Celluminate Blue

rev.10/24

Cat#:	TCT-CLB001-1
Available Size:	5 mg
Form:	Solid
Storage conditions:	-20℃, Protect from Light, Moisture
Shipping conditions:	Room Temperature in continental US; may vary elsewhere
Emission:	355 ± 2 nm
Excitation:	465± 2 nm
Purity:	≥95% by HPLC
Solubility:	DMSO
Sample Type:	Adherent or suspension cells

Application:
Celluminate Blue is a cell-permeant fluorescent dye for monitoring long-term cell location and movement for its stability in live cells. It stably fluoresces for at least 72 hours without affecting cell viability or proliferation, allowing its use in cell tracking. It can also be used for multi generational cell movement tracking. Its blue excitation/emission spectra are ideal for use in the FL1 channel of VisionSort. It can also be used for flow cytometry and fluorescence microscopy applications.

Materials required but not supplied:

- 1X PBS
- Serum free medium
- VS Live Suspension solution

Staining protocol using Celluminate Blue dye:

Preparation of Stock Solution (10mM):
Warm vial to room temperature before opening. To dissolve lyophilised product, add **2.385 mL of** anhydrous DMSO to the vial.
Divide the stock solution into desired aliquots for storage and avoid repeated freeze and thaw cycles.

Preparation of Working Solution:
Dilute stock solution to final working solution of **10 µM** in serum free medium

Preparing Cells for staining :
Culture the cells in the appropriate growth medium beforehand.

Staining of Cells:

1. Centrifuge cells at 500g for 5 min at RT (adjust force and time depends on your cell type)
2. Wash with serum free RPMI medium/ 1X PBS
3. Resuspend 1x10^6 in **1 mL** of pre-warmed serum free medium containing Celluminate Blue working solution (**10 µM)**
4. Incubate cells for 30 mins at 37℃
5. Centrifuge cells at 500g for 7 min at RT and remove Celluminate Blue working solution
6. Wash cells twice with VS Live Suspension solution
7. Add VS Live Suspension Solution and adjust concentration to 1x10^6 cells/mL
8. Run cells on VisionSort. In the example below, 10,000 events were recorded using the FL-1 channel

For best results. Please use three validation controls :

- Stained Cells
- Unstained Cells
- Mix of Stained and Unstained Cell suspension in 1 : 1 ratio

Example Results:

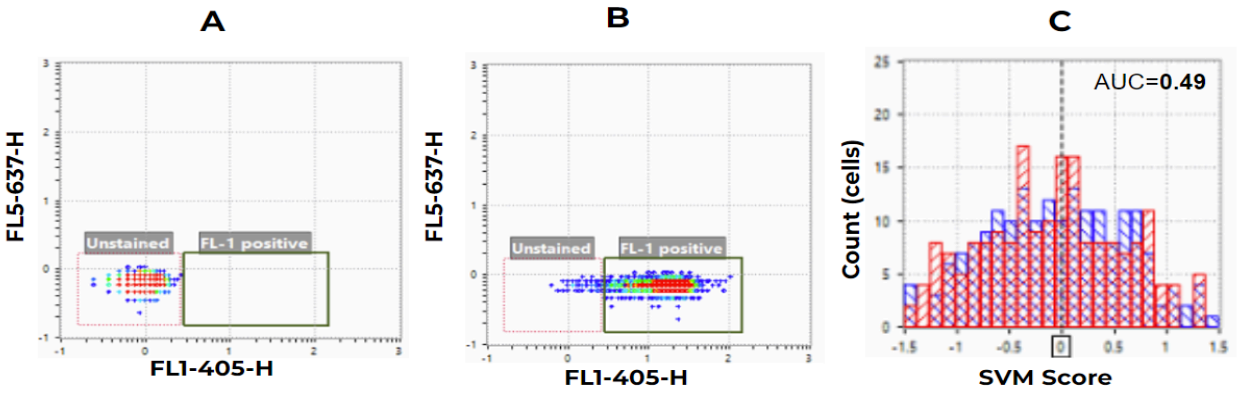

Figure 1. Celluminate dyes do not affect cell morphology and classifier performance. Pan-T cells were stained with Celluminate Blue and used to generate a supervised machine learning classifier on VisionSort. Unstained (A) and stained (B) cell populations were used to define two ground truth cell populations. The resulting classifier (C) shows an AUC score of 0.49 (C) for differentiating stained (red) and unstained (blue) cells, confirming that labeling Pan T cells with Celluminate Blue does not affect cell morphology.

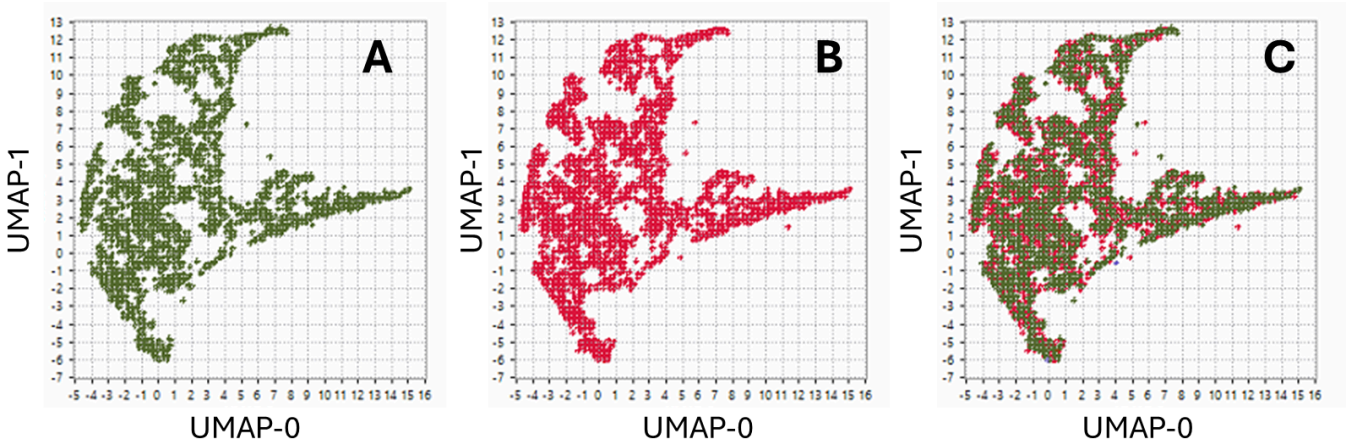

Figure 2. Celluminate Blue does not affect cell morphology. Pan-T cells were stained with Celluminate Blue and used to generate UMAP projections using unsupervised machine learning based on fsGMI waveforms on VisionSort. Stained (A) and unstained (B) cell populations are inseparable (C) when overlaid, indicating Celluminate Blue does not affect cell morphology.

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