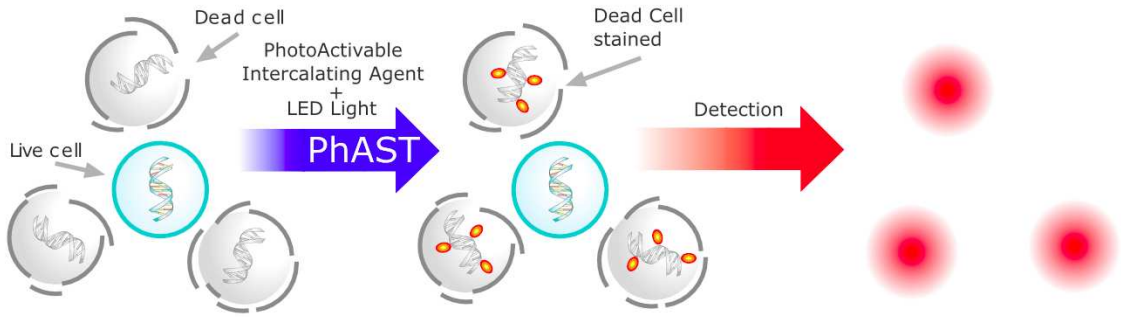
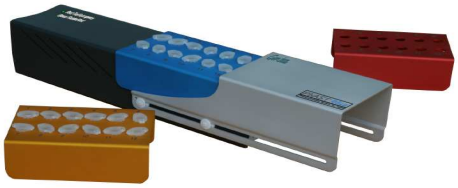
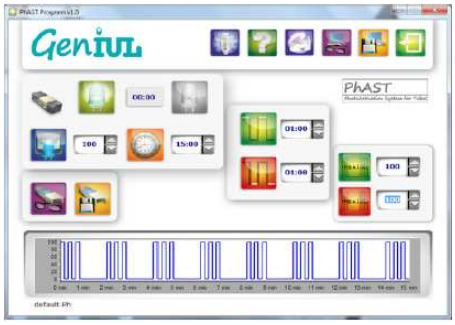
# Nonviable cell staining for microscopy detection

The evaluation of microorganism survival is currently based on cultivation assays or more recently with viable PCR methods. Plate counts are time-consuming and biased by the fact that some microorganisms remains viable but non cultivable. On the other hand, a viable PCR method is a promising approach, but requires a good background in molecular biology. Methods based on fluorescence microscopy, have been successfully used for many years and one of the most used reagents to stain dead cells has been Propidium Iodide. Nowadays their azide derivative, Propidium Monoazide, combines the specificity of staining dead cells with the strong capability to an irreversible bound to DNA by the means of photo -reactive process. Now is possible to stain dead cells prior to fixation and store the sample until the analysis.






## PhAST Blue the solution for precise dead cell staining

### Efficiency

PhAST Blue combines high power LED with the proper optical alignment of the reaction tube to ensure the maximum cross-link efficiency

### Reproducibility and Speed

PhAST Blue improves reproducibility and avoids variations due to manual photoactivation. The PhAST is thermally stable with a constant and uniform light dose, and allows simultaneous photo-activation of 12 samples .

### Flexibility

PhAST Blue allows optimizing staining method by programming different parameters such as light intensities and photo-activation times.

# Protocol

## Propidium Monoazide Staining of Nonviable Cells Prior to Fixation

1) The cell suspension ($10^6$ cells) should be clear as possible, for this reason wash with PBS with at least one step of centrifugation and mix.

2) Incubate in the dark (10-50 min) with Propidium monoazide (50 µM final). During incubation agitate de suspension several times in order to improve the reagent diffusion.

3) Place the tubes in the PhAST Blue and treat the samples at continuous light, 5-10 min at 100% power.

4) Centrifuge the sample and discard the supernatant resuspend with PBS – 1% paraformaldehyde. Incubate 1 hr at room temperature

Sample is ready to be analyzed, stored or for apply a second staining procedure with other reagents if is necessary. Examine by Epifluorescence microscopy using an excitation filter with 546 nm (±12 nm) and a long pass filter (>590 nm) for detection

## ORDERING INFORMATION

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| Cat. No. 9000700 | PhAST Blue Main Unit. |
| Cat No. 90001100 | Pack of 6 Blue Color Holders for 12 microtubes. |
| Cat No. 90001101 | Pack of 6 Red Color Holders for 12 microtubes. |
| Cat. No. 90001102 | Pack of 6 Gold Color Holders for 12 microtubes. |
| Cat. No. 90001103 | Pack of 6 Holders for 12 microtubes, 2 Blue 2 Red, 2 Gold. |
| Cat. No. 900015000 | Box of 50 Ethidium Monoazide mono dose microtubes. |
| Cat. No. 900015004 | Pack of 4 boxes of 50 Ethidium Monoazide monodoses each. |
| Cat. No. 900016000 | Ethidium Monoazide 5 mg: 10 vials of 0,5 mg |
| Cat. No. 900017000 | Propidium Monoazide 1 mg: 2 vials of 0,5 mg |

## REFERENCES

Detecting inactivated endospores in fluorescence microscopy using propidium monoazide. Probst et al (2012). Interational Journal of Astrobiology 11(2):117-123