

# LUMIstar in the production and quality control for ROTAS on-site test kit reagents

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- ROTAS test kit for determination of contaminants
- portable ROTAS luminometer for on-site use
- LUMIstar Galaxy best option for accurate laboratory measurements

## Introduction

Cybersense is a company that specialises in manufacturing and distributing test kits for detecting contamination in soil and water. Its key technology is a kit called ROTAS ('Rapid Onsite Toxicity Audit System'). This is an ecotoxicity sensor that uses a luminescent bacterium as the main component in the test reagent. Chemical contamination in a sample will negatively impact on bacterial metabolism, so that an increased contaminant burden correlates with greater toxicity which is measured as a reduction in light output from the reagent. The luminescent bacterium used in the test reagent is *Vibrio fischeri*, which occurs naturally in marine environments both as a free-living organism and as a symbiont in the luminescent glands of deep sea fish and squid species. The reaction for the light output is shown in figure 1 below:

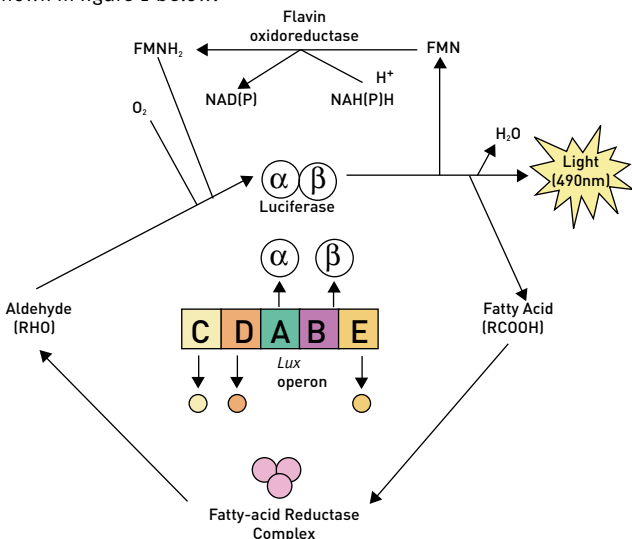


Fig. 1: Reaction principle

The main hardware in the ROTAS test kit is the luminometer manufactured by Cybersense. The key features of this are that it is small and portable. The technology is based on photodiodes, which read only in a narrow range, but have the advantage of being small and light. This is unlike the LUMIstar Galaxy which uses the much larger and more accurate photomultiplier tubes (see figure 2 for comparative readings).

When ROTAS reagent is manufactured it is essential that the finished product is luminescing within the detection range of the ROTAS luminometer. During incubation of the bacterial culture, close monitoring of the luminescence of the bacteria is crucial in getting

good results. Therefore the LUMIstar Galaxy is used to monitor culture luminescence and insure that when the bacteria are harvested for use in the reagent, it is at the correct time and with the correct dilution.

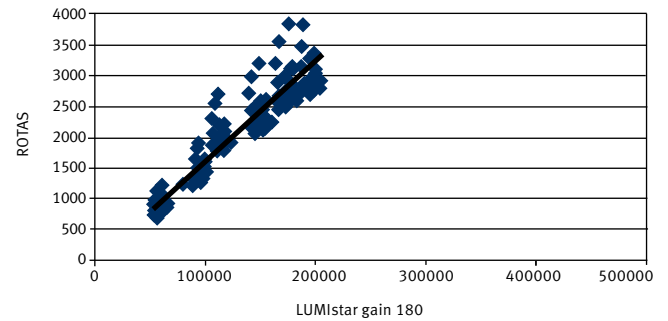


Fig. 2: Luminescence reading on ROTAS luminometer plotted against reading on LUMIstar Galaxy at gain setting 180.

The ROTAS luminometer (Fig. 1) reads between arbitrary values of 0 and 4,080, while the LUMIstar Galaxy (Fig. 2) will read from 0 - 499,999 and has multiple gain settings. The photodiodes in ROTAS have to be individually calibrated and so you get more well-to-well variation in readings. Therefore the LUMIstar Galaxy is the best option for accurate laboratory measurements, where as the portable ROTAS luminometer is designed for on-site use.



Fig. 3: ROTAS Luminometer

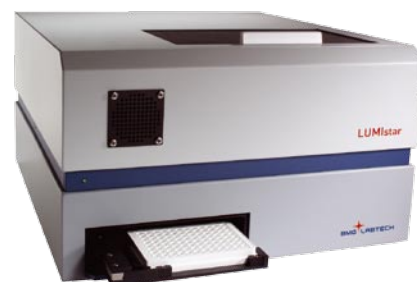


Fig. 4: LUMIstar Galaxy, forerunner of LUMIstar OPTIMA

## Materials and Methods

### Materials and Methods

The following equipment is used.

Up to and including luminescence measurements:

- 5L conical flask
- Shaking incubator
- White 24-well microplates
- Spectrophotometer + cuvettes
- 1% NaCl solution
- Yeast-peptone-salt solution
- Pipettors and tips of various sizes
- Vibrio fischeri* bead or plate stocks

Subsequent to luminescence measurement step:

- Centrifuge
- Cryoprotectant solution
- Bottle-top liquid dispenser
- 1L duran flask
- 80°C
- Freeze-dryer
- 20°C
- ROTAS luminometer + research software

To begin with, a 1500 ml of yeast-peptone-salt solution is inoculated with *Vibrio fischeri* and incubated at 23°C. After approximately 16 hours begin taking optical density (OD) measurements of the culture at wavelength 600nm using the spectrophotometer. Use distilled water as a blank.

When the OD reaches 1 begin taking luminescence measurements. Using the pipettors dispense 0.9 ml of 1% NaCl and 0.1 ml culture into 3 wells of a 24-well microplate. Run the plate on the LUMIstar Galaxy at gain 180 for 1 minute. If the luminescence values are below 150,000 then return the culture to the incubator and retest OD and luminescence in 20 minutes time. If the luminescence values are above 150,000 then the bacteria can be harvested for use as reagent.

If you have to do several successive measurements of the culture on LUMIstar ensure the measurements are labelled as such on the software.

To harvest the bacteria they need to be centrifuged at 3500rpm for 20 minutes. The exact volume of culture to be centrifuged is determined by the luminescence measurement on LUMIstar and the OD of the culture at the same time. For example if the luminescence is in the range 150,000 – 200,000 and the OD is <1.1, measure out 4 x 270ml culture into the centrifuge tubes. The volume of culture needed is less if the luminescence is higher.

After the bacteria has been centrifuged, the supernatant is discarded and the pellets resuspended in a cryoprotectant. The solution of bacteria in cryoprotectant is dispensed into 60 ml vials and these are stored in a –80°C freezer for 24 hours. Finally the vials are freeze-dried for 48 hours in our Virtis freeze-dryer. The finished product is an off-white coloured powder and should be stored in a regular –20°C freezer.

## Results and Discussion

The finished reagent vials need to be QC tested to ensure that they are luminescing at a light level which the ROTAS luminometer can detect. For each batch of reagent, 4 freeze-dried vials are reconstituted with rehydration medium. They are left for 50 minutes to develop and then their luminescence is measured on a ROTAS luminometer. Luminescence values need to be between 1000 and 3500 in order to pass QC. In addition the response of the bacteria to 2 control solutions (spiked with contaminants) is measured to ensure they are sufficiently sensitive for use in the assay.

Manufacturing the reagent to the QC specification can be tricky as there are many variables to take into account. The LUMIstar Galaxy is an essential piece of equipment for the manufacturing process.

Since purchasing in 2001 the instrument has been developed (LUMIstar OPTIMA) and can now be upgraded to a multimode detection reader (FLUOstar OPTIMA or POLARstar OPTIMA) able to measure also fluorescence and absorbance, e.g. for the measurement of optical density.

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