter, insert plunger.
3. Gently push plunger down ensuring that the filter is kept wet, discard filtrate.
4. Detach filter from syringe, remove plunger from syringe, re-attach filter to syringe.
5. Pour 1 ml of *Ultralyse 7* into syringe, insert plunger.
6. Gently pass the liquid through the filter into a 12 ml extraction tube until dryness of filter, discard filter.
7. Pour 9 ml of *Ultralute* into the extraction tube, put cap on tube, invert 3 times.
8. Transfer 100 µl from the extraction tube to a luminometer tube.
9. Add 100 µl of *Luminase* to luminometer tube, gently swirl tube 5 times.
10. Insert luminometer tube into Luminometer, record RLU \Rightarrow RLU_{cATP}

4 FINAL CALCULATIONS

1. Amount of cellular ATP (cATP) is given as : $cATP (pgATP / ml) = \frac{RLU_{cATP}}{RLU_{UC1}} \times \frac{10.000 (pgATP)}{V_{sample} (ml)}$
2. 1 *Escherichia coli* sized bacterium contains 0,001 pg cATP, i.e. the microbial equivalent (ME)
3. The bacterial concentration (BC) in the ballast water sample is then :

$$BC (ME/ml) = \frac{RLU_{cATP}}{RLU_{UC1}} \times \frac{10.000 (pgATP)}{V_{sample} (ml)} \times 1000$$