MitoPT® JC-1 Assay

Catalog #924 & #911

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Not for use in diagnostic procedures.

Assess mitochondrial membrane potential in whole cells

1. INTRODUCTION

Mitochondria play a central role in the biochemical processes associated with the life and death stages of eukaryotic cells[1]. Under normal physiological conditions, a membrane-based proton pump generates an electrochemical gradient, enabling the production of ATP to drive cellular energy-dependent processes[2]. The oxidation of glucose and fatty acids by enzymes associated with the mitochondrial respiratory chain establishes a proton and pH gradient across the mitochondrial inner membrane, resulting in a transmembrane electrical potential gradient ($\Delta\Psi_m$) of -80 to -120 mV and a pH gradient of 0.5-1.0 pH units[3,4].

Depolarization of the inner mitochondrial membrane can lead to an opening of the mitochondrial permeability transition pore (PTP)[5]. This results in the leakage of intermembrane proteins, including cytochrome c, that facilitate the induction of apoptosis through apoptosome formation[6]. Caspase activation has been shown to accelerate the process of $\Delta\Psi_m$ loss[7]. Moreover, a feedback mechanism that results in the generation of reactive oxygen species further accelerates the rate of cell death[7]. Because mitochondrial dysfunction has been closely tied to such neurodegenerative diseases as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis, mitochondria remain an important organelle of study[8].

Loss of mitochondrial $\Delta\Psi_m$, indicative of apoptosis, can easily be detected using lipophilic, cationic fluorescent redistribution dyes such as ICT's MitoPT® reagents: tetramethylrhodamine ethyl ester (TMRE), tetramethylrhodamine methyl ester (TMRM), and 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolocarbocyanine iodide (JC-1)[9]. These dyes have a delocalized positive charge dispersed throughout their molecular structure, and yet their lipophilic solubility enables them to be readily membrane permeant and penetrate living cells[9-11]. They redistribute across cell membranes according to the Nernst equation in a voltage-dependent manner[9-11]. Accordingly, they possess a low membrane partition coefficient; meaning a low tendency to non-specifically associate with intracellular organelles and macromolecules. These excellent potentiometric dyes also exhibit minimal self-quenching, low cytotoxicity, and are reasonably photostable[11]. The MitoPT dyes exhibit very low toxicity and display rapid and reversible membrane equilibration properties[11].

ICT's MitoPT JC-1 assay kits easily distinguish between healthy, non-apoptotic cell populations and those cell populations that are transitioning into an apoptotic state. Inside a healthy, non-apoptotic cell, the lipophilic JC-1 dye, bearing a delocalized positive charge, enters the negatively charged mitochondria where it accumulates[10].

When the mitochondrial $\Delta\Psi_m$ collapses in apoptotic cells, MitoPT JC-1 no longer accumulates inside the mitochondria, instead becoming more evenly distributed throughout the cytosol. When dispersed in this manner, overall cellular fluorescence levels drop dramatically. Healthy cells fluoresce orange and green, whereas cells with depolarized mitochondria fluoresce green and exhibit lower levels of orange fluorescence.

MitoPT JC-1 kits can be used in conjunction with other existing research protocols. Grow cells following the usual cell cultivation protocol. If using an apoptosis induction model system, simply induce apoptosis according to the existing procedure, reserving a non-induced population of cells as a control. Once apoptosis has been induced in the cells, or the mitochondrial membrane has been depolarized by another method, such as using CCCP (included in the kit), spike MitoPT JC-1 dye solution into each sample and control. Incubate the cells for 15-30 minutes at 37°C to allow MitoPT JC-1 to equilibrate within the polarized mitochondria. If the cells are not undergoing some form of metabolic or apoptotic stress, the mitochondrial $\Delta\Psi_m$ will remain intact, and MitoPT JC-1 will accumulate within the slightly negative/alkaline environment of the mitochondria and fluoresce brightly upon excitation. If the cells are apoptotic, the mitochondrial $\Delta\Psi_m$ will break down, causing MitoPT JC-1 to disperse throughout the cell cytosol. This results in a dramatic reduction in the fluorescence of the affected mitochondria, and as a result, overall cellular fluorescence is diminished significantly.

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Learn more about all of ICT's products at www.immunochemistry.com or call 1-800-829-3194.

ImmunoChemistry TECHNOLOGIES LLC

ImmunoChemistry Technologies, LLC
9401 James Ave. S. #155, Bloomington, MN 55431 USA 800-829-3194

immunochemistry.com

2. PROTOCOL OVERVIEW

ImmunoChemistry Technologies' MitoPT JC-1 mitochondrial membrane potential assay kits make it easy to screen cells with a fluorescence microscope, plate reader, or flow cytometer.

When MitoPT JC-1 stained cells are viewed with a fluorescence microscope, non-apoptotic cells will have orange-red fluorescent mitochondrial structures (J-aggregates) as the dye accumulates within healthy, polarized mitochondria. In contrast, apoptotic and metabolically stressed cells will have fewer bright orange-red fluorescent mitochondria and more dim or non-fluorescent mitochondria. In all cells, MitoPT JC-1 will be distributed throughout the cytosol in its monomeric form, exhibiting its distinctive green fluorescence property. However, the overall brightness of apoptotic or metabolically stressed cells will be visibly reduced as a result of the mitochondrial depolarization event.

When cells stained with MitoPT JC-1 are analyzed with a fluorescence plate reader, the instrument will measure the total amount of orange-red fluorescence emitted from the cell population in the microtiter plate well. Healthy control cells bearing mitochondria with normal electrochemical gradients will concentrate the potentiometric dye to a greater extent than will apoptotic cell populations[12-13]. These samples will give a higher relative fluorescence unit (RFU) emissions output of red fluorescence than their apoptotic counterparts. In treated and positive control samples, the mitochondrial membrane potential gradient will collapse, resulting in a lower orange-red fluorescence RFU reading as the dispersed MitoPT JC-1 dye converts to its green fluorescing monomeric form. The difference in fluorescence output of these two populations can be easily distinguished in black 96-well plates using 488 nm excitation and dual emission wavelengths of 527 nm for green fluorescence and 590-600 nm for red fluorescence.

When cells stained with MitoPT JC-1 are run through a flow cytometer, the dye is excited using a common blue argon laser at 488 nm. Monomeric JC-1 exhibits green fluorescence (peak emission 527 nm), which can commonly be measured in the FL-1 emission channel. J-aggregates show a red spectral shift (peak emission 590 nm), commonly measured in the FL-2 emission channel. Therefore, healthy non-apoptotic cells will be detected in both FL-1 and FL-2 channels, and cells with reduced or altered mitochondrial function due to apoptosis or other cellular processes will remain bright in FL-1 and exhibit a reduction in FL-2 fluorescence intensity. MitoPT JC-1 has been used concurrently with other fluorophores in multi-parametric analyses measuring mitochondrial depolarization, caspase activation, phosphatidylserine exposure, and/or cell viability within a single cell population[14-16].

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3. KIT CONTENTS

#924 Trial size kits contain:

- 1 vial MitoPT JC-1 Reagent (100 Tests) #6261
- 1 bottle 10X Assay Buffer (60 mL) #685
- 1 vial CCCP, 50 mM (125 µL) #6257

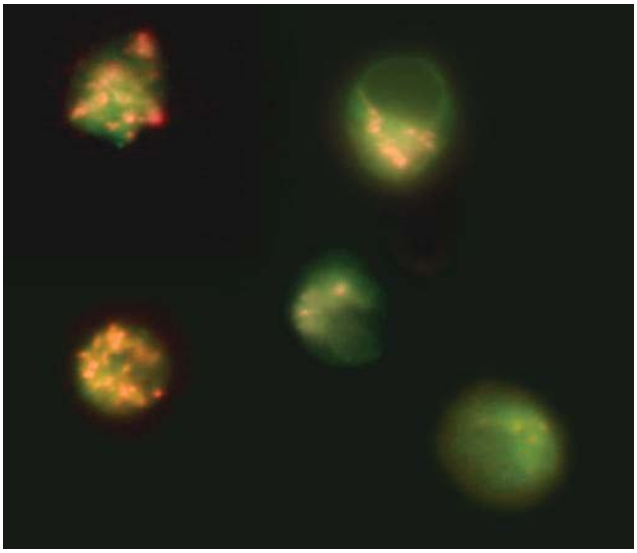
#911 Standard size kits contain:

- 1 vial MitoPT JC-1 Reagent (400 Tests) #6260
- 2 bottles 10X Assay Buffer (2 x 125 mL) #6259
- 1 vial CCCP, 50 mM (600 µL) #6258

4. STORAGE

Store the kit at ≤-20°C. Once opened, some components may be stored at 2-8°C until the expiration date. CCCP should be stored frozen. Once reconstituted with DMSO, dilute and use MitoPT immediately, or store at ≤-20°C for up to 12 months protected from light and thawed no more than twice.

FIGURE 1: MICROSCOPY ANALYSIS OF APOPTOTIC SUSPENSION CELLS



Jurkat cells were treated with 1 µM staurosporine for 2 hours to induce apoptosis, or with DMSO as the negative control. Cells were stained with MitoPT JC-1 for 20 minutes at 37°C , then washed twice.

Normal healthy cells (two cells, upper and lower left), containing mitochondria with polarized inner membranes, concentrate MitoPT JC-1 and fluoresce bright orange. Apoptotic cells (three cells, right), bearing mitochondria of various stages of permeability, exhibit a reduced orange fluorescence relative to the healthy cell population and increased green fluorescence, as the reagent becomes dispersed throughout the cells. (Dr. Brian Lee, ICT).

5. SAFETY DATA SHEETS (SDS)

Safety data sheets are available online at www.immunochemistry.com or by calling 1-800-829-3194 or 952-888-8788.

6. RECOMMENDED MATERIALS

- DMSO, 0.5-1 mL to reconstitute MitoPT
- DiH_20, 540-2250 mL to dilute 10X Assay Buffer
- Cultured cells or tissues treated with the experimental conditions ready to be labeled
- Reagents to create controls by inducing metabolic stress, mitochondrial depolarization, or apoptosis
- Hemocytometer
- Centrifuge at 200 x g
- 15 mL polypropylene centrifuge tubes (1 per sample)
- Black round or flat bottom 96-well microtiter plates
- Microscope slides
- FACS tubes

7. DETECTION EQUIPMENT

- Fluorescence microscope with 488-490 nm excitation and >510 nm long band pass filter capable of detecting both green and red fluorescence simultaneously.
- Fluorescence plate reader with capabilities for 488-490 nm excitation and emission at 590 nm (dual emission at 527 nm and 590-600 nm is best).
- Flow cytometer with excitation laser at 488 nm and emission filter at 527 nm (FL-1) and 590 nm (FL-2).

8. EXPERIMENTAL PREPARATION & CONTROLS

MitoPT JC-1 mitochondrial membrane potential assay kits are compatible with other apoptosis or mitochondrial assessment protocols. Because MitoPT detects mitochondrial membrane depolarization, plan the experiment so that it will be diluted and administered at the time when this event is expected to occur in the cells. The recommended staining procedure detailed below is effective for most applications, but the amount may vary based on the experimental conditions and cell type. An initial experiment may be necessary to determine when and how much MitoPT to use. It is highly recommended that 2 sets of controls be run:

1. A negative population of cells that were not exposed to the experimental conditions.
2. A positive control population that was induced to undergo mitochondrial depolarization or apoptosis, such as:
    - An apoptotic positive control can be created by adding 2 µg/mL camptothecin (catalog #6210) or 1 µM staurosporine (catalog #6212) to cells for >3-4 hours at 37°C.
    - A positive control exhibiting a reduced mitochondrial potential can be created using the CCCP included in the kit (Section 9).

9. MITOCHONDRIAL DEPOLARIZATION WITH CCCP

Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) is a reversible proton gradient uncoupling agent that quickly reduces the electrochemical potential across the inner mitochondrial membrane, resulting in a rapid intracellular mitochondrial depolarization event[17-18]. CCCP can be used to create a positive control cell population containing depolarized mitochondria. ICT's CCCP reagent is a liquid stock at 50 mM in DMSO that must be stored ≤-20°C. Use gloves when handling.

- **Warning:** CCCP contains DMSO, which is a combustible liquid, causes skin irritation, and causes serious eye irritation. In case of exposure, immediately flush eyes or skin with water. See SDS for further information.

1. Determine when the mitochondrial depolarization analysis will be run. Approximately 75 minutes prior to analysis, generate positive and negative mitochondrial depolarization controls using CCCP and DMSO, respectively.
2. Gently warm CCCP to RT; mix or gently vortex.
3. Spike with enough CCCP to obtain 5–50 µM CCCP in the cell culture media. As the CCCP stock is at 50 mM, to stain at 50 µM, simply spike 1 µL CCCP stock per 1 mL cell suspension/overlay medium. If using a lower concentration of CCCP, first dilute the reagent with tissue culture grade DMSO.
4. Prepare a negative control population by spiking with the same volume of tissue culture grade DMSO as was used to spike the CCCP population.
5. Incubate the positive and negative control cell populations for 30–60 minutes at 37°C in a CO_2 incubator to allow time for the depolarization process to occur.
6. Follow the staining protocol. After cells are washed, read the controls immediately, as the mitochondria may revert back to a polarized state once the CCCP is removed.

10. PREPARATION OF 1X ASSAY BUFFER

1. 10X Assay Buffer may form precipitates during cold storage. If this happens, gently warm it until all crystals have dissolved. Do not boil.
2. Dilute 10X Assay Buffer 1:10 in diH_20. For example, add 60 mL 10X Assay Buffer to 540 mL diH_20, for a total of 600 mL. 1X Assay Buffer may be stored at 2-8°C and used within 1 week or frozen and used within 12 months.

11. PREPARARTION OF MITOPT

MitoPT is a lyophilized powder that may be visible as an iridescent or faint red sheen inside the vial. The reconstituted stock should be diluted immediately, or frozen for future use. Protect from light and use gloves when handling.

- **Warning:** JC-1 is harmful if swallowed, in contact with skin, or if inhaled. It causes skin irritation, serious eye damage, and may cause respiratory irritation. In case of exposure, immediately flush eyes or skin with water. See SDS for further information.

1. Reconstitute MitoPT with DMSO:
   a. For 100 Test size vial #6261, add 0.5 mL, **forming a 100X stock.**
   b. For 400 Test size vial #6260, add 1 mL, **forming a 200X stock.**
2. Prepare a 1X working solution:
   a. For 100 Test size, dilute 1:100 in 1X Assay Buffer.
   b. For 400 Test size, dilute 1:200 in 1X Assay Buffer.
3. Vortex the 1X working solution of MitoPT JC-1 thoroughly. If particulate matter is present, the solution can be clarified by centrifugation: 13,000 x g in a microcentrifuge for 3 minutes; or at >1000 x g in a clinical centrifuge for 20 minutes at RT. Transfer the clarified supernatant to a clean tube and discard particulates. **Note: Clarification is especially important when using a fluorescence plate reader or fluorescence microscope for sample analysis.**

12. MICROSCOPY ANALYSIS OF SUSPENSION CELLS

Suspension cells can be readily evaluated using fluorescence microscopy.

1. Prepare experimental and control cell populations. Cells should be at least 5 x 10^5 cells/mL, but the concentration should not exceed 10^6 cells/mL, as cells cultivated in excess of this concentration may become overcrowded and naturally transition into apoptosis. Expose cells to the experimental conditions.
2. Determine cell concentration; a hemocytometer may be used. Transfer the volume of cells needed for 1-2 x 10^6 cells/sample into fresh tubes. The volume of cells and amount of MitoPT should be adjusted to accommodate each particular cell line and research conditions.
3. Staining samples with MitoPT:
   a Centrifuge at 200 x g for 10 minutes at RT to pellet the cells.
   b. Carefully remove and discard supernatant.
   c. Add 0.5 mL 1X working solution (Section 11) per sample and gently mix to resuspend cells.
   d. Incubate 15-30 minutes at 37°C and protect from light.
4. Wash cells to remove excess MitoPT:
   a Centrifuge at 200 x g for 10 minutes at RT to pellet the cells.
   b. Carefully remove and discard supernatant.
   c. Add 2 mL 1X Assay Buffer (Section 10) or culture medium.
   d. Repeat steps a-b; use 0.5 mL 1X Assay Buffer for the second resuspension. Note: if samples cannot be analyzed immediately, add >0.1% BSA to the assay buffer, or use cell culture medium or Dulbecco's PBS to store the samples. Protect from light.
5. Place 50-100 µL on a clean microscope slide and add a cover slip.
6. Observe cells under a fluorescence microscope equipped with the appropriate excitation and emission settings, such as an instrument with 488-490 nm excitation and >510 nm long band pass filter that is capable of detecting both green and red fluorescence simultaneously (Figure 1).

13. MICROSCOPY ANALYSIS OF ADHERENT CELLS

Adherent cell monolayers can be readily evaluated using fluorescence microscopy.

1. Prepare experimental and control cell populations. Culture cells on a sterile coverslip or chamber slide. Cells should not exceed the threshold where spontaneous apoptosis or cell sloughing occurs. Expose cells to the experimental or control conditions.
2. Staining samples with MitoPT:
   a. Remove overlay medium from cell monolayer cultures.
   b. Add enough 1X working solution (Section 11) to cover the cells on the slide.
   c. Incubate 15-30 minutes at 37°C and protect from light.
3. Wash the cells to remove any free dye in the supernatant that may interfere with the analysis:
   a. Gently remove the cell culture supernatant.
   b. Add enough 1X Assay Buffer (Section 10) to cover the cell surface and dilute any remaining free MitoPT dye.
   c. Incubate ~10 minutes at 37°C.
   d. Remove the 1X Assay Buffer and add enough fresh 1X Assay Buffer to cover the cell surface. If samples cannot be analyzed immediately, add >0.1% BSA to the assay buffer, or use cell culture medium or Dulbecco's PBS to store the samples. Protect from light.
4. Cover with a cover slip.
5. Observe cells under a fluorescence microscope equipped with the appropriate excitation and emission settings, such as an instrument with 488-490 nm excitation and >510 nm long band pass filter that is capable of detecting both green and red fluorescence simultaneously.

14. FLUORESCENCE PLATE READER ANALYSIS

The loss of the electrochemical potential gradient across the inner membrane of depolarized mitochondria is easily detectable in most fluorescence plate readers. By comparing the average 590-600 nm signal in stimulated versus non-stimulated samples, loss of $\Delta\Psi_m$ can be detected. Because the loss of red fluorescence is often accompanied by an increase in green fluorescence as more J-aggregates transition to the green fluorescing monomeric form, the dual fluorescence characteristics of the dye can be used in the assessment by comparing the RFU ratios of 590-600 nm (red)/ 527 nm (green). When mitochondrial depolarization occurs, the red/green RFU ratio drops from that of the negative (non-stimulated) control. In other words, the red fluorescence decreases and the green fluorescence remains constant or increases. As fluorescence plate reader spectroscopy is generally less sensitive than flow cytometry (Sections 15 & 16), a higher concentration of cells is recommended. For best results, cell populations should be in excess of 3×10^5 cells/well, which corresponds to a spiked and washed cell suspension of $>3 \times 10^6$ cells/mL in 100 µL/well aliquots.

1. Prepare experimental and control cell populations. Cells should be at least 5×10^5 cells/mL, but the concentration should not exceed 10^6 cells/mL as cells cultivated in excess of this concentration may become overcrowded and naturally transition into apoptosis.
2. Remove a small aliquot and determine the cell density of each population. Concentrate cells to $>3 \times 10^6$ cells/mL:
   a Centrifuge at 200 x g for 10 minutes at RT to pellet the cells.
   b. Carefully remove and discard supernatant.
   c. Add enough cell culture medium to achieve the $>3 \times 10^6$ cells/mL target concentration. Gently vortex to resuspend cells.
3. Place 1 mL per sample into 15 mL polypropylene centrifuge tubes.
4. Staining samples with MitoPT:
   a Centrifuge at 200 x g for 10 minutes at RT to pellet the cells.
   b. Carefully remove and discard supernatant.
   c. Add 0.5 mL 1X working solution (Section 11) per sample and gently mix to resuspend cells.
   d. Incubate 15-30 minutes at 37°C and protect from light.
5. Wash cells to remove excess MitoPT:
   a Centrifuge at 200 x g for 10 minutes at RT to pellet the cells.
   b. Carefully remove and discard supernatant.
   c. Add 2 mL 1X Assay Buffer (Section 10) or culture medium.
   d. Repeat steps a-b; use 0.5-1 mL 1X Assay Buffer for the second resuspension. If samples cannot be analyzed immediately, add >0.1% BSA to the assay buffer, or use cell culture medium or Dulbecco's PBS to store the samples. Protect from light.
6. Pipette 100-200 µL of each sample per well (in triplicate) into a black round or flat-bottom 96-well microtiter plate. Do not use clear or white plates as this would diminish sensitivity and increase background noise.
7. Analyze with a fluorescence plate reader set to perform an end-point read with excitation at 488-490 nm. If possible, measure both a 527 nm emission in the green wavelength and a 590-600 nm emission in the red wavelength. If the plate reader cannot read dual emission wavelengths at the same time, use the red fluorescence setting of 590-600 nm to perform the analysis.

15. FLOW CYTOMETRY ANALYSIS: SINGLE-COLOR (RED)

MitoPT can readily be used to evaluate suspension cells by flow cytometry. MitoPT JC-1 yields excellent results using the common argon blue laser at 488 nm for excitation. Peak emission for red J-aggregates is 590 nm, which is generally read in FL-2.

1. Follow Section 12 (Steps 1-6) to yield a final sample volume of 0.5-1 mL. If samples cannot be analyzed immediately, add >0.1% BSA to the assay buffer, or use cell culture medium or Dulbecco's PBS to store the samples. Protect from light.
2. Run an unstained (autofluorescence control) cell sample and generate a FSC (forward scatter) versus SSC (side scatter) plot. Adjust detector settings so that cells of interest are displayed on scale and can be gated as desired.
3. While gating on the cell population of interest (P1), adjust FL-2 detector settings so that autofluorescence background is roughly within the first decade of the log scale on the fluorescence intensity histogram.
4. Run the brightly fluorescent negative control sample. Generate a histogram with log FL-2 on the X-axis versus the number of cells on the Y-axis (Figure 3). On the histogram, there will appear two cell populations represented by two peaks. The majority of the stained healthy, negative control cells should occur within the higher log fluorescence output decades of the FL-2 (X-axis), whereas the depolarized cell population will appear as a separate peak, or as a shoulder of the first peak, showing decreased fluorescence intensity in the lower log output decades. If possible, adjust the FL-2 PMT voltage to allow the peak of the fluorescent negative control to fall within the third log decade.
5. Run the depolarized positive control sample using the same adjusted PMT voltage as determined for the negative control. The histogram peak should still be observable on the X-axis. If not, increase PMT voltage slightly to achieve positive control staining that reads at least as bright as the first decade of the log scale.
6. Observe the mean fluorescence intensity of all controls and samples at the adjusted settings.

16. FLOW CYTOMETRY ANALYSIS: TWO-COLOR (RED + GREEN)

Flow cytometry makes it possible to measure the dual fluorescence green/red characteristics of MitoPT JC-1 in a sample population. The peak emission wavelength for green monomers is 527 nm (FL-1); for red J-aggregates, it is 590 nm (FL-2).

1. Follow Section 12 (Steps 1-6) to yield a final sample volume of 0.5-1 mL.
2. Follow Section 15 (Steps 2-3) using the controls to adjust FL-1 and FL-2 detector settings to ensure fluorescence measurements are on scale.
3. Create a 2-color dot plot displaying log FL-1 versus log FL-2, and gate the population on P1.
4. Run the MitoPT-stained negative (unstimulated) control and view on 2-color plot. If needed, adjust the P1 region in the initial FSC versus SSC gate.
5. Draw a second gate (P2) around the main population of cells visible in the negative control. If possible, adjust PMT voltages slightly so that the majority of the dual fluorescent population falls within the second and third log decade of FL-1 and FL-2 (Figure 4).
6. Adjust P2 so that >95% of the dual fluorescent population falls within this region. The number will vary depending on the condition of the culture and cell type.
7. Draw a third gate (P3) that falls directly below P2 and includes the events which, compared to P2, have equivalent or increased green fluorescence and decreased red fluorescence.
8. Run the MitoPT-stained postive (depolarized) control and observe the number of cells in the P2 gate. If a change in mitochondrial $\Delta\Psi_m$ occured, a decrease in the number of cells falling in P2 and an increase in the number of cells falling in P3 will be observed (Figure 4).
9. If an unacceptable percentage of cells in the positive control sample fall within P2, fluorescence compensation can be applied to further resolve the FL-2+ (P2) and FL-2- (P3) populations. This is accomplished by subtracting a percentage of the FL-1 signal from FL-2 to account for spectral overlap or "spillover" of fluorescence output from the green channel (FL-1) into the red channel (FL-2).
10. Observe the mean fluorescence intensity of all controls and samples at the adjusted settings.

FIGURE 2: FLUORESCENCE PLATE READER ANALYSIS

Jurkat and HL-60 cells were exposed to DMSO as the negative control (left, dark orange bars) or 50 µM CCCP depolarizing agent (right, light orange bars) for 15 minutes at 37°C, subsequently incubated with MitoPT JC-1 for 20 minutes at 37°C, and washed. Aliquots (100 µL) were analyzed in triplicate in a black 96-well plate using a Molecular Devices Gemini XS fluorescence plate reader set at 488 nm excitation and 590 nm emission filter settings.

The amount of orange fluorescence was measured by the plate reader. Healthy cells in the DMSO control populations exhibited a high level of orange fluorescence; metabolically stressed cells in the CCCP-stimulated samples exhibited a reduced level of orange fluorescence after the mitochondria became depolarized. As the membrane potential gradient collapses, JC-1 will equilibrate out of the mitochondria and into the cytosol, causing cells to lose their red fluorescence (Ms. Tracy Hanson, ICT).

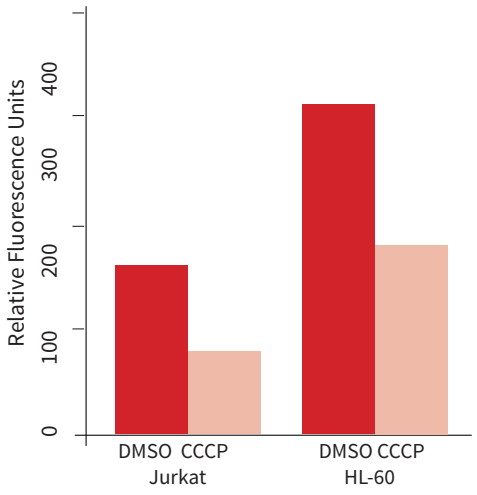


FIGURE 3: FLOW CYTOMETRY ANALYSIS

Jurkat cells were treated with 1 µM staurosporine, an apoptosis-inducing agent (left, black histogram), or DMSO, a negative control (right, red histogram), for 3 hours at 37°C, then stained with MitoPT JC-1 for 15-20 minutes. Cells were analyzed with an Accuri C6 Flow Cytometer.

Apoptotic cells (left, black) exhibit significantly less fluorescence compared to healthy, negative control cells (right, red). Normal healthy cells, containing mitochondria with polarized inner membranes, concentrate MitoPT JC-1 and fluoresce bright orange.

Apoptotic cells, bearing depolarized mitochondria, exhibit a reduced orange fluorescence relative to the healthy cell population. Almost all cells treated with staurosporine exhibit apoptosis-associated mitochondrial depolarization (Ms. Tracy Hanson, ICT, 13A54, 050113).

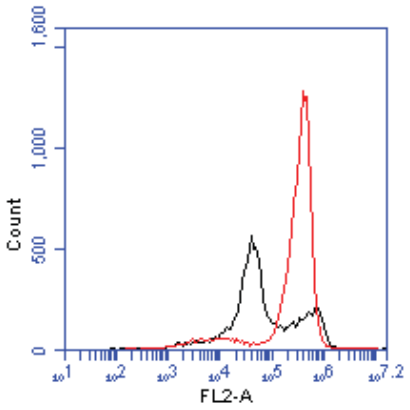
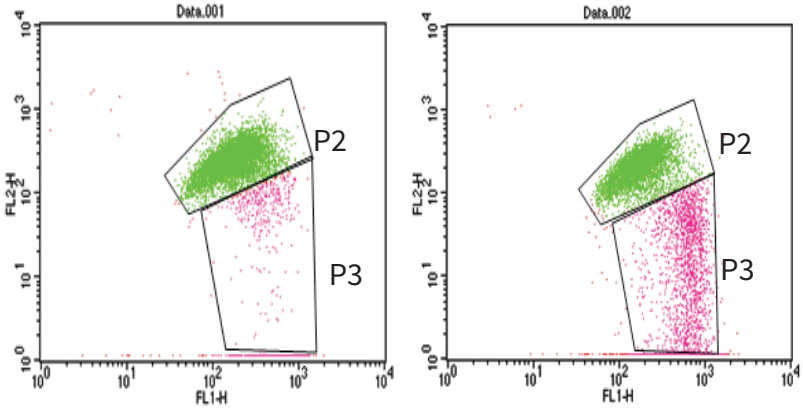


FIGURE 4: TWO-COLOR FLOW CYTOMETRY ANALYSIS

Jurkat cells were treated with either DMSO (Negative Control) or staurosporine (Positive Control) for 3 hours at 37°C and labeled with MitoPT JC-1 for 15 minutes. Cells were analyzed with a FACS Caliber Becton Dickinson Flow Cytometer. Collapse of the mitochondrial membrane potential gradient is indicated by an increase in the number of cells falling from P2 (Green) into P3 (Pink) following induction of apoptosis (Dr. Brian Lee, ICT).



Negative Control Positive Control

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ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

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9401 James Ave. S. #155, Bloomington, MN 55431 USA 952-888-8788, 800-829-3194