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MitoStep™

Flow Cytometry Mitochondrial Membrane Potencial Assay

Reference	Size
MITO-100T	100 test

PRODUCT DESCRIPTION

Membrane potential (ΔΨ) is generated and maintained by concentration gradients of ions such as sodium, potassium, chloride, and hydrogen.

Mitochondrial ΔΨ drives the accumulation in mitochondria of cationic dyes such as cyanines, and the mitochondrial ΔΨ is reduced when energy metabolism is disrupted, notably in apoptosis. Changes in the mitochondrial ΔΨ have been described during necrosis, cell cycle and apoptosis. Mitochondrial uptake of dye is a possible source of fluorescence variance.

Flow cytometry can be used to estimate membrane potential in eukaryotic cells. Methods using cyanines dyes can detect changes in ΔΨ.

PRODUCT	EXCITE (NM)	EMIT (NM)
DiIC1(5)	633	658

Immunostep MitoStep uses a cationic dye DiIC1(5) (1,1´,3,3,3´-hexamethylindodicarbo-cyanine idodide) for the study of mitochondrial ΔΨ. During the apoptosis occurs depolarization of the membrane and as a result there is an increase in cells with less DiIC1(5) fluorescence. MitoStep has been optimized for use in flow cytometry, cells stained with DiIC1(5) are excited using air-cooled Helium-Neon laser emitting at 633nm, cells DiIC1(5) positives emitted at 658 nm. DiIC1(5) mean intensity of fluorescence decreases when cells are treated with reagents that induce apoptosis or reagents that disrupt ΔΨ mitochondrial.

Storage buffer: DiIC1(5), 500 µlof 10µM in DMSO.

Storage conditions: Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. tech@immunostep.com

RECOMMENDATIONS AND WARNINGS ⚠

DMSO is a potentially toxic. It is recommended that the user wear protective clothing, gloves, and eye/face protection in order to avoid contact with the skin and eyes.

Staining cells protocol with DiIC1(5)

1. Harvest the cells after the apoptosis induction or treatment with a disrupt membrane potential reagent and wash in temperate phosphate-buffered saline (PBS).
2. Wash cells twice with temperate PBS and resuspend cells in temperate phosphate-buffered saline (PBS) at a concentration 1 x 106 cells/ml.
3. Add 5 µl of 10µM DiIC1(5).
4. Incubate the cells at 37 °C, 5% CO2, for 15 minutes.
5. After incubation period, add 400 µl of PBS to each tube. Analyze by flow cytometry.

Please, refer to www.immunostep.com technical support for more information.

WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

REFERENCES

1. Howard M. Shapiro. Membrane Potential Estimation by Flow Cytometry. Methods 21, 271-279 (2000).

MANUFACTURED BY

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