



# RST Product Specifications

## PRODUCT NAME

RST

## ASSAY PRINCIPLE

Plate-reader-based analysis of organism respiration for environmental monitoring. The RST assay measures the respiration rates of small organisms in a specialized low-volume, 96-well plate, and measures toxicological changes in small organisms via changes in their oxygen respiration. Test samples can be treated with various effectors/environmental samples (e.g seawater) and then analyzed on a standard fluorescence plate-reader.

The RST assay is based on the ability of O<sub>2</sub> to quench the excited state of MitoXpress<sup>®</sup>, a water-soluble, phosphorescent, oxygen-sensitive probe.

## ASSAY FORMAT

RST (Respirometric Screening Technology) employs a phosphorescence-based oxygen sensing approach to measure oxygen uptake/release in whole organisms. Oxygen uptake informs on Electron Transport Chain (ETC) activity, thus RST provides one of the most advanced means for direct and specific assessment of mitochondrial function and metabolism. The RST platform operates with dedicated phosphorescent oxygen probes and is compatible with standard microplates, fluorescence and time-resolved fluorescence plate readers.

## PRODUCT DESCRIPTION

The MitoXpress<sup>®</sup> oxygen probe is supplied as a dry, stable, soluble reagent that is easily reconstituted in water for simple dispensing with a pipette. One vial of MitoXpress oxygen probe per 96 well plate.

The MitoXpress oxygen probe for RST plate-based extracellular analysis is provided in a complete kit format containing: 1 vial of MitoXpress-1X, 1 Luxcel specialized low-volume plate and 1 adhesive film.

## BASIC CHARACTERISTICS

MitoXpress<sup>™</sup> probe is a chemically stable and inert, biopolymer-based, cell impermeable probe. The probe is excitable at 340-390 or 535 nm and emits at 630-680 nm, Optimal filter combinations are 340 nm for excitation and 642 nm for emission (standard for Victor<sup>™</sup> reader).

## PROBE PREPARATION

To prepare a stock solution of MitoXpress<sup>™</sup> probe, reconstitute MitoXpress-1X in 0.1 ml of water or assay buffer to the vial provided. To prepare assay solution, dilute the reconstituted MitoXpress 1:20 in assay buffer yielding a 0.5 $\mu$ M stock. For measurement add 10 $\mu$ l per well of 0.5 $\mu$ M MitoXpress containing #Number of organisms, then seal carefully using adhesive film, allowing excess volume to be displaced.

## PHOSPHORESCENT MEASUREMENTS

The MitoXpress<sup>®</sup> probe is measured with prompt or time-resolved fluorescence (TR-F) readers, monochromator or filter-based. Optimal wavelengths are 380 nm for excitation and 650 nm for emission, excitation at 532 nm can also be used. For TR-F readers, optimal delay and gate times are 30  $\mu$ s and 100  $\mu$ s respectively. Probe signals should be at least 3 times above blank signal. MitoXpress<sup>®</sup> probe response to oxygen is temperature dependent, so good temperature control of the plate during the measurement is important.

## REFERENCES

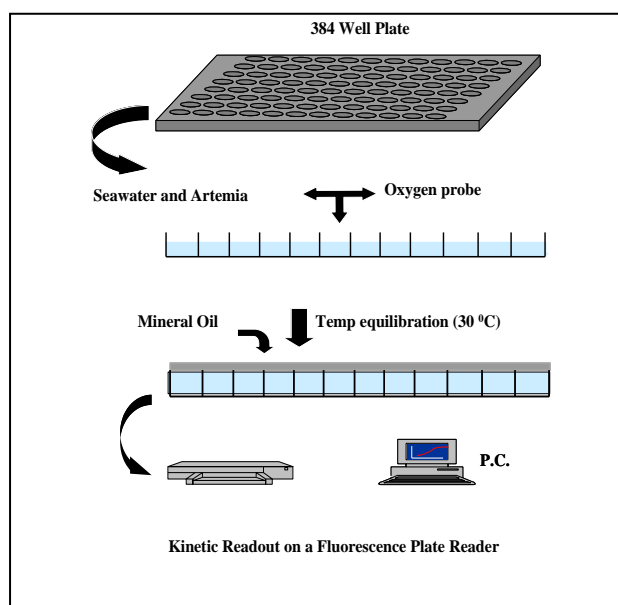
[1] O'Mahony F.C., O'Donovan C., Hynes J., davenport J., and Papkovsky D.B., Optical Oxygen Microrespirometry as a Platform for Environmental Toxicology and Animal Model Studies, *Environ. Sci. Technol.* 2005; 39,5010-5014

[2] Alice Zitova, Fiach C. O'Mahony, Maud Cross, John Davenport and Dmitri B. Papkovsky, Toxicological profiling of chemical and environmental samples using panels of test organisms and optical oxygen respirometry.

## EXAMPLE OF RST ASSAY SET-UP

Platform:	Standard clear 384-well plates or Luxcel RST plates (no mineral oil seal, enhanced performance)
Organism:	<i>Artemia salina</i> (Brine shrimp), instar II-III nauplii
Medium:	Artificial seawater
Oxygen probe:	Luxcel MitoXpress® probe
Seal:	Heavy mineral oil or Luxcel Adhesive Film. (used with RST plate only)
Temperature:	30°C
Detection:	Prompt fluorescence: SpectraMax Gemini plate reader (ex 380/em 650nm) or Time-resolved fluorescence: Victor2 plate reader (ex 340/em 642nm)
Assay Time:	1 hr exposure to treatment + 30 - 120 minute kinetic measurement.

## RST ASSAY SCHEME



## EXAMPLE OF RST ASSAY PROTOCOL AND RESULTS

Artificial seawater (10µL/well), (slight excess) containing MitoXpress oxygen probe (0.5µM) was added to each well containing 4-6 *Artemia salina*.

Menadione, a model chemical known to have superoxide-mediated toxicity, was added to the wells, with or without desferoxamine or dimethylsulfoxide [DMSO].

The plate was incubated at room temperature for 1 hour. After incubation, heavy mineral oil (20µL) was added to each well to seal from ambient air, and the plate was read at regular time intervals (1-5 minutes) for 30-120 min at 30°C.

Time profiles obtained were used to determine the rates of oxygen respiration of *Artemia* (initial slopes of signal) in each sample, with respect to control samples with untreated *Artemia*.

- As shown below, the chemicals induced dose-dependent responses on the respiration/viability of *Artemia salina*:

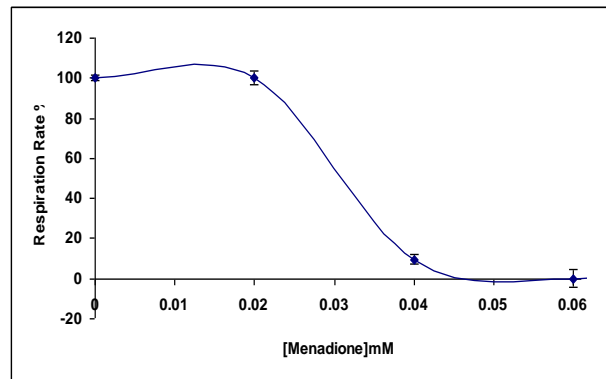
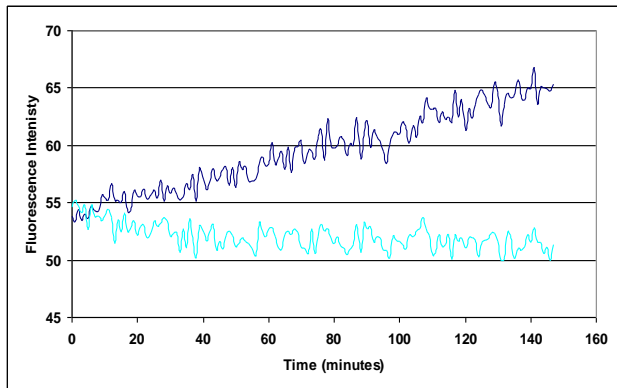


Fig. 1 (left) Respiration profiles of treated (bottom) and control (top) samples with *Artemia salina*.  
 Fig. 2 (right) Effect of menadione (superoxide-mediated toxicity) on the viability of *Artemia salina*.

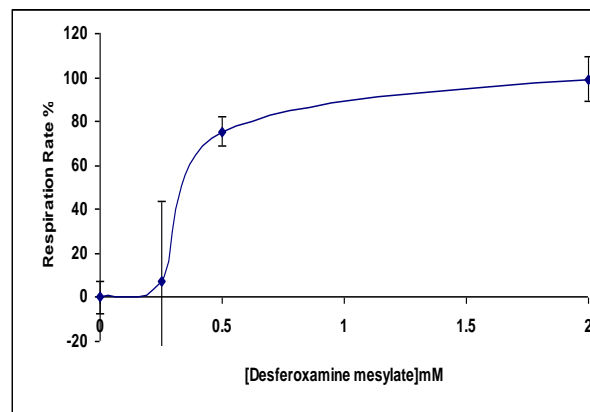
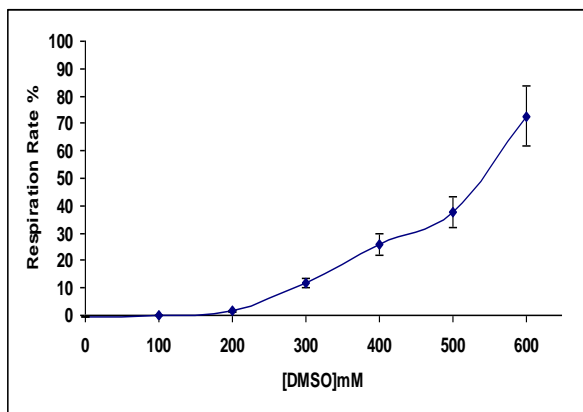


Fig. 3 (left) Inhibition of menadione (0.06mM) toxicity by DMSO (a hydroxy-radical scavenger)  
 Fig. 4 (right) Inhibition of menadione (0.06mM) toxicity by desferoxamine (mimics superoxide dimutase)

## RESULTS

As expected, there was a dose-dependent decrease of the respiration rate of the *Artemia salina* on exposure to menadione. DMSO or desferoxamine protected the *Artemia* from the toxic action of menadione, restoring oxygen respiration of *Artemia* in a dose-dependent manner.



## RST Materials Safety Data Sheet

### PRODUCT NUMBER

RST

### CATALOGUE NUMBER

RST-100

### UNIT SIZE

1 vial of MitoXpress-1X oxygen probe per product

### PROBE MANUFACTURE

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BioInnovation Centre,  
UCC,  
Cork, Ireland.

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### COMPOSITION/INFORMATION ON MITOXPRESS PROBE INGREDIENTS

Dry, water-soluble material comprising a phosphorescent dye conjugated to an inert biopolymer.

### MITOXPRESS HANDLING AND STORAGE

Dry material: store at +4°C (until the indicated expiry date).

Reconstituted product (aqueous stock): store at -20°C.

Diluted probe stock: use on the same day.

Protect products from prolonged exposure to light, may be exposed to light for short time periods.

### OTHER INFORMATION

This material is not considered as hazardous, it is not present above 1%, nor is it a carcinogen above 0.1% as defined in 29 CFR 1910.1200, the OSHA Hazard Communication Standard. Therefore, a Material Data Sheet is not required. We recommend treating all chemicals with caution.

*This material is sold for research purposes only and is not required to appear in TSCA inventory. It is not intended for food, drug, household, agricultural or cosmetic use. It must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. Luxcel Biosciences shall not be liable for any damage resulting from handling or contact with the above product*

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