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Life science solutions

Mitochondrial Membrane Potential Apoptosis Kit manual

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Mitochondrial Membrane Potential Apoptosis Kit manual

Mitochondrial Membrane Potential Apoptosis Kit is a ready-to-use kit designed for fast and convenient analysis of two key indicators of apoptosis: externalization of phosphatidylserine (using Annexin V-AF 488) and changes in mitochondrial membrane potential (using LumiTracker® Mito Red CMXRos).

Annexin V (or Annexin A5) belongs to the Annexin family of intracellular phospholipid-binding proteins. Annexin V is often used to identify apoptotic cells due to its ability to specifically bind to phosphatidylserine (PS), which moves from the inner to the outer side of the cell membrane during the early stages of apoptosis. This kit contains recombinant Annexin V conjugated to AF 488, a bright, photostable green fluorophore with spectral characteristics similar to FITC.

LumiTracker Mito Red CMXRos is a cationic red fluorescent dye that passively diffuses across the plasma membrane and selectively accumulates in active mitochondria depending on their membrane potential. Healthy cells have a high mitochondrial membrane potential, and its decrease is considered a marker of the early stage of apoptosis.

After staining with Annexin V-AF 488 and LumiTracker® Mito Red CMXRos, live cells will have weak green and intense red fluorescence; apoptotic cells, on the contrary, will have high green and low red fluorescence. These two cell populations are easily distinguished using a flow cytometer, and both dyes are excited by the 488 nm line of an argon ion laser.

The kit contains all the necessary reagents for labeling apoptotic cells with Annexin V-AF 488 and determining the mitochondrial membrane potential using LumiTracker® Mito Red CMXRos.

Kit components

Kit component	Count
	21372 50 assays
21515, Annexin V-AF 488 conjugate, 5 ug	1
83215, Annexin V Binding Buffer, 5×, 15 mL	1
15050, DMSO (dimethyl sulfoxide), labeling grade, 1 mL	1
2251-50ug, LumiTracker® Mito Red CMXRos, 50 ug	3

Transportation: at room temperature for 1 week. Store at -20 °C for 9 months.

Shelf life 9 months.

Before you begin

- The commonly used concentration of LumiTracker® Mito Red CMXRos for cell staining is 25–500 nM. The working dilution depends on the cell type and density and should be determined experimentally.
- The recommended concentrations of Annexin V-AF are 2–10 µg/mL, depending on the cell culture being studied. Before the experiment, different dilutions of Annexin V-AF should be tested to determine the optimal concentration.

  Important! Annexin V can be used as an apoptosis marker only in cells with an intact plasma membrane. If the plasma membrane is compromised, Annexin V will bind to PS inside the cell and give a false-positive result.

Preparation of solutions

1. Dissolve the contents of the tube with lyophilized **Annexin V-AF (21515)** in 250 µL of deionized water.

   Important! The diluted recombinant protein must be stored protected from light at 2–8 °C. In solution, the conjugate is stable for one month. For long-term experiments, it is recommended to prepare aliquots and store them at -20 °C. Avoid repeated freezing!

2. Prepare the required volume of 1× Binding Buffer by mixing 1 part of **5× Binding Buffer** with 4 parts of deionized water.

3. Prepare a 10 mM LumiTracker® Mito Red CMXRos stock solution by adding 9.4 µL **DMSO (15050)** to a tube containing **LumiTracker® Mito Red CMXRos (2251-50ug)**. The unused portion of the stock solution can be stored at ≤-20 °C for up to 1 month.

4. Prepare a 10 µM LumiTracker® Mito Red CMXRos working solution by pipetting 1 µL of 10 mM LumiTracker® Mito Red CMXRos stock solution into 1000 µL of medium.

Cell Staining

1. Induce apoptosis in the cells using the desired method. Prepare a negative control by incubating the cells in the absence of the inducing agent. Prepare a positive necrosis control by incubating cells with 2 mM hydrogen peroxide for 4 h at 37 °C.
2. Gently detach adherent cells from the growth surface using a suitable method. For suspension cells, proceed to the next step.
3. Aspirate 1 mL of cell suspension (1×10^5 to 1×10^6 cells/mL) into 1.5 mL microcentrifuge tubes. For flow cytometry, additional tubes with the appropriate controls should be prepared: (1) unstained cells (negative control for instrument

setup); (2) cells stained with Annexin V-AF only; (3) cells stained with LumiTracker® Mito Red CMXRos only (for compensation setup); and tube (4) with experimental cells (double stained with Annexin V-AF and LumiTracker® Mito Red CMXRos).

4. Add 4 µL of 10 µM LumiTracker Mito Red CMXRos solution to tubes (3) and (4), mix well.
5. Incubate cells in the dark for 15–45 min under conditions appropriate for the cell type.
6. Wash cells once with cold PBS (pH 7.4) and once with 1× Binding Buffer.
7. Resuspend cells in 100 µL of cold 1× Binding Buffer.
8. Add 5–10 µL of Annexin V-AF solution, incubate for 10–15 min at room temperature, protected from light.
9. Without prior washing, add 400 µL of 1× Binding Buffer to each tube.
10. Until analysis, store stained cells at 2–8 °C protected from light.

Flow cytometry

1. For flow cytometric analysis of cell apoptosis using Annexin V-AF and LumiTracker® Mito Red CMXRos, in addition to target staining, controls must be prepared (see above).
2. Analyze Annexin V-AF 488 binding using a FITC signal detector.
3. Analyze cells stained with LumiTracker® Mito Red CMXRos using a Phycoerythrin signal detector.
4. Separate compensation settings may be required for correct dilution of dyes in different detection channels.

Fluorescence Microscopy

1. Take a drop of the stained cell suspension and place it on a glass slide. Cover the cells with a coverslip.
2. Alternatively, adherent cells can be stained directly on the coverslip. After staining, invert the coverslip onto the slide so that the cells are between the slide and coverslip.
3. *(Optional)* After staining and before visualization, cells can be washed with 1× Binding Buffer and fixed in 2% paraformaldehyde. Do not fix cells before incubation with Annexin V-AF, since any disruption of the cell membrane can cause non-specific binding of Annexin V to PS on the inner surface of the cell membrane.
4. Examine cells under a fluorescence microscope using an appropriate set of filters.

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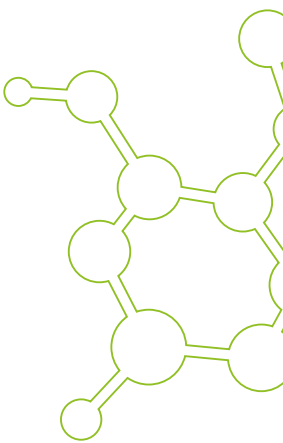

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