ORFLO® | Personal Cell Analysis

MoxiCyte Viability Kit (#MXA055)
User's Guide
(Rev – 20150406)

FOR RESEARCH USE ONLY
on the Moxi Flow or Zepi Flow Instruments

Orflo Technologies, LLC
Ketchum, ID
Tech_support@orflo.com
1-855-879-6694

MoxiCyte Viability Kit (#MXA055)

Overview

The MoxiCyte Viability Kit (#MXA055) is a Propidium Iodide (PI) – based staining reagent designed for use with Orflo's Moxi Flow or Zepi Flow instruments to provide rapid assessment of the viability of a cell sample. The assay is based on the general membrane-integrity/dye-exclusion method of assessing cell viability. PI is a cell membrane-impermeant, DNA-intercalcating dye that increases fluorescence over 50x upon binding to DNA. For healthy cells, the dye is excluded and no fluorescence signal is associated with the cell event. However, as cells lose membrane integrity through necrosis or apoptosis, the PI permeates the cell and a strong fluorescence signal is generated. This kit has been specially formulated for use on the Orflo Moxi Flow and Zepi Flow instruments

Kit Components

- 111 test (15ml) vial of Orflo MoxiCyte Viability Kit Reagent

Storage and Handling

- Store the kit at 2-8°C
- Use product aseptically
- Minimize light exposure.

Protocol

1. Prepare cells into a single-cell suspension free of large (>30um) particulate.
    a. Notes:
        i. Primary harvests (e.g. blood or tissue extractions):
            1. If large extracellular debris is present, pass sample through a 40um cell strainer prior to remove it.
            2. If large amounts of RBCs are present in the sample, an appropriate RBC lyse method must be applied to get proper counts (RBCs will mask the signal of the cell population of interest due to their extremely high concentration levels).
        ii. Cell aggregation/Clustering – Clusters and aggregates can be dissociated through a combination of:
            1. Protease treatment – suspend cells in an appropriate dissociation reagent (e.g. Accumax or Accutase) for a minimum of 5 minutes.
            2. Pipette trituration (repeatedly, e.g. 10x, pull a substantial fraction of the cell sample in and out of a pipette tip). If a dissociation treatment was used, perform this step while cells are still suspended in the dissociation reagent.
2. Mix cell suspension and MoxiCyte Viability Kit reagent (invert several times to mix) according to the following table:

Concentration Of Original Cell Suspension	MoxiCyte Viability Kit Volume	Cell Suspension Volume	Dilution Factor
1 x 10^5 to 3 x 10^6 cells/ml	135 µL	15 µL	10x
3 x 10^6 to 6 x 10^6 cells/ml	190 µL	10 µL	20x
6 x 10^6 to 1 x 10^7 cells/ml	290 µL	10 µL	30x
>1 x 10^7 cells/ml	490 µL	10 µL	50x

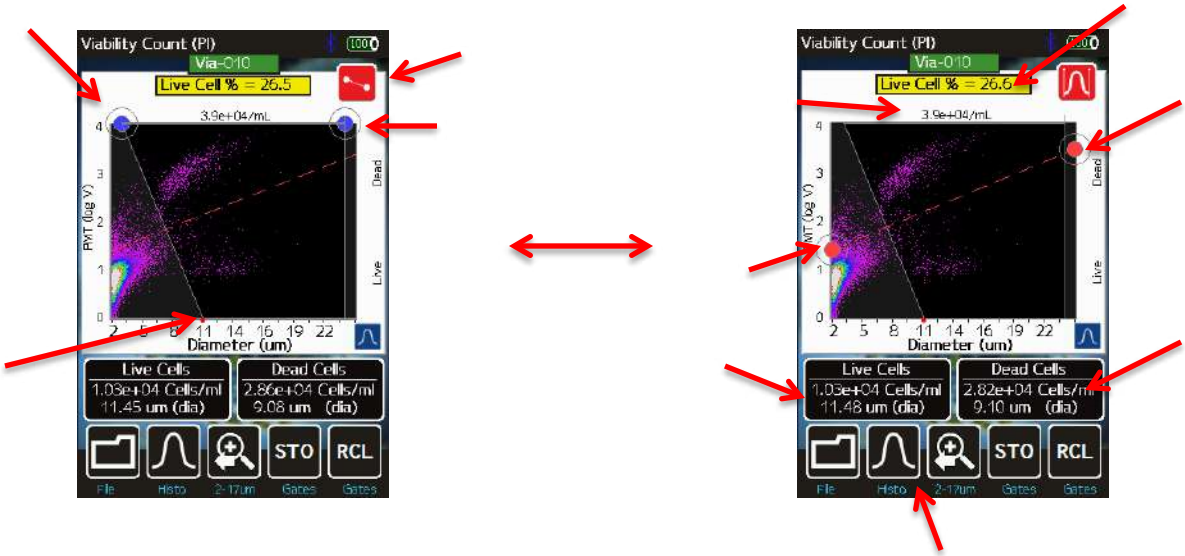
3. Incubate for 5 minutes in the dark at room temperature (25°C).
4. Analyze with the Moxi Flow using the "*Viability Count (PI)*" app within 20 minutes of initial mixing/staining.

Running a Test ***(Note: For detailed Moxi Flow Unit operation instructions and images, please see the latest User Guide at http://www.orflo.com/downloads.html)***

1. Prepare stained cell sample as described above.
2. Turn unit on and select "Viability Count (PI)" assay box.
3. Open both doors of the Moxi Flow unit and insert an unused cassette into the tray.
4. Close both doors and wait for the laser cassette alignment to complete.
5. Open the outer door only and pipette 75 µL (minimum) of sample into the cassette entry well. Mix sample well after incubation to ensure single-cell suspension.
    a. Notes:
        i. Avoid allowing pockets of air interspersed with fluid (pipette fast enough to prevent this).
        ii. It is OK to have a bead of fluid form on the entry well.
6. Close outer door and test will automatically run.

Analyzing Results ***(Note: For detailed Moxi Flow Unit operation instructions and images, please see the latest User Guide at http://www.orflo.com/downloads.html)***

1. Results are initially displayed as a Scatter/Dot Plot, Log[PMT V] vs. Diameter (µm), with cell population gates set from the prior test run.
2. Adjust the size gates to include the cell region by dragging the blue markers to define the appropriate size region (image below/left). Note: The left marker can be angled by toggling the left gate pivot (touch the red or green dot at the bottom of the gate to toggle).



3. Touch the red gate icon (top right of scatter plot, indicated in image to above/left) to enable fluorescent gating.
4. Drag the red markers to position the dashed red live/dead (fluorescence) gating marker (above right) between the live and dead clusters.
5. ***Sample/Test information*** (refer to image on the above/right):
    a. *Total Cell Concentration*: listed above the scatter/dot plot.
    b. *Viability Percentage*: listed in the yellow box above the scatter/dot plot.
    c. *Live Cell Concentration*: Listed in the black box at the bottom-left of the scatter/dot plot.
    d. *Live Cell Mean Diameter*: Listed in the black box at the bottom-left of the scatter/dot plot.
    e. *Dead Cell Concentration*: Listed in the black box at the bottom-right of the scatter/dot plot.
    f. *Dead Cell Mean Diameter*: Listed in the black box at the bottom-right of the scatter/dot plot.
    g. *Mean Fluorescence Intensity (MFI)*: Listed on the PMT histogram display for each cluster (Live/Dead). This can be accessed by touching the "Histo" icon (image on above/right) and selecting the "PMT Histo" icon.

Troubleshooting

- No/poor fluorescent separation –
    a. Check total cell concentration – Increase dilution factor (see table above) to achieve optimal concentrations.
    b. Kit may have expired or degraded – Optimal kit storage conditions are 2-8˚C in the dark. Always use a clean pipette tip to avoid contamination of the reagent. If you suspect the kit integrity may has been compromised, re-order new reagent.

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ncomplete test or poor fluid flow -

a. Over-concentration samples can result in clogging. Increase dilution factor (see table above) to achieve optimal concentrations.
b. Remove large particle/particulate by straining sample with a cell strainer (e.g. 40 µm).
c. Break apart cell clusters/aggregates with dissociation agent (Accutase (MXA020, http://shop.orflo.com/product_p/mxa020.htm) or Accumax (MXA021, http://shop.orflo.com/product_p/mxa021.htm) and/or pipette trituration.

- Cell population not visible/discernible -
  a. Re-scale x-axis after the test to better view smaller-diameter cell populations.
  b. Ensure minimum concentration (>1 x 10^5 cells/ml) of cells are present in the sample.
  c. Reduce potentially interfering cell size populations (e.g. peripheral cells, RBCs) via cell lyse, gradient centrifugation, or pre-plating techniques.