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TOXI-SCREENING KIT

(new) test procedure

Contents of the Toxi-Screening Kit

- 10 vials with freeze-dried *Vibrio fischeri* bacteria - to be stored at -18 °C (± 2 °C)
- 1 flask with reconstitution solution (2% NaCl) - to be stored at 5 °C (± 2 °C)
- 2 flasks with control solution (2% NaCl in Standard freshwater) - to be stored at 5 °C (± 2 °C)
- 1 flask with osmotic adjustment solution (= OAS) (20% NaCl) - to be stored at 5 °C (± 2 °C)
- 40 luminometer cuvettes

Materials needed

- reagents of the Toxi-Screening Kit
- luminometer (Kikkoman PD10, PD20 or PD30)
- adjustable pipettes and pipettes tips
- rack for the cuvettes

TEST PROCEDURE

1. Reconstitution of freeze dried *Vibrio fischeri* bacteria

- take one vial of freeze dried bacteria
- add 400 µl reconstitution solution
- mix the contents by pipetting 3-4 times
- incubate the vial at room temperature for 10 minutes

In the meantime prepare the control and sample cuvettes by setting out 4 luminometer cuvettes in a rack

➔ 2 Control-cuvettes and 2 Sample-cuvettes

2. Control measurement

- pipette 800 µl control solution in Control-cuvette 1
- add 100 µl of the reconstituted bacteria
- mix the contents by pipetting 3-4 times

- measure the luminescence intensity immediately (= RLU control T0)
- repeat the previous steps for Control-cuvette 2

3. Sample measurement

- pipette 800 µl sample solution in Sample-cuvette 1
- add 80 µl of the OAS medium
- add 100 µl of the reconstituted bacteria
- mix the contents by pipetting 3-4 times
- measure the luminescence intensity immediately (= RLU sample T0)
- repeat the previous steps for Sample-cuvette 2

4. Incubate all the cuvettes at room temperature for the chosen contact time (15 or 30 minutes)

5. Measure the luminescence intensity (RLU T15 and/or RLU T30) of all the Control- and Sample-cuvettes

6. Calculation of the results

Calculate the % inhibition for the **Sample** relative to the **Control**

		RLU T0'	RLU T15'	RLU T30'
Control	1			
	2			
Sample	1			
	2			

% inhibition = [(Control T15 - Sample T15) / control T15] *100

and/or

% inhibition = [(Control T30 - Sample T30) / control T30] *100