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MitoView™ Mitochondrial Transition Pore Assay for Imaging

Catalog Number: A064

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	-		
MitoTracker Red CMXRos (Component D)	50 µg	-		
DMSO (Component E)	1.5 mL	-		

Number of assays: 250 based on labeling volumes of 1 mL.
Excitation/emission: 494/517 nm (calcein), 579/600 nm (MitoTracker Red CMXRos).

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 E) 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 200 µM MitoTracker Red CMXRos stock solution.** Dissolve the contents of one vial of Mitotracker Red CMXRos dye (Component D) in 470 µL of DMSO (Component E) for a final concentration of 200 µM.
- **Prepare a 100 µM ionomycin stock solution (optional).** To the Component C vial, add 500 µL of DMSO (Component E) and mix well.

Labeling Protocol

1.1 Prepare the labeling solution. To 1 mL of the modified HBSS prepared above was added 1.0 µL of 1.0 mM calcein AM stock solution, 1.0 µL of 200 µM MitoTracker Red CMXRos stock solution, and 5.0 µL of 80 mM $CoCl_2$ (Component B). Warm the labeling solution to 37ºC, and protect from light.

1.2 Label cells. Wash cells twice in the modified HBSS buffer, aspirate the buffer from the cells, and apply a sufficient amount of labeling solution to cover the cells adhering to a coverslip. Incubate for 15 minutes at 37ºC, protected from light.

1.3 Wash cells. Wash cells in warm modified HBSS buffer to remove residual dye and minimize background, and aspirate buffer.

1.4 (Optional) Prepare a positive control sample. Prepare a 0.5 µM ionomycin solution by making a 200-fold dilution of 100 µM ionomycin stock solution in the modified HBSS buffer. To a previously labeled sample, add a sufficient amount of the 0.5 µM ionomycin solution to cover the cells. As cells experience Ca^{2+} overload from ionomycin treatment, mitochondrial calcein signal should be lost very quickly while MitoTracker Red CMXRos stain signal is preserved.

1.5 Prepare cells for viewing. Mount the cells in warm buffer.