SARTORIUS

Product Guide

iQue® Cell Proliferation and Encoding (V/Blue) Dye

Product Information

Presentation, Storage and Stability

Compatible with iQue® platform with VBR option		
Product Name	**Cat No**	**Format**
iQue® Cell Proliferation and Encoding (V/Blue) Dye	97054	5x384 wells
iQue® Cell Proliferation and Encoding (V/Blue) Dye	97055	20x384 wells
iQue® Cell Proliferation and Encoding (V/Blue) Dye	97056	50x384 wells

Table 1. Product Information

Kit Components	Cat No 97054 5x384 well	Cat No 97055 20x384 well	Cat No 97056 50x384 well	Storage	Stability
iQue® Cell Proliferation and Encoding (V/Blue) Tag-It Violet™ Dye	1 vial 122.25 µg lyophilized	4 vials 122.25 µg lyophilized	10 vials 122.25 µg lyophilized	-20°C	Minimum 6 month shelf life; up to one year
DMSO	1 vial	1 vial	2 vials	-20°C	

Table 2. Kit Components and Storage

Note: Do not open vials until needed. Once DMSO is added to the iQue® Cell Proliferation and Encoding (V/Blue) Tag-It Violet™ Dye use immediately, or store at -20°C, protected from light for no more than one month. A kit manual and a USB key with assay templates are also included in the kit Package

This protocol describes a solution for cell tracking and for proliferation assays. The iQue® Cell Proliferation and Encoding (V/Blue) Tag-It Violet™ Dye passively diffuses into the cell then covalently binds to intracellular proteins enabling its long-term retention. This method has been optimized for the iQue® platform combining high throughput sampling, flow cytometry detection, and multiplexing capabilities.

Required Materials

- Cell Proliferation and Encoding (V/Blue) Tag-It Violet™ Dye
- DMSO
- Target cells of interest and appropriate cell culture media
- Protein-free buffer such as phosphate buffered saline (PBS)
- 96-well v-bottom plate (iQue® #10149) or 384-well v-bottom plates (Greiner #781280)
- iQue® 3 or iQue® platform with violet laser (VBR configuration)

Required Materials

For proliferation studies, it is strongly recommended that a non-proliferation control such as cells treated with mitomycin C (at 10µg/mL, not provided) and an unstained cell control growth medium be included.

Quick Guide – Encoding

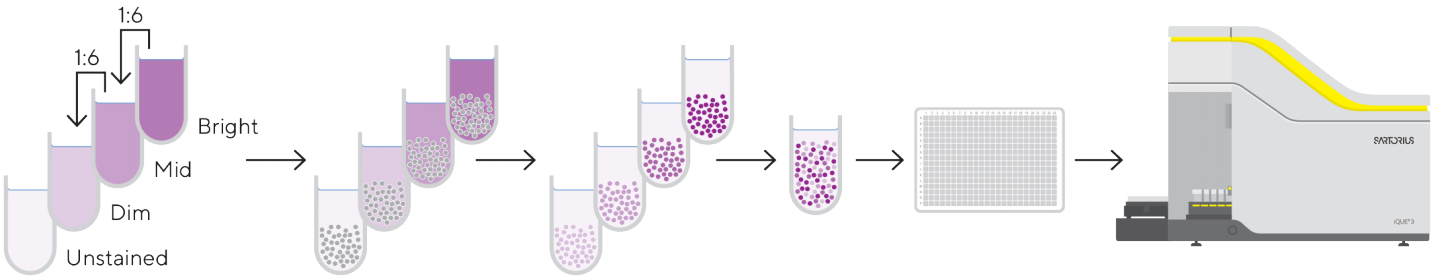

Figure 1.

Fluorescent cell encoding is a technique where different cell populations are labeled with different concentrations of a fluorophore. This establishes a unique signature of fluorescence intensity that enables multiplexing of different cell types in a single well, and up to 4 different cell populations.

Proliferation Principle

	Dead Cells	Latent Cells	Proliferating Cells
Generation 0			
Generation 1			
Generation 2			

Figure 2.

Proliferating cells will have decreasing amounts of dye, corresponding to lower fluorescence intensities. Dead or latent cells will maintain the initial dye intensity, which enables easy discrimination between proliferated and non-proliferated cells.

Cell Proliferation and Encoding (V/Blue) Tag-It Violet™ Dye Protocol

If you anticipate that you will not have enough cell events to reach statistical significance of your cell population of interest, you may have to modify your assay protocol using one or more of the optional protocols below.

1. Dye Preparation

1.1 Bring the reagent to room temperature.

1.2 Briefly centrifuge the vial(s) of lyophilized reagent to ensure the reagent is at the bottom of the vial. Add **50 µL** of anhydrous DMSO to one vial of Cell Proliferation and Encoding (V/Blue) Tag-It Violet™ Dye until fully dissolved to make a **5 mM** stock solution.

2. Labeling Procedure

2.1 Prepare Bright working solution by diluting **5 µL of 5 mM** stock solution in **5 mL** of PBS. Up to two additional working solution intersities (Mid and Dim) can be prepared by further 1:6 dilution in PBS of the Bright and Mid working solutions, respectively.

2.2 Count required cells, centrifugre, and resuspend in PBS at 2 x 10^6 cells/mL.

2.3 Combine an equal volume of cells and Bright working stock. For encoding multiple cell populations, combine an equal volume of desired cells with Mid and Dim working stocks. A fourth cell population can be prepared by leaving cells unstained.

2.4 Incubate cells for 15 minutes at 37°C, protected from light.

2.5 Quench by adding 5x the staining volume of cell culture media containing 10% FBS.

2.6 Centrifuge cells, discard supernatant and repeat wash with cell culture media.

2.7 Resuspend cells in cell culture media to desired assay concentration.

2.8 Verify encoding by analyzing cells on the iQue® platform or iQue® 3 (VBR).

Note: Encoded cells should be used immediately in assay after combination (~4 hours) as the integrity of the discreet encoded populations will degrade with cellular division.

3. Sample Acquisition

3.1 Launch iQue Forecyt® Software.

3.2 If you have not already imported the template (on the provided USB key), import it now and select the correct software version (6.0 or higher) and plate format (96- or 384-well).

3.3 Create a New Experiment using the template:

File → New Experiment→ Use Template → Experiment Name

3.4 Define samples and controls on your plate in the Design tab.

3.5 Click "Run" on the Controller to acquire plate.

4. Data Analysis

4.1 Identify cell populations. The template has gates that are pre-set for different populations: cells. single cells, uncoded (Unstained, Dim, Mid, and Bright), and proliferation (unstained cells, proliferated, and non-proliferated). Below are the gating details for manually drawing the gates. It may be necessary to drag and adjust existing gates to fit cell populations.

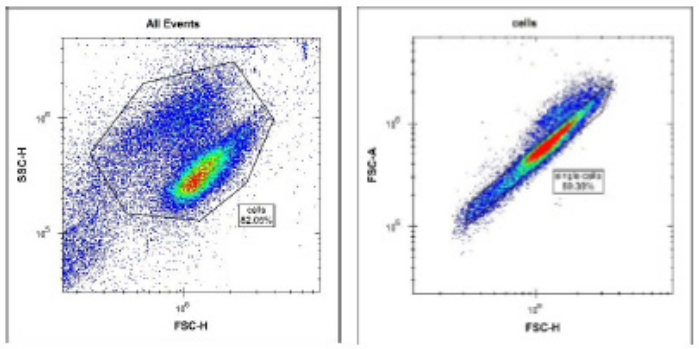

Figure 3.

4.2 In All Events, FSC-H vs. SSC-H, draw gate around "cells."

4.3 Make a new plot, FSC-H vs FSC-A, gating on "cells" draw another gate around "single cells."

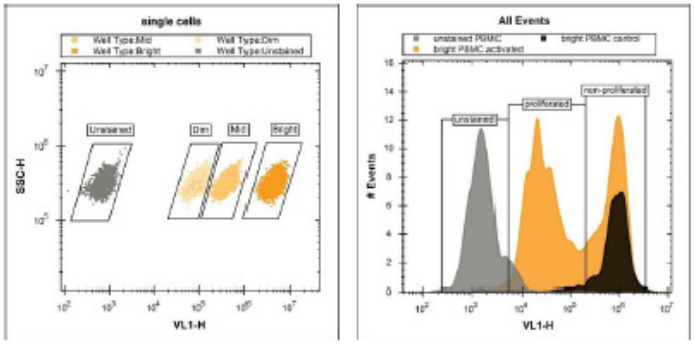

Figure 4.

4.4 For encoding, make a new plot based on "single cells" in VL1_H vs. SSC-H. The Bright, Mid, Dim, and Unstained populations can be separated with the brightest populations furthest to the right.

4.5 For proliferation, make a histogram based on "single cells" and change the x-axis to VL-H. Draw a gate on the unstained cells (furthest to the left) and the non-proliferated cells (furthest to the right). Draw a gate on the proliferated cells between the two gates.

Sales and Service Contacts

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