Protocol for the Analysis of Ballast Water to determine the Concentration of viable Plankton Organisms >50µm 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. para 8 for SOP xx).

3 SAMPLE ANALYSIS

1. Mix the sample in the small beaker, add 1 ml of well-mixed suspension to a grinding tube.
2. Add 5 ml of modified *Ultralyse 30* to the grinding tube.
3. Place the grinding tube in the grinder, run for 2.5 minutes at 6000 rpm.
4. Remove the tube from the grinder, turn it over twice and place it back on the grinder.
5. Run the grinder for 2.5 minutes at 6000 rpm.
6. Remove the tube from the grinder, leave the tube for 5 minutes to let the solids settle.
7. Transfer 100 µl of supernatant to the dilution tube.
8. Close the dilution tube and mix it.
9. Take100 µl of diluted extract and and add it to a luminometer tube.
10. Add 100 µl of Luminase to luminometer tube, gently swirl tube 5 times.
11. Insert luminometer tube into Luminometer, record RLU $\Rightarrow RLU_{cATP}$ after 10 seconds.

4 FINAL CALCULATIONS

1. The obtained RLU values are converted into ATP concentrations using the *Calculate Software*, according to the equation tATP (ng/ml) = (RLU_{tATP} / RLU_{UC1}) x 306.
2. Since the ATP concentrations are proportional to the number of plankton organisms per m^3, the tATP sample concentrations are converted into organism density as follows:
   tATP **/** (SVBW x 1000) = Number of organisms / m^3, where SVBW is the sampled volume of ballast water.