APPLICATION NOTE

FLOW CYTOMETRY

CyAn™ ADP | JC-1 Determination of Mitochondrial Membrane Potential

Mitochondria play an important role in several biological processes, one of which is the involvement in programmed cell death or apoptosis[1-3]. The ability to measure mitochondrial membrane potential (ΔΨ) by flow cytometry has enabled researchers to gain a more detailed understanding of the role of this organelle. As a result of the ΔΨ across mitochondrial membranes being larger than ΔΨ across cytoplasmic membranes, healthy eukaryotic cells exposed to the lipophilic cationic dye 5, 5', 6, 6'-tetrachloro-1, 1', tetraethylbenzimidazolocarbo-cyanine iodide (JC-1) will accumulate the dye within the mitochondria. When excited at 488nm, monomers of JC-1 fluoresce maximally at 527nm. However, at a higher concentration JC-1 forms aggregates which emit maximally at 590nm. Thus cells with high mitochondrial ΔΨ fluoresce orange, and cells with low mitochondrial ΔΨ fluoresce green. Loss of mitochondrial ΔΨ is regarded as an indicator of the onset of apoptosis[1-3].

Materials and Methods

Monolayers of human umbilical vein endothelial cells (HUVEC; Cambrex) were grown in EGM2 cell culture m edia at 37 °C / 98.6 °F with 5% CO_2. The cell culture media was then replaced with fresh media or fresh media containing 5 µM carbonyl cyanide m-chlorophenylhydrazone (mCICCP; a mitochondrial uncoupler) prior to incubation for 45 minutes. After the media was removed, the cells were washed with warmed PBS before incubating with cell culture media containing 1 µM JC-1 and 0.1% bovine serum albumin for a further 45 minutes. Following JC-1 incubation, the cell monolayers were again washed with warmed PBS and suspended in 1 mL cell detachment solution. Cells were then kept in the dark on ice and analysed within 15 minutes.

HUVEC cells were gated using forward and side scatter (Figure 1a) and gated onto plots showing FL1 fluorescence against FL2 fluorescence (Figure 1b, 1c). Green fluorescence (530 ± 20nm) and orange fluorescence (585 ± 15nm) of the JC-1 stained HUVEC cells was measured using a CyAn ADP LX 9 Color flow cytometer*. Compensation settings for green to orange, and orange to green were set at approximately 4% and 10% respectively.

*The FL2 bandpass filter (575 ± 12.5nm) was changed to a 585 ± 15nm filter. The corresponding dichroic mirror (position 3; 595 long pass) was changed to a 625 long pass.

Results

The addition of the mitochondrial poison, mCICCP, resulted in a shift from orange (Figure 1b) to green (Figure 1c) cellular fluorescence. This indicated a drop in mitochondrial membrane potential.

Figure 1a

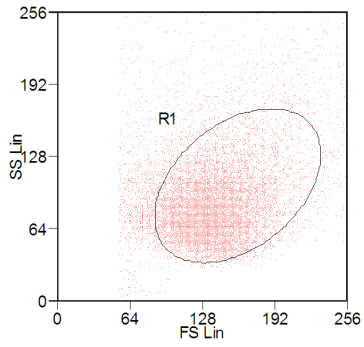


Figure 1b

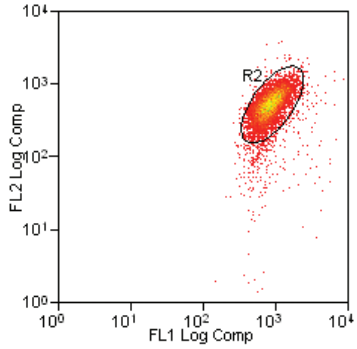
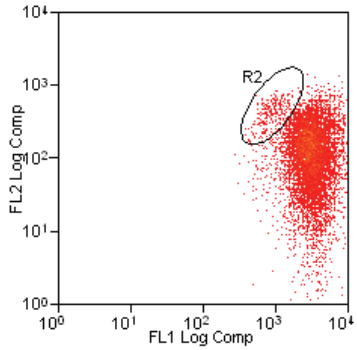


Figure 1c



Assessment of mitochondrial membrane potential using JC-1. HUVEC cells were gated using forward and side light scatter (1a) and gated on to plots showing compensated FL1 against FL2 (1b, 1c). Untreated HUVEC cells (1b). HUVEC cells post mCICCP treatment (1c) showing a higher green fluorescence and lower orange fluorescence than the untreated HUVEC cells. This change in fluorescence is consistent with a loss in mitochondrial membrane potential.

TECHNICAL TIP

> Care should be taken to preserve cell physiology as much as possible before analysis. For example, procedures such as centrifugation should be avoided or minimized.

Discussion

The CyAn ADP LX 9 Color is well suited for the assessment of mitochondrial membrane potential using JC-1. The standard optical filter layout of the CyAn ADP LX 9 Color was configured for the measurement of up to nine commonly used dyes and fluorochromes. However, the optical filters can be simply changed in a matter of seconds to accommodate almost any dye or fluorochrome to fit specific needs, as shown here with JC-1.

References

1. Cossarizza A., Salvioli S., Franceschini M.G., Kalashnikova G., Barbieri D., Monti D., Grassilli E., Tropea F., Troiano L. and Franceschi C. 1995. Mitochondria and apoptosis: a cytofluorimetric approach. *Fund. Clin. Immunol.,* 3: 67-68.
2. Kroemer, G., Zamzani, N., and Susin, S.A. 1997. Mitochondrial control of apoptosis. *Immunol.Today,* 18:44-51.
3. Mathur A., Hong Y., Kemp B.K., Barrientos A.A. and Erusalimsky J.D. 2000. Evaluation of fluorescent dyes for the detection of mitochondrial membrane potential changes in cultured cardiomyocytes. *Cardiovasc. Res,* 46:126-38.

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PRODUCT	CODE
CyAn ADP LX 9 Color	CY201

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Denmark
Corporate Headquarters
Tel +45 44 85 95 00

United States of America
Tel +1 805 566 6655

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www.dako.com

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