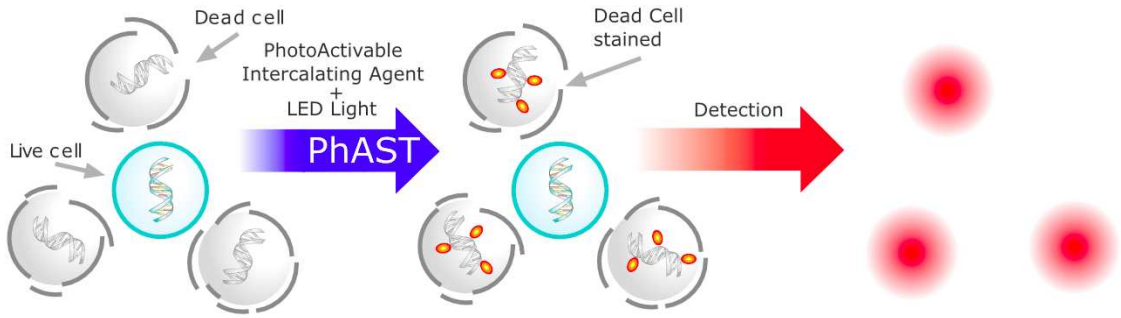
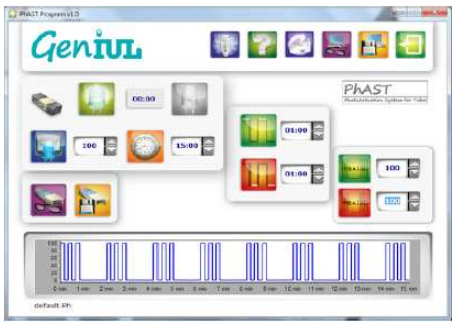
Nonviable cell Staining

Note

Assessment of cell viability in fixed cells by Flow Cytometry

Often, for safety reasons or for convenience, it is frequently necessary to fix cells prior to analysis. Later data analysis will be less ambiguous if nonviable or damaged cells can be eliminated. Flow cytometry determination of viable and non-viable cells in fixed samples can be accomplished by using Ethidium monoazide (EMA) and Propidium monoazide (PMA). They are photo-reactive derivatives from Ethidium Bromide and Propdium Iodide, andboth are positively charged molecules which are excluded by cell with intact membranes, but enter cells with damaged membrane,. These dyes can be photo-chemically cross-linked with short exposure to visible light after the excess dye is washed away, the cells are fixed.





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.

Photolabeling Protocol

Ethidium Monoazide or Propidium Monoazide Staining of Nonviable Cells Prior to Fixation

1) Sample turbidity might affect the labeling efficiency by reducing the light intensity required for dye photoactivation, or capturing dye molecules, so whasing of cells with PBS is advisable.

2) Incubate in the dark (10 min) with Ethidium Monoazide or Propidium monoazide (5-10 μg/ml final). During incubation agitate de suspension several times in order to improve the reagent diffusion.

3) Place the tubes in the PhAST Blue and expose the samples to light during 5-10 min at 100% power.

4) Centrifuge the sample and discard the supernatant. Resuspend the cell pellet in PBS – 1% paraformaldehyde, and incubate at room temperature for 1 hr.

Sample is ready to be analyzed or to be subjected to a second staining procedure with other reagents if it is necessary. Phenanthridium azide derivatives should be analyzed on flow cytometer with excitation at 488 nm, and collect fluorescence emission using a ≥ 630 nm long-pas filter; amplify PMT output logarithmically. Ethidium monoazide does not fluoresce as brightly as Propidium monoazide, so discrimination of nonviable Ethidium Monoazide-bright cells from viable cells may be less obvious.

ORDERING INFORMATION

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 5mg: 10 vials of 0,5 mg
Cat. No. 900017000	Propidium Monoazide 1 mg: 2 vials of 0,5 mg