MicrobMonitor

Biocide Rapide Test

A Device For Monitoring Concentrations Of Biocides And Preservatives

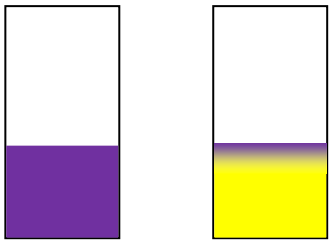
Biocides and Preservatives are essential chemicals for controlling microbial fouling, spoilage and corrosion in industrial fluids and consumer products and for preventing contamination by microbes which threaten health. They progressively lose efficacy if the concentration falls. The ECHA Biocide Rapide Test has been developed to simplify assays of preservative and biocide concentrations on-site or in the laboratory.

1. Principle Of The **Microb**monitor Biocide Rapide Test

An ampoule of gel is supplied which contains the spores of a thermophilic *Bacillus,* growth nutrients and an indicator which changes from purple to yellow. Incubation at 64°C activates the *Bacillus* which will only germinate and grow if incubated at 64°C. The sample to be tested (or a dilution of the sample in deionised water) is added to the ampoule before incubation. After 3.5 hours (or alternate time) at 64°C the growth of the *Bacillus* is assessed as follows:

In most industries, potential spoilage and or harmful organisms will be less sensitive to biocide than the detector organism in the test ampoule. Much more biocide is needed to control these microbes than the MIC for the Biocide Rapide Test. Thus a dilution of the preserved industrial fluid must be made before testing to adjust the biocide level in use to the sensitivity of the detector organism.

INTERPRETATION CHART



Ampoule partially or completely yellow: the sample contains insufficient biocide or preservative to inhibit the growth of the *Bacillus.*

Ampoule purple: The sample contains sufficient biocide or preservative to inhibit the growth of the *Bacillus* i.e. the concentration is at or above the Minimum Inhibitory Concentration (MIC.).

The Biocide Rapide Test Kit consists of:

- 100 or 25 ampoules of a solid agar containing *Bacillus stearothermophilus* spores & nutrients.
- 100 or 25 disposable measuring pipettes.
- Store kits at 6-15°C.

1.1 Method Of Use – A Fluid Sample And Or Dilutions Of The Sample In Distilled Water Are Tested.

- Select sufficient ampoules for use and peel back the foil/ or stab a hole in the foil for sample addition.
- Using a disposable measuring pipette provided, place its tip into the sample to be tested, squeeze and release the upper bulb and allow sample to fill the measuring tube and partially fill the lower bulb.
- Transfer the sample to the ampoule by placing the pipette tip over the ampoule and squeezing the top bulb of the pipette.
- Label and incubate for the predetermined incubation time at 64 °C (± 5 min and ± 0.5 °C). Usually 3.5 hours incubation will be appropriate but it should be ensured that the incubation time employed is sufficient for a control ampoule (sample without biocide) to yield a yellow result. Variations in incubation time or temperatures will yield a different response and so a standard incubation time and temperature must be adopted.

Note. If you are using a water bath you should check the temperature of the water at the level that the ampoules are incubating; Also ensure the ampoule is sealed with its foil lid to avoid excess condensation inside.

1.2 Results

After the specified incubation time assess the colour of the ampoule.

2. Examples.

The following two examples of use are typical. Most other applications can be derived from them.

2.1 Maintaining Target Concentrations Of Biocide Or Preservative In Process Water Or An Industrial Fluid

In controlling microbial growth and spoilage it is usual to have a target concentration at which the biocide should be maintained e.g. 1000 ppm. The monitor can be used to detect whether the biocide/preservative level has fallen below or is in excess of the target level. The Minimum Inhibitory Concentration (MIC) of a biocide is the lowest concentration at which the biocide will inhibit the Biocide Rapide *Bacillus*. This concentration can be determined by experiment and is available from ECHA for common biocides. If the target concentration of biocide is divided by the MIC the result is the number of times the sample must be diluted before testing. For Example: If you are using Biocide X (Biocide Rapide MIC 50 ppm) in a metal working fluid and you wish to maintain a target

concentration of above 1000 ppm then before testing a sample should be diluted by:

$$\frac{\text{Target Concentration}}{\text{MIC}} = \frac{1000}{50} = 20 \text{ x}$$

Thus the sample must be diluted 20 times i.e. add 19 volumes of deionised water to 1 volume of sample and mix thoroughly before testing. A purple ampoule indicates that the target concentration has been achieved (20 x 50 = 1000 ppm).

Other dilutions can be made up and tested if more information is required. For example if a times 10 dilution (9 volumes of water to 1 volume of sample) is also tested at the same time the following results could occur.

X10 and x20 dilutions: Both ampoules purple – no action (Concentration above 1000 ppm)

x10 dilution purple ampoule x 20 dilution yellow ampoule – top-up with 500 ppm biocide. (concentration was between 500 and 1000 ppm)

x10 and x20 dilutions: Both ampoules yellow – top-up with 1000 ppm biocide (Concentration was below 500 ppm)

A greater range of dilutions will allow more precise assays and top-ups and can also detect over-dosing.

2.2 Quality Control Tests And Shelf-Life Of Preserved Products

It is not always necessary to know which preservative is in use or it's MIC. For example, in a shelf-life study, a preserved aqueous product at the time of manufacture is diluted 20, 30, 40, 50, 60 and 70 times and tested with the following results:

X20 – purple; x30 – purple; x40 – purple; x50 – purple; x60 – purple; x70 – yellow
(MIC of preservative is present in the x60 dilution)

After storage for six months the dilutions and tests are repeated with the following results:

X20 – purple; x30 – purple; x40 to x70 – yellow
(MIC of preservative is now present in the x30 dilution) Thus about half of the preservative activity has been lost. A similar procedure can be used to check that a Batch of manufactured preserved product contains the intended biocide concentration.

3 Determining The MIC Of A Biocide

The estimation of the MIC for a biocide is a comparatively simple operation. A range of concentrations of biocide is made in de-ionised water and the Biocide Rapide used to test them. The lowest concentration which yields a purple result is the MIC. In estimating the MIC approximately 5% increments in concentration are adequate.

4 Disposal

Used Biocide Rapide ampoules must be disposed of by incineration, autoclaving or disinfection (pierce or remove seal before immersion).

5 Typical Applications

5.1 Assay of biocides added to industrial fluids during use.

e.g. metal working fluids, process water, detergent baths, electrophoretic paints.

5.2 Shock dosing of biocide.

e.g. cooling water.

5.3 Biocides in fuel.

Test an aqueous extraction or test fuel directly, contact ECHA for advice.

5.4 Biocides in waste effluents.

5.5 Shelf life of preserved products.

e.g. paints, cosmetics, adhesives.

5.6 Disinfectants.

Useful life of disinfectants e.g. hospitals, clean rooms, surgeries, agriculture, food processing.

6 Technical Support

The MIC's and examples given in our technical leaflets are based on dilutions of biocides in water. We cannot guarantee that the Biocide Rapide test will work for all industrial fluids or all anti-microbial chemicals. Our advice is always freely available. Back-up can be provided to calibrate the monitor for unusual fluids and biocides or biocide mixtures.

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