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ab270771 Caspase 3/7 Assay Kit (Magic Red)

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Caspase 3/7 Assay Kit (Magic Red)datasheet:

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For the detection of apoptotic Caspase 3/7 activity in cell populations.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Caspases play important roles in apoptosis and inflammation. Detection of DEVDase enzyme activity (caspase 3 activity) is a reliable method for assessing apoptotic activity in experimental cell populations. The presence of the active form of caspase 3 and 7 in apoptotic cells causes the hydrolysis of the two DEVD target sequences from the Magic Red cresyl violet fluorophore, converting it to the fluorescent form. As apoptosis progresses and caspase activity increases, the red fluorescent signal increases. Magic Red excites at 590 nm and emits at 628 nm and can be analyzed using fluorescence microscopy, a fluorescent plate reader, or flow cytometry. Samples may also be analyzed with Acridine Orange dye or Hoechst stain to visualize lysosomal organelle structure or detect changes in nuclear morphology respectively.

To use Magic Red, add the substrate directly to the cell culture media, incubate, and analyze. Because it is cell-permeant, it easily penetrates the cell membrane and the membranes of the internal cellular organelles - no lysis nor permeabilization steps are required. If caspase-3/7 enzymes (DEVDases) are active, they will cleave the intact (quenched) substrate and release the cresyl violet fluorophore, which will greatly enhance the cresyl violet fluorescence potential. The red fluorescent product will often aggregate inside lysosomes; caspases are not lysosomal enzymes. As protease activity progresses and more substrate is cleaved, the red fluorescent signal potential will intensify, enabling researchers to watch it increase over time and quantify apoptosis.

There is no interference from pro-caspases or inactive forms of the enzymes. If the treatment or experimental condition is causing cell death via apoptosis, apoptotic cells will have elevated levels of caspase-3/7 activity relative to non-apoptotic or negative control cells.

2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt.

Δ Note: Once reconstituted with DMSO, use Magic Red immediately, or store at ≤-20°C for up to 6 months, protected from light and thawed no more than twice during that time.

Item	Quantity		Storage temperature
	25 tests	100 tests	
Acridine Orange (1mM)	1 x 500 µL	1 x 500 µL	+4°C
Hoechst 33342, 200 µg/mL	1 x 1 mL	1 x 1 mL	+4°C
Magic Red Caspase 3/7 Substrate MR-(DEVD)	1 vial	1 vial	+4°C / -20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- DMSO, 100-400 µL to reconstitute Magic Red
- DiH_2O, 400-1600 µL to dilute Magic Red
- Phosphate buffered saline (PBS) pH 7.4, 100 mL
- Cultured cells treated with the experimental conditions ready for staining; do not use paraffin embedded tissues
- Reagents to induce apoptosis and create controls
- Hemocytometer
- Centrifuge at 200 *x g*
- 15 mL polypropylene centrifuge tubes (1 per sample)
- 12 x 75 mm glass or polypropylene tubes
- Black 96-well microtiter plate, flat bottom, non-treated, non-sterile. If using a bottom reading instrument, use a plate with black walls and a clear bottom. If culturing cells in the plate, use a sterile black tissue culture plate.
- Slides and coverslips
- FACS tubes
- Ice or refrigerator
- Green Live/Dead stain
- Fluorescence microscope
- Fluorescence plate reader

Δ Note: Magic Red excites at 550-590 nm and emits >610 nm. It has an optimal excitation and emission wavelength tandem in aqueous solutions of 592 nm and 628 nm, respectively.

Δ Note: Hoechst 33342 can be visualized using a UV-filter with excitation at 365 nm and emission at 480 nm.

Δ Note: Acridine Orange excites at 480 nm and emits >540 nm. Select a filter combination that best approximates these settings.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Experimental Preparation

5.1 Staining cells with Magic Red can be completed within a few hours. However, Magic Red is used with living cells, which require periodic maintenance and cultivation several days in advance. In addition, once the proper number of cells has been cultivated, time must be allotted for the experimental procedure or apoptosis induction (which typically requires a 3-6 hour incubation at 37°C).

5.2 As MR-$(DEVD)_2$ detects caspase-mediated apoptosis, plan the experiment so that it will be diluted and administered at the time when caspases are expected to be activated in the cells.

5.3 The recommended volume of the Magic Red staining solution is 10-20 µL per 300 µL of cells at 10^6 cells/mL, but the ideal amount may vary based on the experimental conditions and method of analysis.

5.4 Culture cells to a density optimal for the specific experiment or apoptosis induction protocol. Cell density should not exceed 10^6 cells/mL as cells cultivated in excess of this concentration may begin to naturally enter apoptosis due to nutrient deprivation or the accumulation of cell degradation products in the media.

5.5 Cells undergoing apoptosis will generate a stronger red fluorescence with MR-$(DEVD)_2$ than non-apoptotic cells of the same lineage. To optimize this assay, adjust the amount of Magic Red substrate used to stain cells and the incubation

time. Determine which parameters produce the greatest difference in the fluorescent signal between induced and non-induced cell populations.

5.6 Hoechst 33342 can be used with Magic Red to label nuclei. Because of the overlap in emissions, dual staining of cells with both Magic Red and Acridine Orange will yield confusing results and is not recommended; these dyes should be used separately. Do not use Magic Red with paraffin-embedded tissues as the chemicals used for paraffin embedding may denature and inactivate the substrate.

6. Controls

It is highly recommended that two sets of controls be run:

One positive control population of cells that was induced to undergo apoptosis or trigger caspase-3/7 activity;

And a placebo population of cells that received just the vehicle used to deliver the apoptosis-inducing agent.

Create positive controls by inducing apoptosis (Section 7). Create negative controls by culturing an equal volume of non-induced cells for every labeling condition. The negative control and induced positive control populations should contain similar quantities of cells.

For example, if labeling with MR-(DEVD)2, Hoechst 33342, and Acridine Orange, make 10 control populations:

Control #	
1 and 2	Unlabelled, induced and non-induced populations.
3 and 4	MR-$(DEVD)_2$-labeled, induced and non-induced populations.
5 and 6	MR-$(DEVD)_2$-and Hoechst-labeled, induced and non-induced populations
7 and 8	Hoechst-labeled, induced and non-induced populations.
9 and 10	AO-labeled, induced and non-induced populations.

7. Apoptosis induction

Prior to commencing the experiment, determine a reproducible method for obtaining a positive control by triggering caspase -3 and -7 activity. This process varies significantly with each cell type.

For example, apoptosis via caspases -3 and -7 may be induced in Jurkat cells with 2-4 µg/mL camptothecin or 1-2 µM staurosporine for >4 hours.

8. Preparation of Magic Red

- Magic Red Caspase-3/7 Substrate is supplied as a lyophilized powder that is dried onto the base of the amber glass vial. It must first be reconstituted in DMSO, forming the stock concentrate, and then diluted 1/5 in diH_2O to form the final staining solution.
- For best results, the staining solution should be prepared immediately prior to use. However, the stock concentrate may be stored at ≤-20°C for future use. Protect from light and use gloves when handling.

8.1 Create the stock solution by reconstituting Magic Red in DMSO. It is vialed in 2 sizes: small (approximately 25-50 Tests); and large (approximately 100-200 Tests). The reconstitution volume will vary based on the vial size:

- Reconstitute the 25-50 tests vial with 100 µL DMSO.
- Reconstitute the 100-200 tests vial with 400 µL DMSO.

8.2 Gently vortex or swirl the vial, allowing the DMSO to travel around the base of the vial until completely dissolved. At room temperature (RT), this should take just a few mins. The stock solution should appear red. Once reconstituted, it may be stored at ≤-20°C for up to 6 months protected from light and thawed no more than twice during that time. If using immediately, dilute in diH_2O to form the staining solution. If not diluting within 1 hour, aliquot and freeze.

8.3 Immediately prior to staining the samples, dilute the stock solution 1/5 in diH2O to form the staining solution. Mix by inverting or vortexing the vial at RT. Use the staining solution

within 15 mins of dilution to prevent non-specific substrate hydrolysis.

- The 25-50 tests vial contains 100 µL of the stock concentrate in DMSO. Dilute 1/5. Add 400 µL diH_2O to it. This yields 500 µL of the staining solution.
- The 100-200 tests vial contains 400 µL of the stock concentrate in DMSO. Dilute it 1:5. Add 1,600 µL diH_2O to it. This yields 2 mL of the staining solution.
- For other amounts, dilute the stock concentrate 1/5 in diH_2O. For example, add 10 µL stock to 40 µL diH_2O; this yields 50 µL of the staining solution.

8.4 Mix by inverting or vortexing the vial at RT.

8.5 Use immediately.

9. Hoechst 33342

Hoechst 33342 is a cell-permeant nuclear stain that emits blue fluorescence when bound to double stranded DNA. It is used to stain the nuclei of living or fixed cells, to distinguish condensed pyknotic nuclei in apoptotic cells, and for cell cycle studies.

Hoechst 33342 is provided ready-to-use at 200 µg/mL. It can be used with Magic Red to label nuclei.

When bound to nucleic acids, it has a maximum absorbance at 350 nm and a maximum emission at 480 nm. It is revealed under a microscope using a UV-filter with excitation at 365 nm and emission at 480 nm.

- Hoechst 33342 contains a low concentration of Bis benzimide H 33342 trihydrochloride (CAS 23491-52-3) which is below the threshold for reporting. Hoechst is a suspected mutagen at high concentrations. Prolonged skin contact may cause redness and irritation. Because of the small quantity of product, the health hazard is small. See SDS for further information.

10. Acridine Orange

Acridine orange (AO) is a chelating dye and can be used to reveal lysosomes, nuclei, and nucleoli. The acidic pH of the lysosome results in the concentration and aggregation of AO.
It is provided ready-to-use at 1 mM. AO may be used neat or diluted in diH_2O or media prior to pipetting into the cell suspension.
Always protect AO from bright light.
Lysosomal structures can be visualized by staining with AO at 0.5 to 5.0 µM. This concentration range can be obtained by diluting the AO reagent stock 1/2,000-1/200 (0.05-0.5% v/v) into the final cell suspension.
For example, if using AO at 1.0 µM in the final cell suspension, it must be diluted 1/1,000. First dilute it 1/100 in diH_2O; e.g., put 10 µL AO into 990 µL diH_2O. Pipette the diluted AO into the cell suspension at approximately 1/10; e.g., put 50 µL diluted AO into 450 µL cell suspension.
As AO exhibits a very broad emission range, several filter pairings can be used to view this stain. The same excitation/emission filter pairings used to view Magic Red can be used: an excitation filter of 550 nm (540-560 nm) and a long pass >610 nm emission/barrier filter pair. With this pairing, the lysosomes appear red.
When illuminating with a blue light (480 nm) excitation filter, a green light (540-550 nm) emission/barrier filter combination works well. Lysosomes will appear yellowish green. As this filter combination is very close to the maximum emission of AO, the slide may appear too bright. Excess AO may be removed by washing cells prior to viewing.
Because of the overlap in emissions, dual staining of cells with both MR-$(DEVD)_2$ and AO will yield confusing results. Therefore, these dyes should be used separately

- Acridine Orange contains a concentration of 3,6-Acridinediamine, N,N,N',N'-Tetramethyl-, monohydrochloride (CAS 65-61-2, or CAS 494-38-2 free base) at less than 0.1% which is below the threshold for reporting. This product may be a potent mutagen at high concentrations and probable carcinogen. Because of the small quantity of product, the health hazard is small. See SDS for further information.

11. Microscopy analysis of suspension cells

11.1 Prepare cell populations. Initial cell concentrations should be 3-5 x 10^5 cells/mL and should not exceed 7 x 10^5 cells/mL, as cells cultivated in excess of this concentration may begin to naturally enter apoptosis.

11.2 Expose cells to the experimental conditions and create positive and negative controls, see Sections 6 & 7.

11.3 When ready to label with the staining solution, cell concentrations should be 1-2 x 10^6 cells/mL for best viewing. Density can be determined by counting cell populations on a hemocytometer. If necessary, concentrate cells by gentle centrifugation at 200 x g for 5 - 10 mins. Remove the supernatant and resuspend with cell culture media or PBS.

11.4 Transfer 300 µL into 12 x 75 mm glass or polypropylene tubes. If desired, larger cell volumes can be used, but additional MR-$(DEVD)_2$ staining solution may be required.

11.5 Reconstitute MR-(DEVD)2 with DMSO to form the concentrated stock solution (Section 8):

- Reconstitute the 25-50 tests vial with 100 µL DMSO.
- Reconstitute the 100-200 tests vial with 400 µL DMSO.
- When ready to stain cells, dilute the stock 1/5 in diH2O to form the MR-$(DEVD)_2$ staining solution (Section 8):
- Add 400 µL diH_2O to the 25-50 tests vial.
- Add 1,600 µL diH_2O to the 100-200 tests vial.

11.6 Add 10 µL of the MR-$(DEVD)_2$ staining solution to each 300 µL cell suspension and mix thoroughly. If different cell volumes are used, add the MR-$(DEVD)_2$ staining solution at a ratio of approximately 1/30. For example, add 35 µL MR-$(DEVD)_2$ staining solution to 1,000 µL of cell suspension forming a final volume of 1,035 µL.
Δ Note: Do not add MR-$(DEVD)_2$ to cells that are to be labeled with AO; add a placebo instead, such as diH_2O µL.

11.7 Incubate cells for 60 mins at 37°C protected from light. Cells may settle on the bottom of the tubes; gently resuspend by swirling cells every 20 mins during the incubation to ensure even distribution of Magic Red substrate. After the incubation, cells can be stained with Hoechst 33342 (Section 9), or unstained cells may be labeled with AO (Section 10).

11.8 If cells are to be labeled with Hoechst 33342, add it at approximately 0.5% v/v. For example, if the cell suspension is

310 µL, add 1.55 µL Hoechst 33342. Incubate 5 mins at 37°C. Go to Step 11.11.

11.9 Because of the overlap in emissions, dual staining of cells with both Magic Red and AO is not recommended; the dyes should be used separately. To stain cells with AO:

- Dilute AO to 1/2,000-1/200 (0.05-0.5% v/v) into the final cell suspension. For example, if using AO at 1.0 µM in the final cell suspension, first dilute it 1/100 in diH_2O; e.g., put 10 µL AO into 990 µL diH_2O. Pipette the diluted AO into the cell suspension at 1/10; e.g., add 35 µL to 315 µL cell suspension.
- Incubate for 30 mins at 37°C.
- If viewing under the same filters used for MR-$(DEVD)_2$ (excitation at 550 nm; emission >610 nm), cells may be viewed immediately after staining without a wash step, go to Step 11.11.
- If viewing under blue (480 nm) excitation and green (540-550 nm) emission wavelengths, any excess AO may have to be washed away as the cells may appear too bright at this range. Brightness will depend on the type of microscope used and the cell line. To wash cells:
  - Gently pellet cells at 200 *x g* for 5-10 mins at RT.
  - Remove and discard supernatant.
  - Resuspend cells in 300 µL or a similar volume of PBS in which the cells were originally suspended.

11.10 Place 15-20 µL of cell suspension onto a microscope slide and cover with a coverslip.

11.11 Observe cells using a fluorescence microscope equipped with an excitation filter of 550 nm (540-560 nm) and a long pass >610 nm emission/barrier filter pairing. Select a filter combination that best approximates these settings. Using these filters, positive cells will appear red with brightly stained vacuoles and lysosomes.

If the samples were stained with both Magic Red and Hoechst 33342, and if a multi-wavelength filter option is available on the fluorescence microscope, the dual staining properties can be examined. Hoechst 33342 can be seen using a UV-filter with excitation at 365 nm and emission at 480 nm.

As AO exhibits a very broad emission range, one of several filter pairings can be used. The same excitation/emission pairing filters used to view MR-$(DEVD)_2$ can be used: a 550 nm

(540-560 nm) excitation and long pass >610 nm emission/barrier filter pairing. With this pairing, the lysosomes appear red. When illuminating with a blue light (480 nm) excitation filter, a green light (540-550 nm) emission/barrier filter combination works well. Lysosomes appear yellowish green instead of red.

12. Microscopy analysis of adherent cells

12.1 Seed 10^4-10^5 cells onto a sterile coverslip in a 35 mm petri dish or onto chamber slides or grow in a plate.

12.2 Grow cells until 80% confluent. This usually takes about 24 hrs but will vary with each cell line.

12.3 Expose cells to the experimental conditions and create positive and negative controls (Sections 6 and 7).

12.4 Reconstitute MR-$(DEVD)_2$ (Section 8) to form the concentrated stock solution at.

- Add 400 µL diH_2O to the 25-50 tests vial.
- Add 1,600 µL diH_2O to the 100-200 tests vial.

12.5 Add Magic Red staining solution at approximately 1/30 and gently mix to ensure an even distribution of MR-$(DEVD)_2$. For example, add 10 µL staining solution to 300 µL cells forming a final volume of 310 µL.

Δ Note: Do not add Magic Red to cells that will be stained with AO: add a placebo instead, such as diH_2O.

12.6 Incubate 30-60 mins at 37°C protected from light.

12.7 Remove the media from the cell monolayer surface and rinse twice with PBS, 1 min per rinse. At this point, cells can be analyzed or stained with Hoechst 33342 (Step 9). Unstained cells can be labeled with AO (Step 10).

12.8 If cells are to be labeled with Hoechst 33342, add it at approximately 0.5% v/v. For example, add 1.55 µL Hoechst 33342 to 310 µL cells labeled with MR-$(DEVD)_2$ and control samples. Incubate 10-20 mins at 37°C. Go to Step 12.11.

12.9 Because of the overlap in emissions, dual staining of cells with both MR-$(DEVD)_2$ and AO is not recommended; the dyes should be used separately. To stain cells that have not been exposed to MR-$(DEVD)_2$:

a. Dilute 1 mM AO at 1/2,000-1/200 (which is 0.05-0.5% v/v) into the final cell volume. For example, if using AO at 1.0 µM in the final cell volume, it must be diluted 1/1,000. First dilute it 1/100 in diH_2O; e.g., add 10 µL AO to 990 µL diH_2O. Pipette the diluted AO to the cells at 1/10; e.g., add 35 µL diluted AO to 315 µL cell media.
b. Incubate 30 mins at 37°C.
c. Remove the media from the cell monolayer surface. Rinse twice with PBS, 1 min per rinse.

12.10 Mount the coverslip with cells facing down onto a drop of PBS. If a chamber-slide was used, pull off the plastic frame and add a drop of PBS to the cell surface and cover with a coverslip.

12.11 Observe MR-$(DEVD)_2$-stained cells using a fluorescence microscope equipped with an excitation filter of 550 nm (540-560 nm) and a long pass >610 nm emission/barrier filter pair. Select a filter combination that best approximates these settings. Using these filters, positive cells stained with MR-$(DEVD)_2$ will appear red with more brightly stained vacuoles and lysosomes.

12.12 If samples were stained with both MR-$(DEVD)_2$ and Hoechst 33342, and if a multi-wavelength filter option is available on the fluorescence microscope, the dual staining properties of the sample can be examined. Hoechst 33342 can be seen using a UV-filter with excitation at 365 nm and emission at 480 nm.

As AO exhibits a very broad emission range, one of several filter pairs can be used. The same excitation/emission pairing filters used to view Magic Red can be used: an excitation filter of 550 nm (540-560 nm) and a long pass >610 nm emission/barrier filter. With this pairing, the lysosomes appear red.
When illuminating with a blue light (480 nm) excitation filter, a green light (540-550 nm) emission/barrier filter combination works well. Lysosomes will appear yellowish green instead of red.

13. Fluorescence plate reader analysis

13.1 Prepare cell populations. Cell concentrations should be 2-8 x 10^6 cells/mL. If this is too dense for the cell line, induce apoptosis first, then concentrate the cells and stain with MR-$(DEVD)_2$. Cell concentrations can be achieved by low speed centrifugation (<200 x *g* at RT) for 5-10 mins.

13.2 Expose cells to the experimental conditions and create positive and negative controls (Section 6).

13.3 Transfer 300 µL cell suspension into 12 x 75 mm glass or polypropylene tubes or a black microtiter plate. If using a bottom reading instrument, use a plate with black walls and a clear bottom. Avoid bubbles. Larger cell volumes may also be used, but additional MR-$(DEVD)_2$ substrate will be required per sample.

13.4 When ready to label with the Magic Red staining solution, cells should be at least 2 x 10^5 cells/100 µL aliquot (equal to 2 x 10^6 cells/mL) for each microtiter plate well.

13.5 Reconstitute MR-$(DEVD)_2$ with DMSO to form the concentrated stock solution (Section 8):

- Reconstitute the 25-50 test vial with 100 µL DMSO.
- Reconstitute the 100-200 test vial with 400 µL DMSO.

13.6 When ready to stain cells, dilute the stock 1/5 in diH^2O to form the MR-$(DEVD)_2$ staining solution (Section 8):

13.7 Add 20 µL MR-$(DEVD)_2$ staining solution directly to 300 µL cell sample and gently mix. If different cell volumes are used, add MR-$(DEVD)_2$ staining solution at approximately 1/15. Due to sensitivity limitations, plate readers require a higher concentration of Magic Red for detection compared to microscopes.

13.8 Incubate cells for 60 mins at 37°C protected from light. As cells settle to the bottom, gently resuspend them approximately every 10-20 mins to ensure MR-$(DEVD)_2$ is evenly dispersed among all cells.

13.9 Read the 300 µL sample as one sample or split it into 3 wells of 100 µL each. If cells were stained in a tube, transfer to a black microtiter plate.

13.10 Measure the fluorescence intensity of the red fluorescent Magic Red cresyl violet fluorophore. Set the plate reader to perform an endpoint read. Magic Red has an optimal excitation and emission wavelength tandem of 592 nm and

628 nm, respectively. Select the filter pairings that best approximate these settings. If available, use a cut-off filter at 630 nm to filter out shorter wavelength excitation interference.

14. Single Color Flow Cytometry Analysis of Suspension cells

14.1 Expose cells to the experimental conditions and prepare control cell populations; also prepare an unstained control for gating.

14.2 Initial cell concentration should be 1 x 10^5 - 1 x 10^6 cells/mL. The optimal cell concentration for staining will vary based on the experimental conditions. In general, flow cytometric analysis has lower cell density requirements than analyzing with a fluorescence plate reader or microscope.

14.3 Transfer 300 µL cell suspension per sample into FACS tubes. Different sample volumes may be used; however, this will change the amount of Magic Red needed for optimal staining and alter the number of tests per vial.

14.4 Reconstitute MR-$(DEVD)_2$ with DMSO to form the concentrated stock solution (Section 8):

- Reconstitute the 25-50 test vial with 100 µL DMSO.
- Reconstitute the 100-200 test vial with 400 µL DMSO.

14.5 When ready to stain cells, dilute the stock 1/5 in diH_2O to form the MR-$(DEVD)_2$ staining solution (Section 8):

- Add 400 µL diH_2O to the 25-50 tests vial.
- Add 1,600 µL diH_2O to the 100-200 tests vial.

14.6 Add MR-(DEVD)2 staining solution at approximately 1/30 and gently mix to ensure an even distribution of MR-(DEVD)2. For example, add 10 µL staining solution to 300 µL cells forming a final volume of 310 µL.

14.7 Incubate 30-60 mins at 37°C protected from light. Gently resuspend cells approximately every 20 mins throughout the staining process.

14.8 For single-color analysis, a 640 nm red excitation laser or comparable can be used with an emission filter pairing that best approximates 675/25 (often FL4). If available, use laser filter pairings that more closely resemble the excitation and

emission optima of Magic Red (592 nm and 628 nm, respectively).

14.9 Run the unstained control and generate an FSC-A vs. SSC-A plot to gate whole cells from cellular debris. Generate a histogram displaying log fluorescence on the channel being used to detect Magic Red® (X-axis) versus the number of cells (Y-axis). If possible, adjust the voltage to place the unstained sample in the first decade of the log scale. Voltage adjustment is not possible on some instruments, such as the Accuri C6.

14.10 Run the experimental samples and observe data on the above histogram. Caspase negative/MR-(DEVD)2 (-) cells will fall within the lower log fluorescence output decades of the X-axis, whereas caspase positive/MR-DEVD)2 (+) cells will appear as a shoulder or as a separate peak on the right side of the negative peak histogram.

15. Dual Color Flow Cytometry Analysis of Suspension cells

Because of minimal spectral overlap with the green/FITC channel (often FL1), Magic Red can be easily combined with green fluorochromes for dual color analysis studies. When MR-(DEVD)2 is used in combination with ICT's Green Live/Dead Stain four populations can be identified.

- Early apoptosis
  MR-$(DEVD)_2$ (+)/ Green Live/Dead Stain (-)
  Early to mid-stage apoptotic cells Intact cell membranes
  Active caspase-3/7 enzymes
- Late apoptosis
  MR-$(DEVD)_2$ (+)/ Green Live/Dead Stain (+)
  Late stage apoptotic cells Compromised cell membranes
  Active caspase-3/7 enzymes

- Necrosis
  MR-$(DEVD)_2$ (-)/ Green Live/Dead Stain (+)
  Dead cells
  Compromised cell membranes

- **Live**
  MR-$(DEVD)_2$ (-)/ Green Live/Dead Stain (-)
  Live, healthy cells

Follow Section 14 steps 14.1 to 14.8; include single-color compensation controls:

Then follow the steps detailed, below.

15.1 Perform the staining procedure for the second fluorochrome according to the manufacturer's protocol.

15.2 Run the unstained control and generate an FSC-A vs. SSC-A plot to gate whole cells from cellular debris. Generate two histograms displaying:

a. Log fluorescence on the channel being used to detect Magic Red® (X-axis) versus the number of cells (Y-axis).

b. Log fluorescence on the FL channel being used for the second fluorochrome (X-axis) versus the number of cells (Y-axis).

Δ Note: If possible, adjust the voltages to place the unstained sample in the first decade of the log scale on both histograms. The voltages are not hanged after this step or the compensation made would be invalid.

15.3 Run single color controls. While monitoring dual color density plots, adjust compensation to remove spectral overlap from interfering FL channels. A red 640 nm excitation laser or comparable can be used with an emission filter pairing that best approximates 675/25 (often FL4) to measure Magic Red. If available, use laser filter pairings that more closely resemble the excitation and emission optima, e.g. 592 nm and 628 nm, respectively. Read the single-color control for the second dye using laser/filter pairings recommended by the manufacturer. Transfer 300 µL cell suspension per sample into FACS tubes. Different sample volumes may be used; however, this will change the amount of Magic Red needed for optimal staining and alter the number of tests per vial.

15.4 Run dual color experimental samples and analyze.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)