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Product Information

Isolated Mitochondria Staining Kit

Catalog Number **CS0760**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Mitochondria, the site of most energy production in eukaryotic cells, have a double membrane structure: an outer membrane and a folded inner membrane.[1] Across the inner membrane of intact mitochondria there is a voltage gradient (membrane potential = $\Delta\psi$) with the inside negative and the outside positive. Mitochondrial membrane potential dissipation is known to be an early event in apoptosis. Thus, an effective distinction between apoptotic and healthy cells can be achieved by measuring the inner membrane potential. This can be done by observing the uptake of the cationic carbocyanine dye JC-1 into the mitochondrial matrix,[2] according to the membrane potential. In healthy cells, this dye concentrates in the matrix, where it forms bright red fluorescent agglomerates. Any event that dissipates the mitochondrial membrane potential prevents the accumulation of the JC-1 dye in the mitochondria and thus, the dye is dispersed in the cytoplasm, leading to a shift from red (agglomerated JC-1) to green fluorescence (JC-1 monomers).

This kit enables a fast and simple staining of isolated mitochondria from animal tissues and cell lines. It includes valinomycin, a mitochondrial membrane-dissipating agent, for control experiments.

Components

The kit is sufficient for 50 staining assays of 2 ml using cuvettes or 1,000 assays of 0.1 ml using 96 well plates.

- JC-1 Stain 25 µg
  Catalog Number J4519
- DMSO 1 ml
  Catalog Number D8418
- JC-1 Assay Buffer 5× 25 ml
  Catalog Number J4394
- Valinomycin Ready Made 0.1 ml
  Catalog Number V3639

Reagents and Equipment Required but Not Provided.

- Ultrapure water (17 MΩ·cm or equivalent)
- Mitochondria Isolation Kits (For tissues - Catalog Number MITOISO1 and for cells - Catalog Number MITOISO2)
- Fluorimeter cuvettes (Catalog Number C0793) or 96 well plates (Catalog Number P8741)
- Fluorimeter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Isolated Mitochondria

Prepare isolated mitochondria according to the Mitochondria Isolation Kit protocol (For tissues - Catalog Number MITOISO1 and for cells - Catalog Number MITOISO2). The initial protein concentration for the staining procedure should be 0.1–1 mg/ml (1 mg/ml is preferred) for assays performed in 96 well plates or 2–20 mg/ml for assays performed in cuvettes.

JC-1 Stain

Dissolve the contents of the vial of JC-1 Stain (Catalog Number J4519) in 25 µl of DMSO, vortexing vigorously. **Make sure the dye is completely dissolved**. Store the JC-1 Stock Solution (1 mg/ml) at –20 °C, preferably in working aliquots. Before use, prepare the JC-1 Working Solution (0.2 mg/ml) by diluting the JC-1 Stock Solution 5-fold with DMSO. Keep the JC-1 Working Solution at room temperature.

Note: JC-1 is extremely sensitive to light and should be protected from light at all stages of its preparation.

1× JC-1 Assay Buffer
Dilute an aliquot of the JC-1 Assay Buffer 5× (Catalog Number J4394) 5-fold with ultrapure water. Keep the diluted buffer at 4 °C before use. The concentrated buffer may be refrozen.

Valinomycin Working Solution
Before use, dilute an aliquot of the Valinomycin Ready Made (1 mg/ml, Catalog Number V3639) 10-fold in DMSO to 0.1 mg/ml.

Storage/Stability
The kit is shipped on dry ice and stored at –20 °C.

Procedure
Fluorimeter analysis
1. Prepare a JC-1 Staining Solution – Immediately before starting the assay, dilute the JC-1 Working Solution (0.2 mg/ml) 1:1,000 in 1× JC-1 Assay Buffer to a final concentration of 0.2 μg/ml. If completely dissolved, use immediately. If not, incubate on ice until completely dissolved, up to 20 minutes while vortexing every few minutes.
2. For a control assay, add valinomycin to the mitochondrial sample to a final concentration of 0.5 μg/ml (200-fold dilution of the Valinomycin Working Solution). Keep the mitochondrial sample containing valinomycin on ice for ~10 minutes to allow complete dissipation of the membrane potential.

The procedure was performed using a Perkin-Elmer LS 50 B fluorimeter (excitation wavelength = 490 nm; slit = 5 nm, emission wavelength = 590 nm; slit = 7.2 nm) and a BIO-TEK (Synergy HT) plate reader (excitation wavelength = 485 nm, emission wavelength = 590 nm, sensitivity = 100). If a 490 nm filter is not available, the excitation may be performed in the range of 475–520 nm.

Assay in cuvettes
The following assay is designed for a 2 ml assay reaction volume in 4 ml cuvettes.

1. Add 1.8 ml of the JC-1 Staining Solution to a 4 ml fluorimeter cuvette.
2. Add a volume (up to 200 μl) of the isolated mitochondrial sample equivalent to 10–100 μg of protein (100 μg is preferred) and mix by inversion.
3. If required, bring the total reaction volume to 2 ml with JC-1 Staining Solution. Mix by inversion.
4. Read the fluorescence of the sample in a fluorimeter using time-drive method with the following settings:
   Excitation wavelength = 490 nm
   Emission wavelength = 590 nm
5. For assaying the valinomycin treated mitochondrial control sample, repeat steps 1-4 with the valinomycin treated sample.

Assay in 96 well plate
This assay is designed for a total volume of 100 μl.

1. Add 90 μl of the JC-1 Staining Solution per well.
2. Add a volume (up to 10 μl) of the isolated mitochondrial sample or valinomycin treated mitochondrial sample equivalent to 0.5–5 μg of protein (5 μg is preferred) per well.
3. If required, bring the total reaction volume to 100 μl with JC-1 Staining Solution.
4. Read the fluorescence of the sample in a fluorimeter using time-drive method with settings as follows:
   Excitation wavelength = 490 nm
   Emission wavelength = 590 nm

See Appendix, Figure 1 for typical results with this kit of mitochondria staining using a multiwell plate.

Fluorescence microscopy
JC-1 aggregates in the intact mitochondria can be visualized as bright red staining using standard broad-pass filters that are used routinely for propidium iodide or Cy3™ visualization.

Trouble Shooting
1. If the fluorimeter used does not support time-drive assays, an end point measurement may be used. In this case, it is important to run a valinomycin control sample. For an end point assay, follow steps 1-2 in the protocol. Then incubate the cuvette/plate containing the sample and the dye for 7–10 minutes (the time required to reach uptake saturation) in the dark at room temperature and measure the fluorescence (excitation = 490 nm, emission = 590 nm).
2. If the apparatus does not include a 490 nm filter, the excitation may be performed in the range of 475–520 nm.
3. In order to monitor real-time membrane dissipation, the valinomycin can be added directly to the assay sample (final concentration of 0.5 μg/ml) during the fluorescence measurement, after reaching uptake saturation.

4. If the signal is off scale (too high or too low), consider adjusting the slit width or the sensitivity accordingly.
5. If the uptake signal is not sufficient:
   - Use a fresh aliquot of the 1 mg/ml JC-1 Stock Solution and make sure that the dye is completely dissolved.
   - Calibrate the assay using different concentrations of isolated mitochondrial protein. The volume of the assay sample should not exceed 10% of the total reaction volume.

References

1. Rice, J.E., and Lindsay, J.G., in *Subcellular Fractionation: A Practical Approach*, Graham, J.M., and Rickwood, D., eds., Oxford Univ. Press Inc. (New York, NY: 1997), pp 107-142.
2. Reers, M. et al., J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. Biochemistry, **30**, 4480-4486 (1991).

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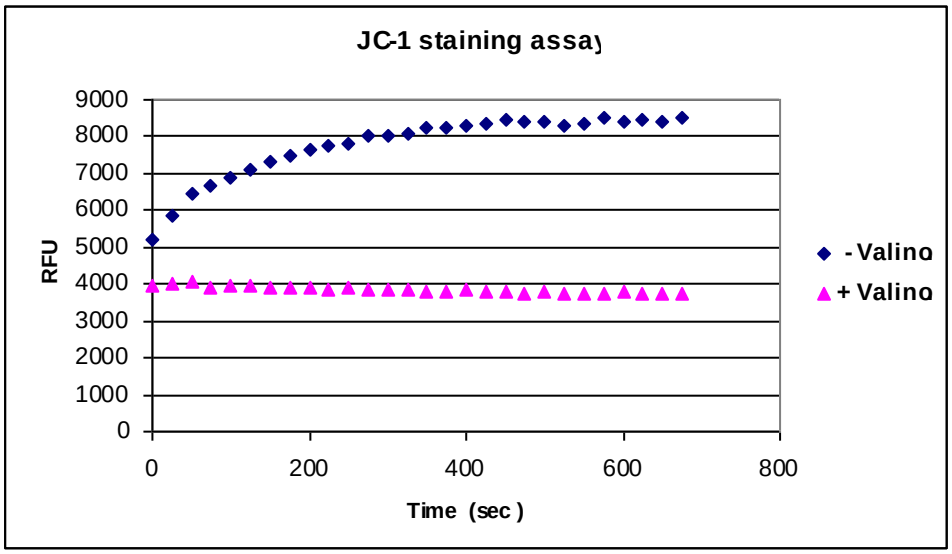
Appendix

Figure 1.
Typical Mitochondria Staining using JC-1 Stain in a Multiwell Plate Format



RFU – Relative Fluorescence units.
Mitochondria were isolated from CHO cells using the Cell Mitochondria Isolation Kit (Catalog Number MITOISO2) and stained in a multiwell plate using the Isolated Mitochondria Staining Kit. The upper line represents the JC-1 dye uptake of an intact mitochondrial sample. The lower line represents the dye uptake of the valinomycin treated mitochondrial control sample.