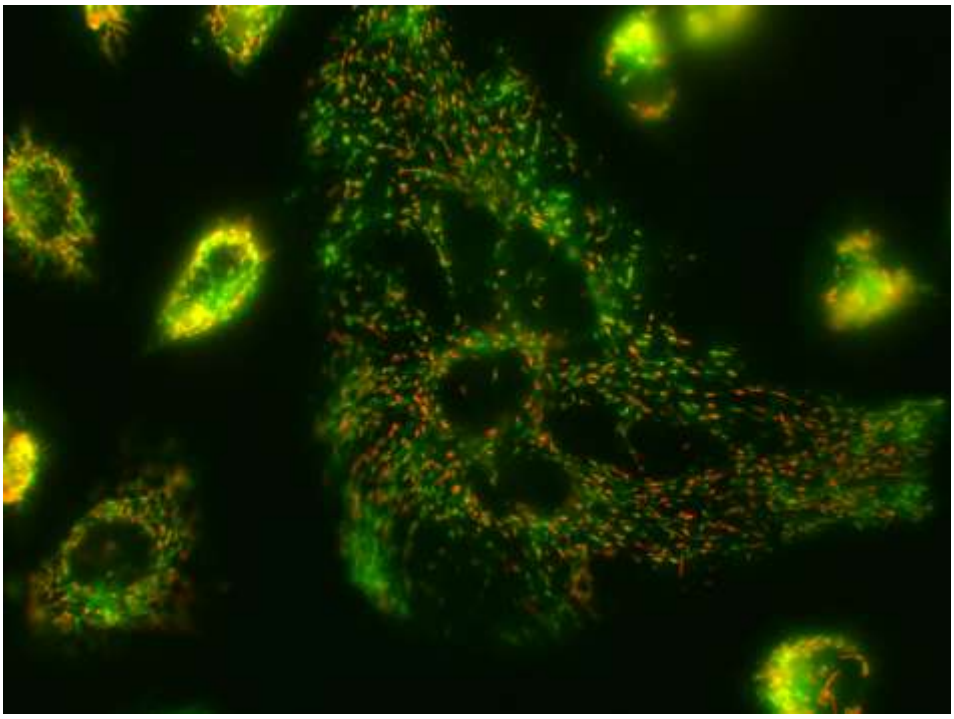
PRODUCT INFORMATION SHEET



MARKERGENE™ *DYRECT JC-1* MITO HEALTH ASSAY KIT
Product M1890

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MARKERGENE™ *DYRECT JC-1* MITO HEALTH ASSAY KIT

(Product M1890)

NOTE: The following information is given as a viable methodology for use of the **MarkerGene™ Dyrect JC-1 Mito Health Assay Kit**. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment. For exact concentrations and incubation times we recommend reading the literature for specific cell type information as the fluorescent dye used in this assay has a long history as a direct and simple measurement of delta-psi changes in mitochondria of live cells.

I. OVERVIEW

Mitochondria and their functional status provide an early indication of cellular toxicity and thus have emerged as a critical target in drug discovery and toxicity profiling. Apoptosis, necrosis and slow degenerative disease all exhibit changes in the electrochemical gradient across the mitochondrial membranes, ΔΨm, which give rise to the creation of the electronic potential necessary for ATP production. Introduced in 1991, the live cell carbocyanine dye JC-1 indicates this change in ΔΨm by reversibly changing its emission from green in non-polar mitochondria to orange/red in hyperpolarized mitochondria. The red signal is a result of the aggregation of green emitting JC-1 monomers into orange/red emitting aggregates – so called J-aggregates. Useful in nearly all cell types tested, it remains one of the most reliable markers of mitochondrial health in live cells.

This kit provides instructions that allow for flexibility in the *in situ* delivery of the fluorescent reagent with minimal perturbations to the cell where disruption of the native conditions could alter results. Or where a media change would disrupt sensitive drug dosing formulations. This is accomplished by using an alternative protocol that allows adding the reagent at 2X concentration directly *in situ* to cells in complete media. While some background will be evident, the signal to noise is sufficient for most analyses.

Alternatively, the Opti-Klear™ Live Cell Imaging Buffer (M1898) provided will reduce the background and can maintain your cells in the absence of $C0_2$ for at least 1-4 hours. This buffer is ideal for maintaining culture media pH outside of CO_2 incubators for cells normally grown in bicarbonate buffered media formulations.

This kit provides convenient single use vials, with enough materials to perform 500 assays based on labeling volumes of 100 μL for 96 well plates – adjust accordingly for your particular plate or vial format.

II. MATERIALS

1 JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) (5 single use vials)

2 DMSO (dimethylsulfoxide) 1 mL

3 Opti-Klear™ Live Cell Imaging Buffer 5X: 1 x 30 mL

Required but not provided:

Tissue culture media, sterile distilled H_20.

Storage and Handling. Fluorescent reagents should be handled with care, kept cold when not in use, and stored frozen (-20°C). In case of contact with skin or eyes, wash thoroughly with soap and cold-water. Reagents should be stable for at least 6 months following purchase. High background fluorescence readings for blank samples will indicate decomposition. Before opening, each vial must be at room temperature.

Store Opti-Klear™ Live Cell Imaging Buffer 3 at 4°C in the amber bottle provided to avoid the creation of toxic hydrogen peroxide from light exposure.

III. STAINING PROTOCOL FOR ADHERENT CELLS IN COMPLETE MEDIA

1. JC-1, **Solution A** (100X or 0.5M stock): Add 100 μL of DMSO 2 to a vial of JC-1 1, briefly vortex and transfer to a sterile 15 mL centrifuge tube. This solution is unstable in solution for long periods, so prepare as needed from our convenient single use vials.

2. JC-1, **Solution B** (2X or 10μM): Add 5 mL complete media at 37°C to **Solution A** and vortex for 30 seconds to prevent JC-1 red aggregate/precipitate formation. If aggregates persist, filter through a 0.22 μm filter.

3. Remove 50% of the cell culture media from cells, and replace with an equal amount of **Solution B**.

4. Incubate for 30 minutes to 1 hour at 37°C.

5. Observe fluorescence staining at both Em/Ex 514nm/529nm (monomer) and Em/Ex 585nm/590nm (J-aggregates) by plate reader, flow cytometer or epi-fluorescence microscope. (FITC, rhodamine filter sets or equivalents are acceptable as well.)

6. Total mitochondria show as green in FITC channel and J-aggregates show as orange/red in rhodamine channel.

IV: STAINING PROTOCOL FOR ADHERENT CELLS WITH COMPLETE MEDIA REMOVAL

1. JC-1, **Solution A**: (100X or 0.5M stock). Add 100 µL of DMSO 2 to a vial of JC-1 1, briefly vortex and transfer to a sterile15 ml centrifuge tube (not provided). JC-1 is unstable in solution for long periods, so prepare as needed from our convenient single use vials.

2. JC-1, **Solution B**: (1X, 5µM) Add 10 mL serum-free media at 37°C to **Solution A** and vortex for 30 seconds to prevent JC-1 red aggregate/precipitate formation. If aggregates persist, filter through a 0.22 µm filter.

3. Remove cell culture media from cells by aspiration, wash once with serum free media and replace with **Solution B**.

4. Incubate for 30 minutes to one hour at 37°C.

5. To improve signal to noise replace media with 1X Opti-Klear™ Live Cell Imaging Buffer at 37°C supplied in the kit as a 5X concentrate prior to analysis. To prepare 1X Opti-Klear™ Imaging Buffer add 2 mL of 5X Opti-Klear™ 3 to 8 mL sterile water and briefly mix.

6. Observe fluorescence staining at both 529nm (monomer) and 590nm (J-aggregates) using excitation at 514 and 585 by plate reader, flow cytometer or epi-fluorescence microscope. (FITC, rhodamine filter sets or equivalents are acceptable as well.)

7. Total mitochondria show as green in FITC channel and J-aggregates show as orange/red in rhodamine channel.

Note:

1.) In HeLa cells green staining of total mitochondria is visible by 1-5 minutes, and by 10 minutes, J-aggregates form and are at a maximum by 30 minutes. Length of incubation and concentrations may vary for other cell types – optimize for your specific cell type.

2.) For non-adherent cells, pellet cells and resuspend in **Solution B**.

FIGURES:

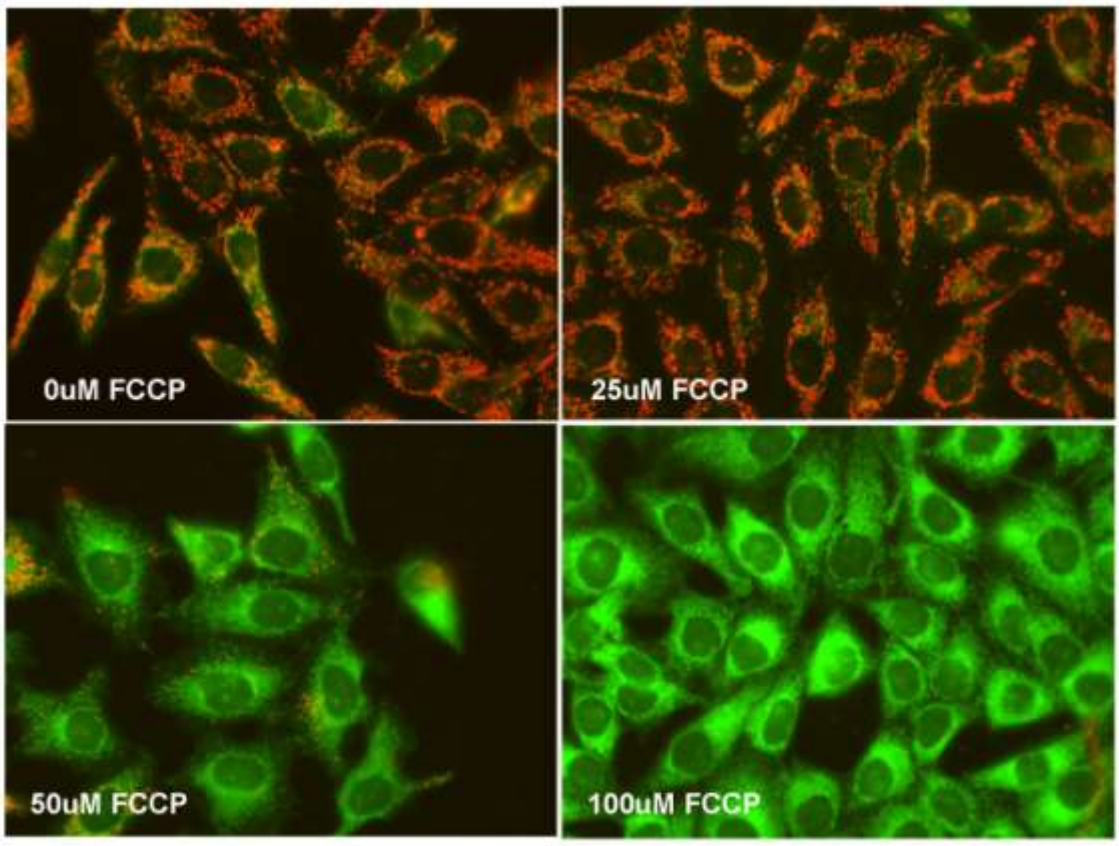

Figure 1: Reduction in red J-aggregate formation in HeLa cells with increasing concentrations of the protonophore carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) an uncoupler of mitochondrial oxidative phosphorylation. HeLa cells in complete media were treated with FCCP for 30 minutes, then JC-1 added for 30 minutes and imaged with a 40x, 1.3NA oil immersion objective using standard FITC/TRITC filter sets.

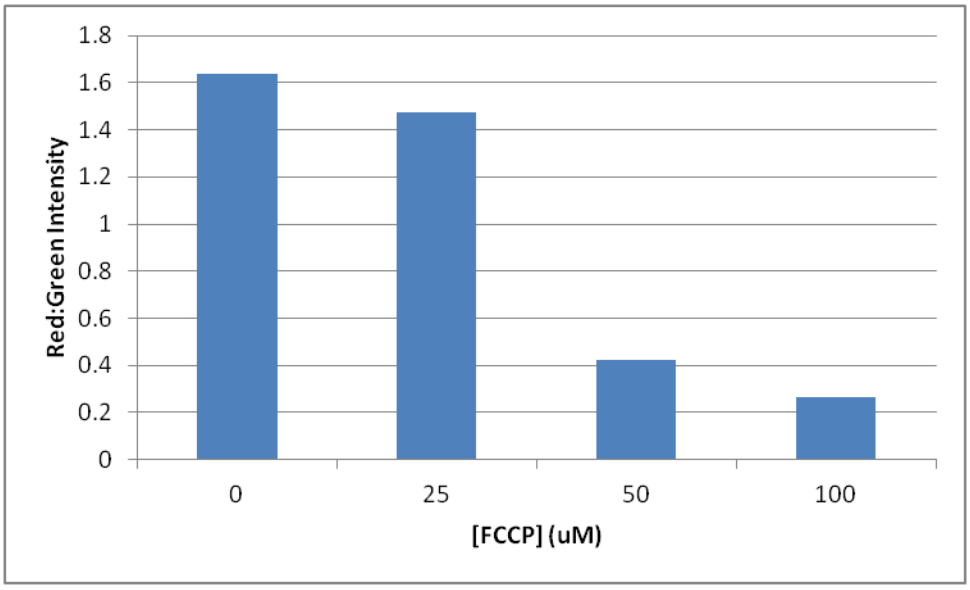

Figure 2: Reduction in J aggregates with increasing concentrations of FCCP. Cells were treated as described in Figure 1. Image intensity of red and green signal were collected and converted to the ratios shown.

M1890 KIT CONTENTS

DESCRIPTION	QUANTITY	PART NO.	STORAGE
SAMPLE PREPARATION			
1 JC-1	5 VIALS	M1890-001	F, L, D, B
2 DMSO	1 mL	M1890-002	FL,G, B
3 5X Opti-Klear™Live Cell Imaging Buffer	30 mL	M1890-003	C
DOCUMENTATION			
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Notes: F=store at or below -18^{o}C; R=store at room temperature; C=store cold (4^{o}C); L=light sensitive; D=store desiccated; FL=flammable; G=wear protective clothing/gloves/safety glasses when using; B=avoid breathing dust/fumes.

REFERENCES:

1.) Smiley ST, Reers M, Mottola-Hartshorn C, Lin M, Chen A, Smith TW, Steele GD Jr, Chen LB. (1991) "Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1." Proc Natl Acad Sci U S A 88(9):3671-5.

2.) Reers M, Smith TW, Chen LB. (1991) "J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential." Biochemistry. 30(18):4480-6.

3.) Reers M, Smiley ST, Mottola-Hartshorn C, Chen A, Lin M, Chen LB. (1995) "Mitochondrial membrane potential monitored by JC-1 dye." Methods Enzymol. 1995;260:406-17.

4.) Wallace DC. (1999) "Mitochondrial diseases in man and mouse." Science, 283 (5407): 1482-88.

5.) Mathur A, Hong Y, Kemp BK, Barrientos AA, Erusalimsky JD. (2000) Evaluation of fluorescent dyes for the detection of mitochondrial membrane potential changes in cultured cardiomyocytes." Cardiovascular Research 46 (1): 126-138.

6.) Salvioli S, Ardizzoni A, Franceschi C, Cossarizza A. (1997) "JC-1, but not DiOC(6)(3) or rhodamine 123, is a reliable fluorescent probe to assess Delta Psi changes in intact cells: Implications for studies on mitochondrial functionality during apoptosis." FEBS Letters, vol. 411 (1): 77-82.

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