

NutriCulture JC-1 Mitochondrial Membrane Potential Assay Kit

Troubleshooting Guide

100 tests

Cat No: JMM100

Use this guide for common staining and readout problems with the NutriCulture JC-1 Mitochondrial Membrane Potential Assay Kit. The datasheet specifies separate storage conditions for JC-1/CCCP and Assay Buffer, complete dissolution and 1:10 dilution of the buffer, complete thawing of JC-1 without centrifugation, strict light protection, controlled temperature/pH, and immediate analysis after staining.

Before You Start - Quick Checks

1. Store JC-1 (100x) and CCCP at -20°C protected from light; store 10x Assay Buffer at 2-8°C.
2. Warm 10x Assay Buffer until salt crystals dissolve, then dilute 1:10 with molecular-grade nuclease-free water.
3. Use diluted 1x Assay Buffer within one week when stored sealed at 2-8°C.
4. Completely thaw and warm JC-1 (100x) to room temperature before dilution; do not centrifuge the reagent.
5. Keep assay reagents at 25°C and pH 7.4 during the experiment.
6. Analyze samples immediately after completing staining.

Problem	Most likely cause	Recommended action
Salt crystals remain in the Assay Buffer	The 10x buffer was not warmed sufficiently before dilution.	Warm the 10x Assay Buffer until all salt crystals are completely dissolved, then prepare 1x buffer by 1:10 dilution.
JC-1 forms particulates after dilution	The 100x dye may not have been completely thawed and warmed before dilution.	Completely thaw and warm JC-1 (100x) to room temperature before adding it to the aqueous assay system. Do not centrifuge the reagent.
Red/orange aggregate signal is unexpectedly weak	Staining conditions, temperature, pH, or sample handling may differ from the protocol.	Keep reagents at 25°C and pH 7.4, stain at the specified cell density and time, and analyze immediately after staining.
Green monomer signal is unexpectedly dominant	Technical variables such as temperature or pH may have shifted the JC-1 response.	Verify the required temperature and pH conditions and use the CCCP control to confirm that the assay can detect a membrane-potential decrease.
Positive CCCP control does not show the expected decrease	CCCP may not have been added according to the protocol.	For the flow protocol, add 1 uL CCCP to the positive-control sample in 1 mL assay buffer and incubate 5 minutes at 37°C. For microscopy/plate-reader format, use 1 uL per 1 mL 1x buffer.
Flow-cytometry samples contain too many cells	The cell density may exceed the datasheet limit.	Resuspend samples in warm 1x Assay Buffer at no more than 1×10^6 cells/mL.
Flow-cytometry staining is weak	JC-1 amount or staining time may be below the recommended setup.	Add 10 uL JC-1 (100x) to 1 mL sample and incubate 15-30 minutes at 37°C, 5% CO ₂ . The datasheet notes that dye amount may be optimized if needed.

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Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Plate-reader signal is inconsistent	Plate format or filter selection may not match the assay.	Use a black 96-well culture plate for fluorescence plate-reader analysis and appropriate filter sets for green monomer and orange-red aggregate signals.
Microscopy or flow signal changes during handling	The datasheet states that JC-1 is sensitive to temperature and pH and that samples should be analyzed immediately.	Standardize handling time, maintain specified temperature/pH, protect from light, and begin measurement immediately after the final wash/resuspension.
JC-1 signal is lost after storage	The dye may have been exposed to light or repeated freeze-thaw cycles.	Prepare small aliquots, store at -20°C protected from light, and avoid repeated freeze-thaw cycles.
Diluted Assay Buffer is old	The one-week storage period after dilution may have been exceeded.	Prepare fresh 1x Assay Buffer if the diluted buffer has been stored longer than one week at 2-8°C.
Healthy and unhealthy populations are difficult to separate	Detection channels may not match the fluorophore states.	Use rhodamine-type filters for J-aggregates and FITC-type filters for monomers as described in the datasheet.
Cell culture is too confluent at staining	The datasheet recommends cultures no more than 80% confluent at visualization.	Standardize cultures below or at 80% confluency before staining and compare matched cultures.
Wash conditions differ between samples	Unequal washing may alter final signal.	Use warm PBS and the same centrifugation/wash sequence for all flow-cytometry samples or the same wash sequence for adherent-cell formats.

Still having trouble? Recheck buffer dissolution and 1:10 dilution, JC-1 thawing without centrifugation, light protection, 25°C/pH 7.4 handling, cell density, staining time, the CCCP positive control, filter sets, and immediate analysis. JC-1 is highly sensitive to handling conditions.