SPIRODELA DUCKWEED TOXKIT MICROBIOTEST

Growth inhibition test on the higher aquatic plant *Spirodela polyrhiza*



Practical and sensitive 72h <u>stock culture free</u>* microbiotest based on area measurement of the first fronds by Image Analysis

* The kit contains tubes with "turions" (= vegetative buds) which can be stored and germinated "on demand"



Each *Spirodela* duckweed Toxkit contains all the materials for performance of 2 complete bioassays

SPIRODELA DUCKWEED TOXKIT

A simple and practical 72h growth inhibition microbiotest

with the higher aquatic plant Spirodela polyrhiza

Each SPIRODELA DUCKWEED TOXKIT contains all the materials to perform 2 growth inhibition tests on Spirodela polyrhiza, in a 48 cups multiwell test plate, in 5 test concentrations, each with 8 replicates. Easy to follow instructions and detailed illustrations are provided in the kits for the conduct of range-finding and definitive tests. The test organisms are included in the kits as "dormant" vegetative buds (turions) which can be stored for several months and germinated "on demand" for the performance of the microbiotest.

Test criterion

• The Spirodela duckweed Toxkit microbiotest is a 72h assay based on the growth inhibition of the "first frond" of the plant after 3 days exposure to a dilution series of the toxicant, with subsequent calculation of the 72h EC50. Growth is determined by measurement of "the area" of the first frond, at t0h and t72h with the aid of Image Analysis.

Reproducibility

- Turions of high quality produced in strictly controlled conditions preclude variability associated with recruitment/maintenance of live stocks in conventional duckweed bioassays.
- High uniform quality of the growth and dilution medium is achieved by simple dilution in deionized water, of concentrated solutions of "Steinberg medium".
- Standardized microplate test containers constructed of biologically inert materials ensure uniform exposure conditions.
- A Quality Control Test with a reference chemical is described in detail, for accuracy and reproducibility check.

Cost-effectiveness

- The dormant "turions" can be germinated "on demand", eliminating the need and the costs of continuous culturing and maintenance of stocks of test organisms.
- Minimal equipment needed for test performance : a laboratory incubator (or a temperature conditioned room) provided with illumination, and conventional laboratory glassware.
- The shelf life of the turions is guaranteed for several months when stored properly, reducing test scheduling constraints.

Contents

- Tubes with dormant turions, concentrated growth and dilution medium, germination/test containers and a spatula for the transfer of the germinated turions to the test plate.
- Detailed Standard Operational Procedure, abbreviated Bench Protocol, Data treatment sheets
- Specification sheet with batch number of the turions and the media, and the 72h EC50 value for a Quality Control Test on the reference chemical potassium chloride (KCl).

User-Friendliness

- Simple handlings and scorings, which allows to handle multiple tests concurrently
- Total performance time of the assay and the measurements is less than 3 hours.
- The area measurements of the first fronds are made on a photo of the multiwell test container, without any prior manipulation of the organisms.
- Area measurements of the first fronds (on the stored photo) can be postponed to any appropriate timing.
- An Excel programme for easy data treatment and calculation of the 72h EC50 can be obtained on demand.

Sensitivity

• Comparable to the sensitivity of the *Lemna* duckweed species used in conventional duckweed assays.

Validation

- Duckweed assays are used in many laboratories worldwide for toxicity screening of environmental samples or chemicals.
- The precision of the standardized *Spirodela* duckweed microbiotest has been determined in an extensive "International Interlaboratory Comparison" with participation of more than 50 laboratories from 20 countries.
- Validity criteria have been selected for the *Spirodela* duckweed microbiotest.

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