TECHNICAL MANUAL

JC-10 Mitochondrial Membrane Potential Assay Kit

- **SKU CODE:** ARMA00351
- **DETECTION PRINCIPLE:** Ratiometric Fluorescence Assay
- **RUO:** Research-Use-Only

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JC-10 Mitochondrial Membrane Potential Assay Kit

Please read entire manual carefully before starting experiment.

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1. Intended Use

The JC-10 Mitochondrial Membrane Potential Assay Kit provides a highly sensitive, ratiometric method for detecting early stages of apoptosis and cellular stress in live cells. By monitoring changes in the mitochondrial membrane potential, researchers can quantify mitochondrial health across diverse platforms, including fluorescence microscopy, flow cytometry, and microplate readers.

The primary advantage of JC-10 over its traditional analogue, JC-1, lies in its superior aqueous solubility. While older generation dyes rapidly precipitate out of standard buffers at functional working concentrations, JC-10 remains completely soluble, eliminating erratic artifactual signals, reducing hands-on wash steps, and delivering a robust, reproducible signal-to-noise ratio optimised for high-throughput screening applications.

This manual must be read in its entirety before using this product. **For Research Use Only. Not to be used for diagnostic or therapeutic purposes.**

2. Principle of the Assay

JC-10 is a dual-emission ratiometric probe that measures mitochondrial membrane potential. In healthy cells, JC-10 concentrates inside highly polarised mitochondria, forming J-aggregates that emit orange fluorescence (540 nm / 590 nm). In stressed or depolarised cells, as the membrane potential collapses, the dye disperses into the cytoplasm as monomers, shifting the signal to green fluorescence (490 nm / 525 nm).

The ratio of orange-to-green fluorescence (F590 / F525) is a direct measure of mitochondrial depolarisation.

3. Materials Provided

Component	Amount	Cap Colour	Storage	Reconstitution
JC-10 Dye (Dry Powder)	0.5 mg	Red	-20°C (Desiccated/Foil)	245 µl Anhydrous DMSO
FCCP Control (Dry Powder)	50 µg	Violet	-20°C	50 µl Anhydrous DMSO
Dye Loading Buffer	25 ml	WM	4°C	Ready-to-use 1X HBSS
Masking Buffer Matrix	25 ml	NM	4°C	Used to dissolve Mask Powder
Brilliant Black BN (Powder)	175 mg	–	Room Temp or 4°C	Dissolve in Masking Buffer

4. Storage and Handling

Store the unopened kit at -20°C. Bring all components to room temperature before use.

- **JC-10 Dye:** Ready to use once reconstituted. Store at -20°C.
- **FCCP Control:** Ready to use once reconstituted. Store at -20°C.
- **Dye Loading Buffer:** Ready to use as supplied. Store at 4°C.
- **Masking Buffer:** Ready to use as supplied. Store at 4°C.

Protect all JC-10 solutions from direct light.

5. Other Supplies Required

- Black-walled, clear-bottom 96-well microplates.
- Ratiometric fluorescence microplate reader with bottom-read capability.
- Anhydrous DMSO for reconstitution of the dye and control.
- HBSS or serum-free medium for dilution of the FCCP control.
- 37°C, 5% CO_2 cell culture incubator.
- Vortex mixer, pipettes and pipette tips.

6. Reagent Preparation

Reconstitution: Reconstitute the JC-10 Dye with 245 µl of anhydrous DMSO and the FCCP Control with 50 µl of anhydrous DMSO. Dissolve the Brilliant Black BN powder in the Masking Buffer Matrix to prepare the Masking Solution.

The volumes below are per 1 plate. Warm all buffers to room temperature or 37°C before use. Protect all JC-10 solutions from direct light.

Dye Loading Solution:

1. Add 50 µl of the JC-10 solution to 5.0 ml of Dye Loading Buffer.
2. Vortex briefly to mix. The solution will transition from pink to near-colourless when homogeneously dissolved.

FCCP Positive Control Solution (40 µM):

1. Add 2.0 µl of FCCP to 198 µl of HBSS or serum-free media. Vortex to mix.

7. Assay Procedure

Bring all reagents to room temperature and prepare the working solutions as described above before starting. Protect the plate from direct light throughout the procedure.

1. **Cell Setup:** Seed cells in a black-walled, clear-bottom 96-well plate. Plan for a final volume of 100 µl per well (80 µl of medium + 20 µl of test compound).
2. **Positive Control Pre-Treatment:** To designated positive control wells, add 10 µl of diluted FCCP solution directly to 100 µl cell volume. Incubate for 30 minutes at 37°C prior to dye addition to completely uncouple the mitochondria.
3. **Dye Loading:** Add 50 µl of prepared Dye Solution directly to every well. Do not remove culture medium or pre-treatment liquids.
4. **Incubation:** Incubate the cells for 30 to 60 minutes at 37°C in a 5% CO_2 incubator. Protect the plate from direct light.
5. **Masking:** Add 50 µl of Masking Solution directly to all wells containing cells and dye loading solution. Do not remove any liquid from wells.

6. **Measurement:** Immediately measure fluorescence using a ratiometric microplate reader in Bottom-Read mode.

Note: Removal of serum-containing medium is not required.

8. Microplate Reader Optical Settings

Parameter	Monomer Channel (Green)	J-Aggregate Channel (Orange/Red)
Excitation Wavelength	~490 nm	~540 nm
Emission Wavelength	~525 nm	~590 nm
Target State	Depolarised / Compromised Mitochondria	Polarised / Healthy Mitochondria
Instrument Profile	FITC / Fluorescein settings	Texas Red® / TRITC settings

9. Calculation of Results

Calculate the ratio of Aggregate to Monomer fluorescence (F590 / F525). A reduction in this ratio indicates mitochondrial membrane depolarisation and compromised cellular health.

10. Important Notes

- This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
- Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
- Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
- If the signal from your sample falls outside the range of the assay, please adjust the sample by performing further dilution or concentration as needed.

- If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
- Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.