405 E Gude Dr, STE 214
Rockville, MD 20850, USA
Web: www.abpbio.com
Email: info@abpbio.com

MitoView™ Mitochondrial Transition Pore Assay for Flow Cytometry

Catalog Number: A065

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
Calcein, AM (Component A)	5×50 µg	-	• **-20 °C,** • **Protect from light**	**The product is stable for 1 year when stored as directed.**
Cobalt (II) chloride (Component B)	1.2 mL	80 mM in H_2O		
Ionomycin (Component C)	37 µg	-		
DMSO (Component D)	1.5 mL	-		

Number of assays: 250 based on labeling volumes of 1 mL.
Excitation/emission: 494/517 nm (calcein).

Introduction

The mitochondrion plays a vital role in the processes of apoptotic and necrotic cell death. The mitochondrial permeability transition pore is a nonspecific channel formed by components from the inner and outer mitochondrial membranes, and appears to be involved in the release of mitochondrial components during cell death. The MitoView™ Mitochondrial Transition Pore Assay is based on published experimentation for mitochondrial transition pore opening. This assay employs calcein AM, a colorless and nonfluorescent esterase substrate, and $CoCl_2$, a quencher of calcein fluorescence, to selectively label mitochondria.

The MitoView™ Mitochondrial Transition Pore Assay provides a direct method of measuring mitochondrial permeability transition pore opening. Cells are loaded with calcein AM, which passively diffuses into the cells and accumulates in cytosolic compartments, including the mitochondria. Once inside cells, intracellular esterases cleave the acetoxymethyl esters to liberate the very polar fluorescent dye calcein, which does not cross the mitochondrial or plasma membranes. The fluorescence from cytosolic calcein is quenched by the addition of $CoCl_2$, while the fluorescence from the mitochondrial calcein is maintained. As a control, cells that have been loaded with calcein AM and $CoCl_2$ can also be treated with an ionophore, ionomycin, to allow entry of excess Ca^{2+} into the cells to trigger mitochondrial pore activation and subsequent loss of mitochondrial calcein fluorescence.

Experimental Protocol

Buffer Requirements and Recommendations

This assay was developed using Hanks' Balanced Salt Solution (HBSS) with sodium bicarbonate, calcium, and magnesium that also included HEPES (10 mM), L-glutamine (2 mM) and succinate (100 µM) to support healthy mitochondrial function in live cells. This protocol is compatible with common buffers used in live-cell imaging, but the buffer used for the optional ionomycin positive control must contain Ca^{2+} in order to trigger mitochondrial transition pore activation.

Preparation of Stock Solutions

Aliquots of stock solutions can be frozen at ≤-20˚C for up to 6 months.

- **Prepare a 1.0 mM calcein AM stock solution.** Dissolve the contents of one vial of calcein AM (Component A) in 50 µL of DMSO (Component D) for a final concentration of 1.0 mM. Once prepared, the 1.0 mM calcein AM stock solution should be used within a short time period and should not be frozen and thawed repeatedly.
- **Prepare a 100 µM ionomycin stock solution (optional).** To the Component C vial, add 500 µL of DMSO (Component D) and mix well.

Labeling Protocol

1.1 Resuspend cells of interest in prewarmed HBSS at a final concentration of 1×10^6 cells/mL.

1.2 Prepare 1 mL aliquots of the cell suspension in separate flow cytometry tubes. For each cell sample, prepare 3 aliquots: one will contain calcein AM only (tube 1), one will contain calcein AM and $CoCl_2$ (tube 2), and the final one will contain calcein AM, $CoCl_2$, and ionomycin (tube 3). Also prepare a sample of the cells containing no added reagents for instrument set up.

1.3 To tubes 1, 2, and 3, add 1 µL of 1.0 mM calcein AM stock solution and mix well.

1.4 To tubes 2 and 3, add 5 µL of $CoCl_2$ (Component B) and mix well.

1.5 To tubes 3, add 5 µL of 100 µM ionomycin stock solution and mix well.

1.6 Incubate the samples at 37ºC for 15 minutes, protected from light.

1.7 Add ~3.5 mL of HBSS to the tubes and pellet the cells by centrifugation. This step serves to remove excess staining and quenching reagents.

1.8 Resuspend the pellet in ~400 µL of buffer suitable either for flow cytometric analysis or for further staining, if appropriate.

1.9 After staining, place samples on ice and analyze within one hour.

1.10 Analyze the samples using a flow cytometer with 488 nm excitation and emission filters appropriate for fluorescein. The sample containing no added reagents is useful for instrument set up.