Celluminate Kit

rev.11/24

Cat# TCK003

I. INTRODUCTION:

The Celluminate kit includes a group of versatile fluorescent dyes used for cell labeling and cell tracing. The dyes are non-toxic and stably fluoresce for at least 72 hours without affecting cell viability or proliferation. The dyes are retained within cells, allowing for the study of cell division, migration, and lineage tracking over extended time periods. Their wide range of Excitation/Emission spectra are ideal for use with multiplex channels and lasers.

II. MATERIALS PROVIDED:

Cat#	Name	Volume/Units	Ex/Em(nm)	Fluorescence Channel	Storage Temp
TCT-CLG001-1	Celluminate Green	20 x 50 ug	492/517	FL-2	-20℃
TCT-CLB001-1	Celluminate Blue	5 mg	371/464	FL-1	-20℃

III. Key Features:

- Detection Method: Fluorescence
- Platforms: Flow cytometry, Ghost Cytometry, Fluorescence microscopy

IV. Sample type:

- Live Cells

V. Shipping and Storage:

- Shipping Temp: -4℃
- Storage Temp: -20℃

VI. Preparation of Stock and Working Solution

- **For Celluminate Blue:**
  Add **2.385 mL** of anhydrous DMSO to **5 mg** of Celluminate Blue to make 10 mM stock solution, final working solution is 10 nM (1/1000 dil)

- **For Celluminate Green :**
  Add **10.8 uL** of anhydrous DMSO to **50 ug** of Celluminate Green to make a 10 mM stock, final working solution is 2 nM (1/5000 dil)

Note:
Warm vial to room temperature before opening. Divide the stock solution into single aliquots for storage and avoid repeated freeze and thaw cycles.

Preparing cells for staining :
This protocol can be used for a wide range of cell types and culture conditions (adherent and suspension cells). Culture the cells in the appropriate growth medium beforehand.

VII. Cell Staining:

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
3. Resuspend $1x10^6$ cells in CellTracker dye diluted in 1mL of serum free medium
4. Incubate cells for 30 mins at 37°C
5. Wash cells with serum free medium once
6. Resuspend cells in VS Live Suspension Solution
7. Adjust concentration to $1x10^6$ cells/mL
8. Run cells on VisionSort

The use of three validation controls is suggested:

- Stained cells
- Unstained cells
- Mix of Stained and Unstained cells in a 1 : 1 ratio

VIII. EXAMPLE RESULTS:

For Celluminate Blue

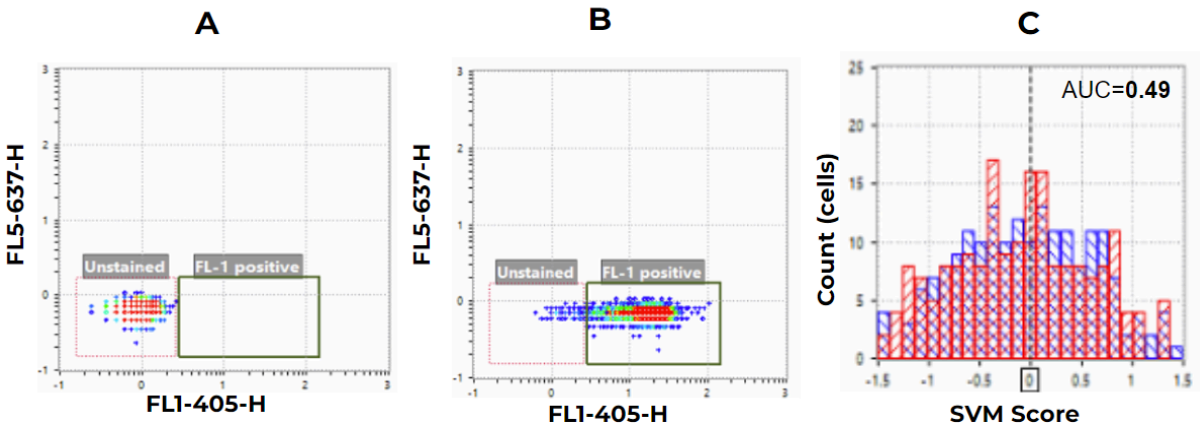

Figure 1. Celluminate dyes do not affect cell morphology and classifier performance. Pan-T cells were stained with Celluminate Blue and used to generate a supervised machine learning classifier on VisionSort. Unstained (A) and Stained (B) cell populations were used to define two ground truth cell populations. 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.

For Celluminate Green

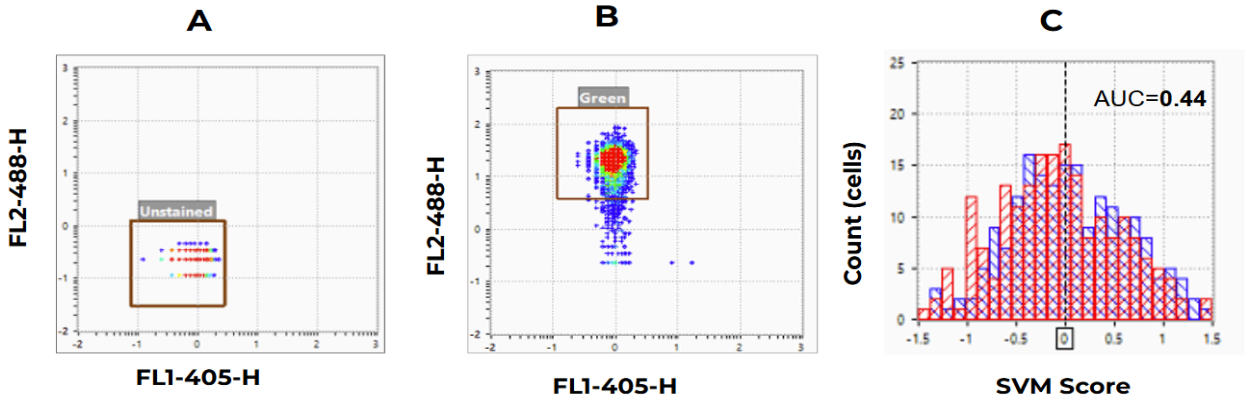

Figure 2. Celluminate dyes do not affect cell morphology and classifier performance. Pan-T cells were stained with Celluminate Green and used to generate a supervised machine learning classifier on VisionSort. Unstained (A) and Stained (B) cell populations were used to define two ground truth cell populations. The resulting classifier (C) shows an AUC score of 0.44 (C) for differentiating stained (red) and unstained (blue) cells, confirming that labeling Pan T cells with Celluminate Green does not affect cell morphology.

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