

## Application Note No. 994-4007

# Xcyto<sup>®</sup> 5

## Mitochondrial Potential Assay: Analysis of the Mitochondrial Membrane Potential using MitoView™ dyes and the Xcyto<sup>®</sup> 5 system

### Product description

The Xcyto<sup>®</sup> 5 image cytometer and XcytoView™ software integrate image acquisition, image analysis, feature extraction and data presentation in one step for seamless quantitative data of individual cells and cell populations within heterogeneous samples.

### Application

This application note describes a protocol to measure changes in the mitochondrial membrane potential of cells. Loss of mitochondrial membrane potential is known to precede apoptosis and chemical-hypoxia-induced necrosis. This assay provides a simple fluorescent-based method to distinguish polarized (healthy) cells from depolarized (apoptotic) cells. Since mitochondrial membrane potential is a key parameter of mitochondrial function, this assay can serve as an indicator of overall cell health.

### Introduction

Cell death can occur by two distinct mechanisms: necrosis or apoptosis. Necrosis occurs when cells are exposed to harsh physical or chemical stress (e.g., hypothermia, hypoxia) while apoptosis is a tightly controlled biochemical process by which the cell is active participant in its own termination (“cellular suicide”). Apoptosis is one of the main types of programmed cell death which occur in multicellular organisms. It is characterized by a series of events that lead to a variety of morphological and biochemical changes, including membrane blebbing, cell shrinkage, alteration of membrane asymmetry and permeability, condensation of chromatin and nucleus, DNA

fragmentation, and formation of membrane-bound vesicles (apoptotic bodies).

Disruption of mitochondrial membrane potential is often associated with the early stages of apoptosis. This application note describes a method to detect disruption of mitochondrial membrane potential in individual cells using MitoView™ dyes (Biotium). MitoView™ dyes are cell membrane permeable and become brightly fluorescent upon accumulation in the mitochondrial membrane. The dyes rapidly accumulate in mitochondria and can be imaged without washing. The MitoView™ 633 dye is dependent on mitochondrial membrane potential, thus making it suitable for monitoring changes in mitochondrial membrane potential during apoptosis. The MitoView™ Green dye is not dependent on mitochondrial membrane potential and is thus used for estimating the total mitochondrial mass in individual cells.

MitoView™ 633 is a far-red fluorescent dye with absorbance/emission at 622/648 nm. MitoView™ Green is a green fluorescent dye, spectrally similar to FITC, and with absorbance/emission at 490/523 nm.

The advantages of mitochondrial potential assay are that it is easy and fast. However, the assay may not always be specific for apoptosis. Thus, cells with a decreased red/green fluorescence intensity ratio may also represent chemical-hypoxia-induced necrotic cells. Hence, we recommend using another apoptosis assay to confirm apoptosis.

## Procedure

The following protocol was developed using Jurkat, HeLa, U2OS and MCF-7 cells with an optimized concentration of MitoView™ 633 and MitoView™ Green, but it can be adapted for most mammalian cell types. Growth medium, cell density, cell type variants and other factors may influence labelling. We recommend testing a concentration range of the MitoView™ dyes to establish the optimal conditions for your preferred cell model.

## Materials needed

- Cell sample to be stained in suspension <sup>1, 2</sup>
- Complete medium for the cell type used <sup>1</sup>
- MitoView™ 633 <sup>3, 4</sup>
- MitoView™ Green <sup>3, 4</sup>
- DAPI (500 µg/ml, [Solution 12](#), Cat. No. 910-3012)
- OPTIONAL: phosphate buffered saline (PBS) <sup>1</sup>
- Xcyto® 2-Chamber Slide Type 100-A (Cat.no. 942-0010)

## Protocol

Induce apoptosis in cell culture by desired method. Prepare a negative control by incubating cells in the absence of an apoptosis-inducing agent, but in the presence of solvent (e.g. DMSO). Perform staining in complete growth medium for the cell type used.

1. Harvest cells, determine cell concentration and adjust the cell concentration to  $2 \times 10^6$  cells/ml. Transfer a representative cell sample from the cell suspension into a tube (e.g. Eppendorf)
2. NOTE: If comparing two or more samples, it is important that they are at the same concentration ( $2 \times 10^6$  cells/ml recommended)
3. Add MitoView™ 633 to the tubes at a final concentration of 100 nM  
NOTE: The optimal dye concentration should be determined empirically
4. Add MitoView™ Green to the tubes at a final concentration of 100 nM  
NOTE: The optimal dye concentration should be determined empirically
5. Mix thoroughly by pipetting or gently vortexing
6. Incubate cells at 37°C for 15 minutes in the dark
7. Add DAPI to a final concentration of 1 µg/ml to stain dead cells
8. Mix thoroughly by pipetting or gently vortexing
9. Analyze immediately or within 15 minutes for best results  
NOTE: Cells can be imaged directly in medium containing stains; washing steps are not required
10. Load the Xcyto® 2-Chamber Slide Type 100-A with 50 µl of cell suspension
11. Engage Xcyto® 5 image cytometer by opening XcytoView™ Software
12. Eject sample tray and place slide on tray
13. Select *Mitochondrial Potential* protocol in the Protocol Browser or drop-down menu; both are available from the Main Window
14. In the data acquisition control section in the main window, select your *Sample media*, enter *Sample ID*, and press the *Run* icon

OPTIONAL: Create user-defined stop criteria in the acquisition limits section, available from the

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<sup>1</sup> Provided by the user

<sup>2</sup> An untreated control sample should be included. Preferable, use logarithmically proliferating cells as control

<sup>3</sup> Recommended manufacturers: [www.biotium.com](http://www.biotium.com); MitoView™ 633 (Catalog no. 70055); MitoView™ Green (Catalog no. 70054)

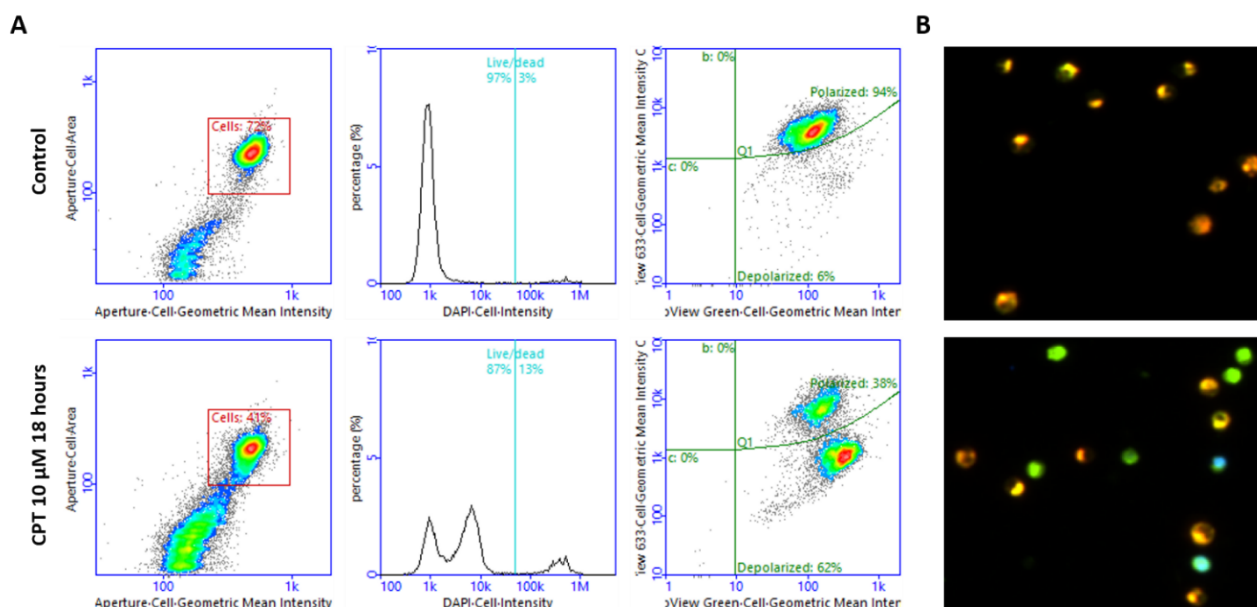
<sup>4</sup> Follow manufacturer's instructions for staining cells with MitoView™ 633 and MitoView™ Green

main window, by the number of either counts or views. Criteria can be edited when limits are disabled

- a. To define a gate for the counts limit, wait for the file to be displayed in the plot manager, create a gate or use a template-defined gate, select the name in the drop-down menu and enable the counts limit
15. Cells are imaged, segmented, and analyzed automatically. Numerical cell data is presented in the plot manager
- NOTE: Inspection of the gates “Cells”, “Live/dead”, and Q1 is crucial and may require adjustment for proper analysis

During image acquisition and analysis, the results will be displayed in the Plot Manager, when the analysis of the first view has been completed. For every analyzed view, result data will be added incrementally to the displayed results.

Cells are identified based on transmitted light microscopy. Mitochondrial depolarization is revealed as a decrease in the red/green fluorescence intensity ratio (see examples below).



**Figure 1.** Jurkat cells stained with MitoView™ 633, MitoView™ Green and DAPI. **A)** Cells were grown in the absence (control) or in the presence of 10  $\mu$ M camptothecin (CPT) for 18 hours at 37°C. Cells were harvested, stained according to the protocol, imaged and analyzed with the Xcyto®5 image cytometer and XcytoView™ software using the Mitochondrial Potential protocol. Aperture masking plots (left panel) display cells identified based on transmitted light microscopy and the gate “Cells”. The “Cells” population were gated on in the histogram and density plots. Histograms (middle panel) show the DAPI intensity and the divider gate “Live/dead”. Density plots (right panel) show the MitoView™ 633 intensity versus the intensity of the MitoView™ Green dye excluding dead cells. Quadrants in the density plots displayed were used to mark various cell populations. Inspection of the gates “Cells”, “Live/dead”, and Q1 is crucial and may require adjustment for proper analysis. In this example, CPT causes a dramatic increase in the depolarized population (decreased MitoView™ 633 intensity and MitoView™ Green positive). NOTE: Only single cells are included in the analysis. **B)** Representative images of the stained control (upper) and CPT treated (lower) samples, displaying the various cell populations acquired using the Xcyto® 5 image cytometer and XcytoView™ software.

## Notes

The MitoView™ 633 dye is for use in live cells and cannot be fixed. The MitoView™ Green dye can be used to stain mitochondria in both live and fixed cells.

For samples containing small cell types or particles, such as PBMCs, we recommend a settling time of 2-5 minutes after loading the Xcyto® slides to avoid movement of sample components during data acquisition. Movement during data acquisition can cause errors in image overlay and thereby quantification of cell data.

Analysis time is highly correlated with cell density of the sample; Lower cell densities increase acquisition and analysis times.

## Handling and storage

For handling and storage of ChemoMetec® instruments, reagents and Xcyto® slides refer to the corresponding product documentation. For other reagents, refer to the material data sheet from the manufacturer of the reagents and chemicals.

## Warnings and precautions

For safe handling and disposal of the ChemoMetec® reagents and Xcyto® slides refer to the corresponding product documentation and the Xcyto® Software User Guide. For other reagents refer to the safety data sheet from the manufacturer of the reagents and chemicals required for this protocol. Wear suitable eye protection and protective clothes and gloves when handling biologically active materials.

## Limitations

The Xcyto® 5 system, consisting of the Xcyto® 5 image cytometer and XcytoView™ software, is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the Xcyto® 5 system depend on correct use of the reagents, Xcyto® slides and the Xcyto® 5 instrument and might depend on the type of cells being analyzed. Refer to the Xcyto® 5 Software User Guide for instructions and limitations.

## Liability disclaimer

This application note is for RESEARCH PURPOSES ONLY. It is

not intended for food, drug, household, or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. ChemoMetec A/S shall not be held liable for any damage resulting from handling or contact with the above product.

## Product disclaimer

ChemoMetec A/S reserves the right to introduce changes in the product to incorporate new technology. This application note is subject to change without notice.

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