MARINE ALGALTOXKIT™

MARINE TOXICITY TEST WITH MICROALGAE

BENCH PROTOCOL

Principle :

The **Marine Algaltoxkit** contains all the materials, including the test species ***Phaeodactylum tricornutum*** to perform 2 complete algal growth inhibition assays, according to internationally accepted Standard Methods.

Optical density (at 670 nm) is used as the parameter for algal growth/inhibition. The tests are performed in disposable cells of 10 cm path-length which allow for direct and rapid scoring of the OD in the "long cell" test vials, using any conventional spectrophotometer equipped with a holder for 10 cm cells.

1. Preparation of the algal culturing medium :

Preparation of syntethic Seawater
Fill a 2 liter volumetric flask with approximately 1500 ml deionized water and add the contents of vial number 1 containing pure NaCl. When dissolved, add the contents of the other vials with concentrated salt solutions, in sequence 2 to 7 as indicated on the flasks.
Addition of Nutrient Stock Solutions
Add 30 ml of Stock Solution A, 1 ml of Stock Solution B and 2 ml of Stock Solution C.
Add deionized water up to the 2000 ml mark and shake to homogenize the contents.

2. Preculturing of the algae :

Take one tube containing the microalgae, handshake it vigorously and pour the contents into the preculturing cell. Rince the tube **twice** with 7.5 ml algal culturing medium and transfer this liquid into the preculturing cell to make sure that all the microalgae are transferred.
Close the preculturing cell and incubate it **for 3 days** in an incubator or a temperature controlled room at 20 °C (+/- 2 °C), with a constant uniform illumination supplied by cool white fluorescent lamps; make sure to obtain 10000 lux for sideway illumination or 3000-4000 lux for bottom illumination.

3. Preparation of the concentrated algal inoculum :

After 3 days of incubation take the culturing cell and shake to homogenize the algal suspension*. Take the long cell marked "Calibration cell" and filll it with 25 ml algal culturing medium, close with the lid and measure the OD at 670 nm.

* *SHAKING PROCEDURE OF LONG CELLS WITH ALGAL SUSPENSIONS*

- *Place the thumb in the middle on the bottom of the cell and all other fingers on the on the lid at equal distance from each other. Press firmy to ensure a tight closing of the cell. Turn the cell upside down and shake gently for approx. 10 seconds and turn the cell upwards again. Score the OD in the spectrophotometer after 10 seconds.*

Put the culture cell in the spectrophotometer and read the optical density (OD1). Take the OD/N sheet and look up the number of algae (N1) corresponding with OD1. With N2 equal to 1.10^6 algae/ml, calculate from the N1/N2 ratio the dilution factor needed to reach an optical density equal to OD2, corresponding to an algal density of 1.10^6 cells/ml. Transfer the algal suspension from the culture cell into a 100 ml container and add the volume of algal culturing medium needed to make up a 1.10^6 cells/ml suspension. Stopper and shake the container to distribute the algae evenly.
Rinse the culture cell, transfer 25 ml algal suspension into this cell, close and shake, and read the OD. Check on the OD/N graph

whether the OD corresponds with the desired OD2 value (1.10^6 algae/ml)*

4. Preparation of the toxicant dilution series

Prepare the dilution series of the effluent or chemical according to the detailed instructions given in the Standard Operation Procedure Manual. Inoculate each flask with microalgae from the concentrated suspension to obtain 1.10^4 cells/ml as the start concentration.

5. Transfer of the algae-toxicant dilutions into the test vials :

Take one of the two holding trays with long cells, remove the rubber bands and the two plastic strips, and mark all the cells in sets of 3 (a,b,c), corresponding to the concentration range CO-C5. Open all cells by hinging one half of the lid over the other half till it clicks over the small catch. After thorough shaking, transfer 25 ml of the algae-toxicant dilutions from each flask into the corresponding long cells. Close all cells and shake them immediately before reading the OD's in the spectrophotometer. Score the T_0 data on the Results Sheet.

6. Incubation of the test vials :

Put all the cells back into the holding tray in a random way (*i.e. not in the sequence CO-C5, and not all 3 parallels next to each other*). Open each cell half and make sure that all open halfs face the same side of the tray. Slide the plastic strip over the open part of the long cells, taking care to leave some opening near the middle of the long cells for gas exchange. Incubate the cells (in their tray) for 3 days in an incubator or in a temperature controlled room with constant uniform illumination (10000 lux for sideway illumination or 3000-4000 lux for bottom illumination), at 20 (+/- 2°C).

7. Scoring of the results :

Determine the OD in each cell after 24h, 48h and 72h of incubation, after applying the shaking procedure outlined above, and score the data on the Results Sheets.

8. Data treatment :

Calculate the mean daily OD values for the 3 replicate cells. Determine the EbC_{50} or ErC_{50} according to internationnally accepted procedures (e.g. ISO/CD Guideline 10253). A floppy disk with a linear regression programme can be obtained, for automatic data treatment.

***Validity of the test** :*

Besides all other prerequisites, the number of algae in the control test vials must have increased by at least a factor 16 during the 72h test period for the toxicity test to be acceptable.

9. Reference test :

It is recommended that every 5 to 10 assays, a quality control test be carried out to check proper adherence to the test protocol, as well as the test sensitivity.

A reference test can be carried out with potassium dichromate ($K_2Cr_2O_7$). The 72h EC_{50} of the quality control test should be within the 95% confidence limits stipulated on the specification sheet.

The dilution series to be prepared for the reference test with potassium dichromate is : 1.8 - 3.2 - 5.6 - 10 - 18 mg/l.