Protocol Booklet

Product Code(s)	HB13032
Product Name(s)	JC-10 Mitochondrial Membrane Potential Assay Kit
Purpose	Measurement of mitochondrial membrane potential in cultured cells.

Please note: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

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Product Overview

JC-10 is a highly soluble fluorescent probe ideal for assessing mitochondrial membrane potential. In healthy cells with polarized mitochondria, JC-10 aggregates, emitting a strong orange fluorescence (Ex/Em: 540nm/590nm). However, in cells with depolarized mitochondria, a hallmark of apoptosis and other cellular stresses, JC-10 reverts to its monomeric form, resulting in a shift to green fluorescence (Ex/Em: 490nm/525nm). This reversible, ratiometric change in fluorescence emission provides a reliable indicator of mitochondrial health. JC-10's superior aqueous solubility to JC-1 makes it a convenient and robust tool for various applications, including fluorescence microscopy, flow cytometry, and high-throughput screening. This kit contains everything needed to make 25 mL of working solution which is suitable for five 96-well plates or 500 flow cytometry samples.

Components & Storage

HB13032 JC-10 Mitochondrial Membrane Potential Assay Kit contains:

SKU	Component	Quantity	Storage Temperature
HB19967	100x JC-10 in DMSO (≈3.5mM)	250µl	-20°C
HB11290	JC-10 Mitochondrial Membrane Potential Assay Kit Loading Buffer	25ml	-20°C
HB14754	JC-10 Mitochondrial Membrane Potential Assay Kit Masking Buffer	25ml	-20°C

Protocol

This protocol provides a starting point for experiments and will need optimising for the specific details of each assay. The optimal dye concentration and loading time will vary depending on cell type and application with a recommended range being 1-15µM.We recommend using either CCCP (HB5062) or FCCP (HB2903) as a positive control with this kit using a concentration of 2-5µM and a pre-incubation of 30 minutes before adding dye to cells.

Plate Reader Assay

1. Seed cells in a 96-well plate and treat with test compounds prior to the addition of dye loading solution.
    a. The incubation time of test compound will depend upon the precise agent used and therefore may need optimisation. For CCCP or FCCP a 30-minute incubation is sufficient.
    b. Aim for a final volume of 100 µL/well (80 µL of cell culture medium and 20 µL of 5X test compound).
    c. This assay is compatible with serum containing media however some test compounds show lower potency in the presence of serum. If a serum-free experiment is required, then the serum-containing media should be replaced with HEPES buffered Hanks balanced salt solution.

2. Remove all reagents from freezer and allow to warm to room temperature. Protect from light.

3. Prepare dye loading solution by adding 50 µL of 100X JC-10 stock solution to 5 mL of Dye Loading Buffer. Vortex briefly to mix, the solution should transition from pink to almost colourless when dissolved.

4. Add the 50 µL/well of dye loading solution directly to cells.

5. Incubate cells for 30 - 60 min at 37 °C. Protect from light.

6. Add 50 µL/well masking solution directly to wells containing cells and dye loading solution.
    a. The volume of masking solution may need optimising dependent upon the experiment.

7. Measure fluorescence using a microplate reader for ratiometric analysis.
    a. To measure JC-10 monomer fluorescence, use Excitation = 490nm, Emission = 525nm or filters designed for FITC/GFP.
    b. To measure JC-10 aggregate fluorescence, use Excitation = 540nm, Emission = 590nm or filters designed for Texas Red.
    c. It is possible to measure the fluorescence ratio for up to 8 hours following dye addition for kinetic experiments. We recommend using an imaging interval of >5 minutes to reduce photobleaching.

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Microscopy Assay

1. Seed cells in an imaging plate and treat with test compounds prior to the addition of dye loading solution.
    a. It is possible to load JC-10 dye prior to the addition of test compounds for real-time visualization of apoptosis if desired.
    b. The incubation time of test compound will depend upon the precise agent used and therefore may need optimisation. For CCCP or FCCP a 30-minute incubation is sufficient.
    c. This assay is compatible with serum containing media however some test compounds show lower potency in the presence of serum. If a serum-free experiment is required, then the serum-containing media should be replaced with HEPES buffered Hanks balanced salt solution.

2. Remove all reagents from freezer and allow to warm to room temperature. Protect from light.

3. Prepare dye loading solution by adding 50 µL of 100X JC-10 stock solution to 5 mL of Dye Loading Buffer. Vortex briefly to mix, the solution should transition from pink to almost colourless when dissolved.

4. Add the dye loading solution directly to cells. The volume required will vary depending on well size. We recommend a dilution of 1 part working solution to 2 parts cell culture media (e.g. 50 µL of dye loading solution to 100 µL of cell culture medium).

5. Incubate cells for 30 - 60 min at 37 °C. Protect from light.

6. Add masking buffer directly to cells, the volume required will vary depending on the well size. We recommend a dilution of 1 part masking buffer to 3 parts volume in well (e.g. 50 µL of masking buffer to the 150 µL per well from previous steps).

7. Image cells using a fluorescence microscope. JC-10 monomers can be visualized with standard FITC or GFP filters, and JC-10 aggregate fluorescence can be viewed using Texas Red® or Propidium Iodide filters.
    a. It is possible to design an imaging configuration to capture the monomer and aggregate fluorescence simultaneously using a 490nm excitation filter paired with a 530nm long pass emission filter.
    b. It is possible to measure the fluorescence ratio for up to 8 hours following dye addition for kinetic experiments. We recommend using an imaging interval of over 5 minutes to reduce the photobleaching of dye.

Flow Cytometry Assay

1. Remove all reagents from the freezer and allow to warm to room temperature. Protect from light.

2. Prepare dye loading solution by adding 50 µL of 100X JC-10 stock solution to 5 mL of Dye Loading Buffer. Vortex briefly to mix, the solution should transition from pink to almost colourless when dissolved.

3. Collect and suspend cells exposed to test reagents in 100 µL of Hank's balanced salt solution, or equivalent buffer, at 1-5 x 10^5 cells/tube.

4. Add the dye loading solution directly to tubes. We recommend adding 50 µL/tube.

5. Incubate cells for 15 - 30 min at room temperature or 37 °C. Protect from direct light.

6. Analyse cells using a flow cytometer. To detect cells with healthy mitochondria, use phycoerythrin (PE) settings. To detect cells with compromised mitochondria, use FITC settings. Use positive controls (e.g. FCCP-treated cells) to perform compensation corrections.

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Guidelines, precautions, troubleshooting

Please contact our technical support team at technicalhelp@hellobio.com for advice on how to resolve any problems encountered when using this product. Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the product.

Contact

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