Dyes and	TMRE (Perchlorate)	STENCELL T E C H N O L O G I E S
Stains	Cell-permeant, cationic fluorescent dye used to measure mitochondrial membrane potential	Scientists Helping Scientists [™] WWW.STEMCELL.COM
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Catalog #100-0992	25 mg	INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

TMRE (Perchlorate) is a cell-permeant, cationic compound that emits a highly fluorescent orange signal. It readily accumulates in the mitochondria due to the high mitochondrial membrane potential, whereas depolarized mitochondria fail to retain the dye. TMRE has a sufficient retention time that is optimal for cell sorting for further purification and characterization (Barteneva et al.). TMRE does not inhibit mitochondrial function, and can be used to identify apoptotic cells (Barteneva et al.). TMRE is well suited for high-throughput screening and is compatible with flow cytometry.

CAS Number:
Chemical Formula:
Molecular Weight:
Excitation Wavelength:
Emission Wavelength:
Structure:

115532-52-0 C₂₆H₂₇ClN₂O₇ 514.95 g/mol 552 nm 574 nm



Properties

Storage: Shelf Life: Format: Store at -20°C. Stable until expiry date (EXP) on label. Protect product from prolonged exposure to light. Red powder



Directions for Use

Please read the entire protocol before proceeding. Treat cells as desired before preparing the TMRE (Perchlorate) working solution.

Preparation of TMRE (Perchlorate) Working Solution

- To prepare a stock solution, dissolve TMRE (Perchlorate) in dimethyl sulfoxide (DMSO) at 1 mM. Mix thoroughly. NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date as indicated on the label. After thawing aliquots, use immediately; do not re-freeze.
- To prepare the TMRE (Perchlorate) working solution, dilute the stock solution (prepared in step 1) to 0.2 2 µM with Hanks' Balanced Salt Solution with 20 mM HEPES (HHBS) or buffer of choice. Mix thoroughly. Use the working solution immediately; do not store.
 NOTE: The optimal concentration of the working solution should be determined for different cell types.

Staining Samples

- 1. For each sample, prepare 5 x 10^5 1 x 10^6 cells in 1 mL of warm (37°C) culture medium or buffer of choice. NOTE: The optimal cell density to induce apoptosis should be determined for different cell lines.
- 2. To induce apoptosis, treat the cells with test compounds and incubate at 37°C and 5% CO₂ for an appropriate length of time. Prepare untreated cells as a control.

Example: Jurkat cells treated with 5 µM staurosporine require ~2 hours of incubation for apoptosis to occur.

- 3. Prepare additional controls as follows:
 - a. Unstained control
 - b. Uncoupling control: carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP) or carbonylcyanide-3-chlorophenylhydrazone (CCCP) at 5 - 50 μM

NOTE: The optimal concentration should be determined for different cell lines.

- 4. Add the TMRE (Perchlorate) working solution to the cells (excluding unstained control) as follows:
 - Non-adherent cells: Add 2 µL of the TMRE (Perchlorate) working solution to each sample.
 - Adherent cells: Gently lift the cells with 0.5 mM EDTA or suitable cell dissociation reagent and wash once with serum-containing medium before resupending the cells in 1 mL of warm culture medium or buffer of choice and adding 2 µL of the TMRE (Perchlorate) working solution to each sample.
- 5. Incubate the cells at 37° C and 5% CO₂ for 15 30 minutes.
- Centrifuge the cells at 100 300 x g for 4 minutes. Remove supernatant and resuspend the cells in 1 mL of HHBS or a buffer of choice. NOTE: Centrifugation speed may need to be optimized to ensure apoptotic cells are sufficiently pelleted.

Fluorescence Detection

Monitor the fluorescence intensity at Ex/Em = 552/574 nm as follows:

- Fluorescence microscope: Cy3 or appropriate filter set
- Flow cytometer: PE detection channel

References

Barteneva NS et al. (2014) Mitochondrial staining allows robust elimination of apoptotic and damaged cells during cell sorting. J Histochem Cytochem 62(4): 265–75.

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