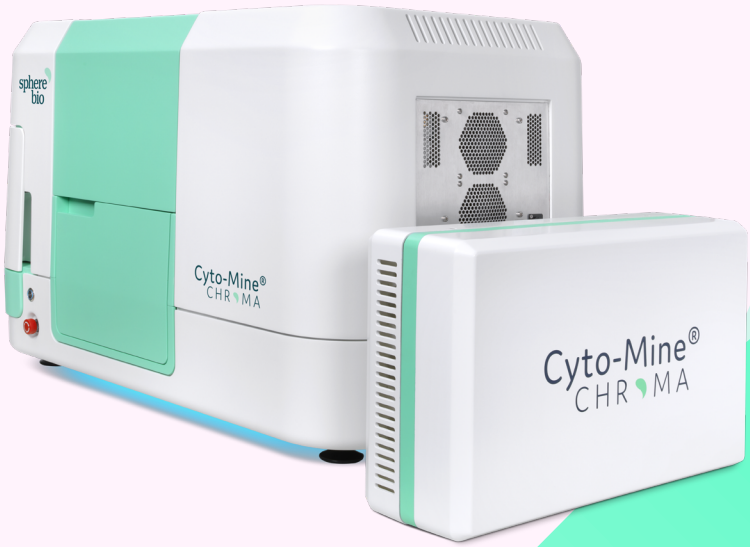
User Guide

Viability Probe User Guide





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1. Introduction

1.1 About this Guide

This user guide provides comprehensive instructions for performing cell viability staining using the Fluidic Sciences C315 Viability Probe. It includes detailed information on the assay principle, workflow, required materials, step-by-step protocols, data analysis guidance, and technical support resources.

1.2 Product Information

Product Code	Product Name	Contents	Storage	Detection Target	Platform Compatibility	Shelf Life	Animal Origin
C315	Viability Probe	5 vials of Viability Probe	Store at 4-8°C	Apoptotic/ Dead Cells	Cyto-Mine® Chroma	See Product Expiry Date	Animal Origin Free

1.3 Overview of the Viability Probe assay

The Viability Probe is an assay kit designed for use with the Cyto-Mine® Chroma platform. It contains a fluorescent probe that enables detection of cell viability at the single-cell level within picodroplets.

The Viability Probe can be used in conjunction with other cellular markers or assays. When used in conjunction with FRET Cyto-Cellect® assay kits and Cyto-Mine® Chroma, the assay enables high-throughput identification and selection of single cells that secrete IgG antibodies, while concurrently assessing their apoptotic state. This dual-parameter analysis supports more informed clone selection, improving both productivity and cell health screening in one automated workflow.

Note: *If the Viability Probe is used in combination with FRET Cyto-Cellect® assay kits, please refer to the User Guide for each Cyto-Cellect® assay kit to ensure compatibility with your secreted molecule of interest.*

1.4 Intended Use and Applications

The assay is intended for research use only, by enabling rapid exclusion of apoptotic or unhealthy cells, thereby improving the efficiency and success rate of the downstream process. It is not intended for medical diagnostic, therapeutic or prophylactic purposes.

1.5 Storage and Stability

Store at 4-8°C

Note: *Always check expiry dates before use and avoid using expired materials.*

2. Assay Principle and Workflow

2.1 Assay Concept

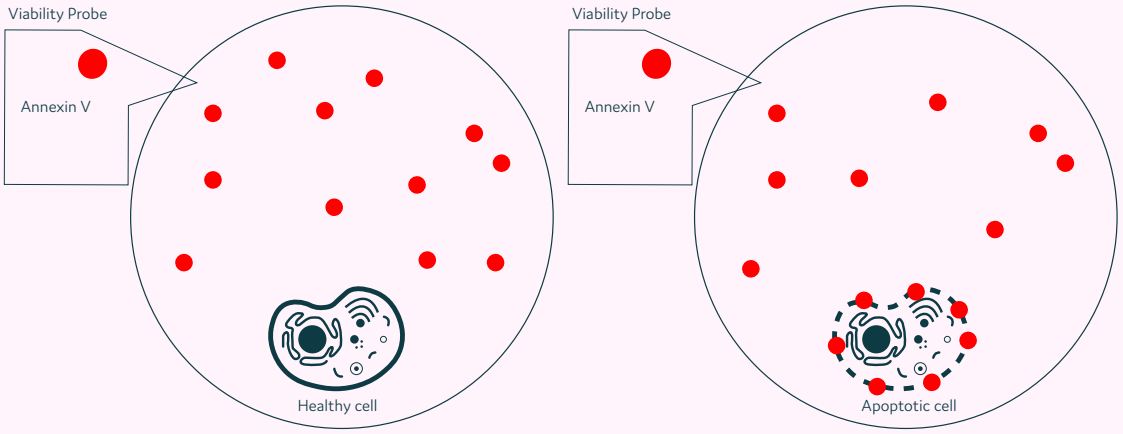

Figure 1. Schematic diagram for Viability Probe assay on Cyto-Mine® Chroma.

The Viability Probe consists of Annexin V molecules conjugated to a far-red fluorophore, designed to detect apoptotic and dead cells. During apoptosis, phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane. The exposed PS is specifically bound by the fluorescently labelled Annexin V, allowing apoptotic cells to be detected through localization of far-red fluorescence signal at the cell surface (**Figure 1**). These probes are co-encapsulated with individual cells into picodroplets for a subsequent incubation. During analysis and sorting on the Cyto-Mine® Chroma system, each droplet is assessed for localized far-red fluorescence. This enables evaluation of cell viability at the single-cell level. Users can apply gating strategies to select droplets that are far-red-negative, thereby enriching for healthy cells while excluding those that are apoptotic.

2.2 Key Components and Reagents

Assay kit contents:

- 5 x vials of Viability Probe

Other materials and reagents (not included in the kit):

Product Name	Supplier	Product Code
Cyto-Mine® Chroma	Fluidic Sciences	S008
Cyto-Cartridge® (Pack of 5)	Fluidic Sciences	C302
Cyto-Surf® A (500 mL)	Fluidic Sciences	C303
Cyto-Surf® B (500 mL)	Fluidic Sciences	C304
OptiPrep™ Density Gradient Medium	Sigma-Aldrich Merck	D1556
Poloxamer 188 Non-ionic Surfactant (10%)	Thermo Fisher Scientific	24040-032
Disposable cell strainers, 30 µm, sterile	Celltrics, Sysmex Partec	04-004-2326
Preferred culture medium (fresh)	User Preference	User Preference
Centrifuge	User Preference	User Preference
Cell Counter	User Preference	User Preference
Pipettes	User Preference	User Preference

2.3 Protocol for use of Viability Probe in Cyto-Mine® Chroma

Before starting :

- Make sure the pipettes used have a valid calibration.
- Use best practice when pipetting, to ensure precision and accuracy.
- All steps involving handling any stock solution should be carried out in a Class II Microbiology Safety Cabinet (MBSC).
- Refer to the Cyto-Mine® Chroma User Manual for detailed operating instructions.

Step 1: Preparing the Encapsulation Medium

1. Prepare Encapsulation Medium, according to the table below.

Note: *We recommend preparing at least 2 mL of the Encapsulation Medium to allow for cell counting and sample loss during preparation.*

Reagent	% of total	For 2 mL
Preferred culture medium (fresh)	83 % (v/v)	1,660 µL
OptiPrep™ Density Gradient Medium	16 % (v/v)	320 µL
Poloxamer 188 Non-ionic Surfactant (100X)	1 % (v/v)	20 µL

2. Keep the Encapsulation Medium cold while preparing the cells.

Step 2: Preparing the Cell Sample

1. Harvest cells from the culture flask and determine cell density and viability
2. Calculate the volume containing the desired number of cells for the experiment.

Note: *We recommend an input cell density of 100,000-300,000 (0.1×10^6 - 0.3×10^6) cells/mL to minimize the number of picodroplets containing > 1 cell. This concentration should be optimized for the individual experiment. Please contact support@fluidic.com for further information.*

3. Pipette the required volume of cell suspension into a sterile microcentrifuge tube
4. Centrifuge cells at **300 x g** for **5 minutes** at room temperature

Note: *the centrifugation speed may vary depending on the cell line and should be optimized accordingly*

5. Remove and discard supernatant
6. Resuspend cells in fresh culture medium equilibrated to room temperature
7. Repeat centrifugation at room temperature
8. Remove and discard supernatant
9. Resuspend cells in **100 µL Encapsulation Medium**
10. Take one vial of the **Viability Probe** from the fridge and spin briefly.

11. Add the **100 µL** cell suspension to the **Viability Probe** and mix.
12. Incubate for **15 min** at **room temperature** in the dark.
13. Add **900 µL encapsulation medium** to the sample.
14. Filter the cell suspension through a 30 µm cell strainer.
15. Determine the cell concentration (cells/mL) and viability (%).
16. If the Viability Probe is combined with one of the Cyto-Cellect® FRET assay kits, add the 1 mL cell suspension containing the Viability Probe to the vial containing FRET Probes (refer to the User Guides of the relevant Cyto-Cellect® assay kits for more information about the FRET probes).
17. Gently mix the cell suspension by pipetting up and down.

Step 3: Loading Cell Suspension

1. Transfer the 1 mL of cell suspension with the assay probes into the sample chamber on a Cyto-Cartridge®.
2. Load the Cyto-Cartridge® into the Cyto-Mine® Chroma.

Step 4: Picodroplet Generation

Refer to the Cyto-Mine® Chroma User Manual.

Step 5: Incubation

Refer to the Cyto-Mine® Chroma User Manual.

Step 6: Sorting

1. Setting detection conditions on Cyto-Mine® Chroma.

Using the Sensors tab (refer to the Cyto-Mine® Chroma User Manual), set up detection parameters as detailed below, aiming for 0.5-1V for the Viability Probe (Far-Red, see **Figure 2**). If Cyto-Cellect® FRET probes are used in combination to the Viability Probe, set up the signal height of the FRET probes (Green and Orange) to ~1-1.5V.

Viability Probe Only	Lasers	Red Laser (638 nm) for excitation of viability probe
	Detection Channels	678/30 (Far-Red) for detection of viability probe emission
	PMT Gains	Far-Red - starting from 0.4V, adjust so that picodroplet peaks reach Signal ~0.5V
	Droplet Definition	Far-Red Mode: Default Auto calculate Thresholds
Viability Probe with Cyto-Cellect® Probes	Lasers	Blue Laser (488 nm) for excitation of FRET Donor
		Red Laser (638 nm) for excitation of viability probe
	Detection Channels	520/18 (Green) for detection of FRET Donor emission
		605/20 (Orange) for detection of FRET Acceptor emission
		678/30 (Far-Red) for detection of viability probe emission
	PMT Gains	520/18 (Green) - starting from 0.4V, adjust so that picodroplet peaks reach Signal 1-1.5V
		605/20 (Orange) - starting from 0.4V, adjust so that picodroplet peaks reach Signal 1-1.5V
		678/30 (Far-Red) - starting from 0.4V, adjust so that picodroplet peaks reach Signal ~0.5V
	Droplet Definition	Green Mode: Default Auto calculate Thresholds

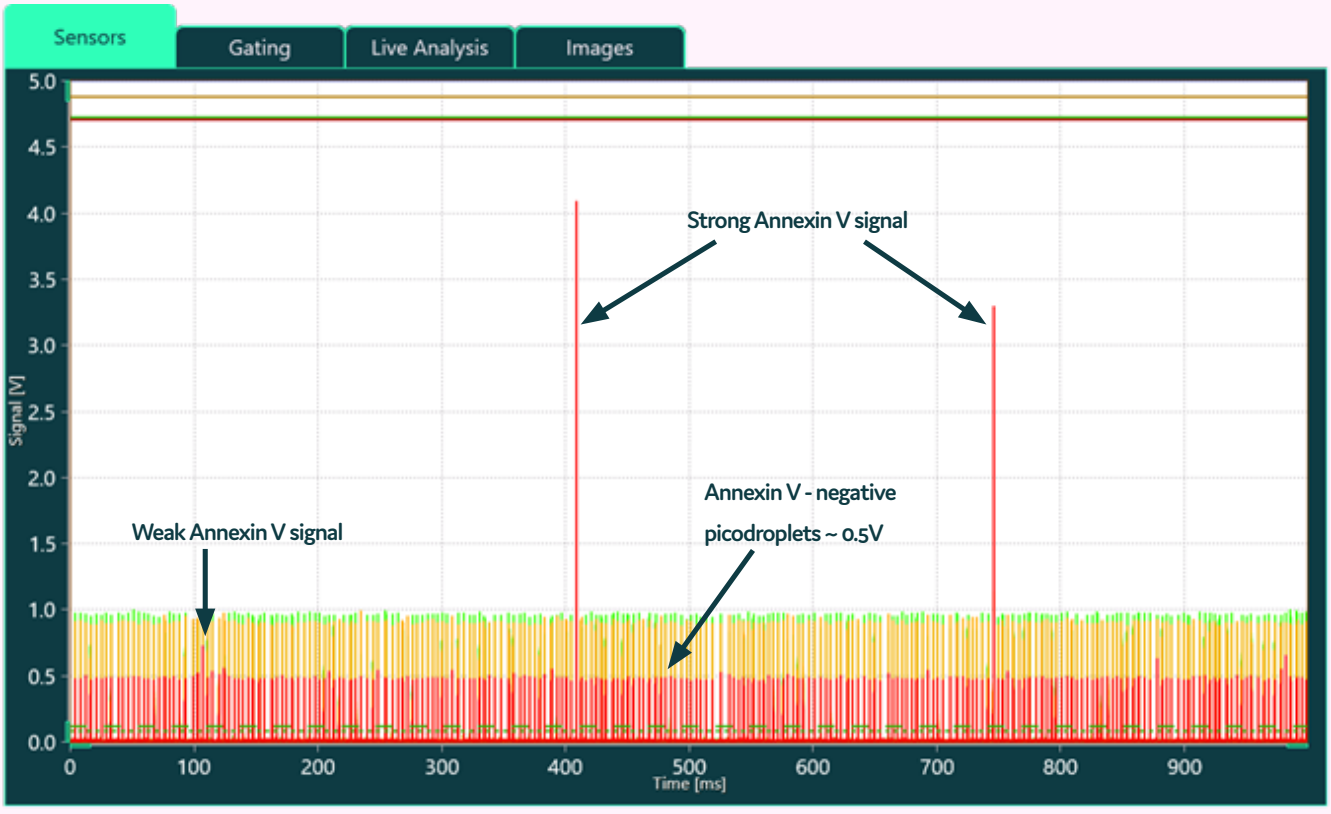

Figure 2. Screenshot of Peak signal height in the sensors tab.

2. Gating:

Use **Gate 1** if the **Viability Probe** is used alone. If **Cyto-Cellect® FRET assay kits** are also included, use both **Gates 1 and 2**.

Gate 1: Excluding non-viable cells

Gate 2: Include FRET$^+$ cells

Gate #	X-Axis	Y-Axis	Gating logic
1	Far-Red Peak (V)	Orange Average (V)	Exclude
2	Green Average (V)	Orange Average (V)	Include

Non-viable cells are Annexin V$^+$ (Far-Red Peak$^+$) and will be detected as localized fluorescence in the Far-Red PMT. They are best visualized in the Scatter Plot by plotting Orange Average vs Far Red Peak (see **Figure 3A**). The viable, antibody secreting cells are defined as Annexin V$^-$ (Far-Red Peak$^-$) and FRET$^+$ population. To gate on these cells, exclude the Far-Red$^+$ population (**Figure 3A**) before gating on FRET$^+$ population (**Figure 3B**).

Note: *The position of the viable (Annexin V negative) population on the Far-Red peak axis is dependent on the gain setting for the Far-Red PMT, which determines the signal height in the Sensors tab (**Figure 2**).*

*In the example above, as the main population of Annexin V negative picodroplets was set to a signal height of ~0.5 V (**Figure 2**), the corresponding gate extended from ~ 0.6 V – 4.7 V on the Far-Red Peak axis (**Figure 3B**). Setting the gate closer to 0.5 V would include also very weak Annexin V signals, but has a higher risk of gating false positives in Gate 1. Conversely, setting the gate farther to the right (>0.6 V) may exclude true Annexin V positive events*

Ultimately, the gating strategy should be tailored to the experimental objective. For example, if only a small number of FRET positive cells is expected, a more permissive gating approach may be useful to capture more potential candidates. However, if the goal is to optimize colony outgrowth, a stricter gating strategy could be more appropriate.

The FRET positive population typically shifts to the upper left of the FRET negative population, characterized by a decrease in Green and a corresponding increase in Orange Average fluorescence.

Step 6: Sorting

Refer to the Cyto-Mine® Chroma User Manual.

(A)



(B)

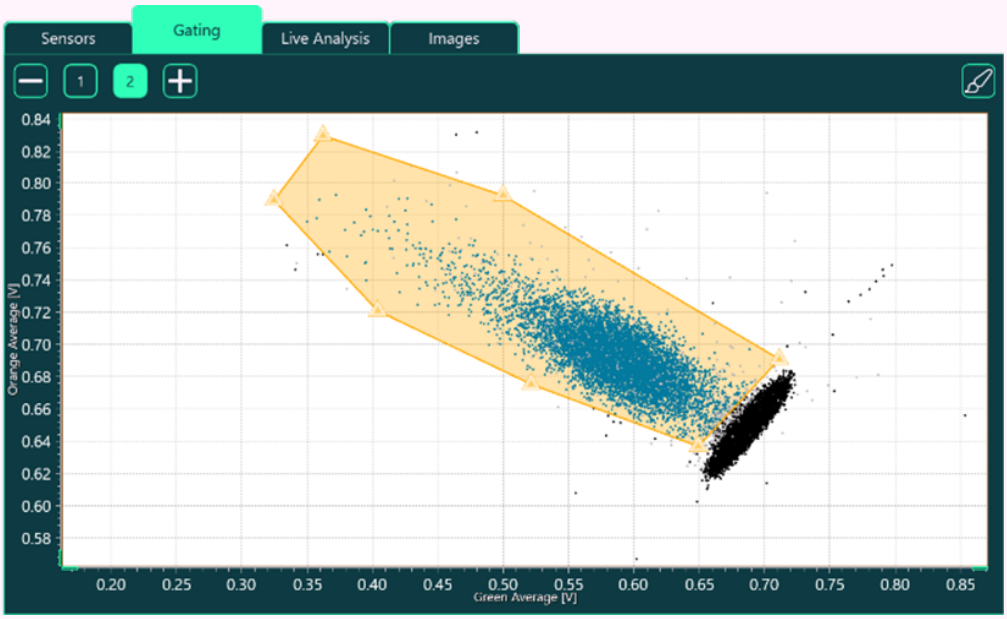

Figure 3. Gating strategy during sorting. A) Screenshot of a representative Sorting gate excluding picodroplets containing non-viable cell. B) Screenshot of a representative Sorting gate including the FRET⁺ population

Finish

3. Technical Support

For technical enquiries and support questions, please email support@fluidic.com.

Notes

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