

Magic Red Caspase 3 & 7 Assay Kit

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CATALOG #
AAA52705

PRODUCT SYNONYMS

Magic Red Caspase 3 & 7; Magic Red Caspase 3 & 7 Assay Kit; Magic Red Caspase 3 & 7 assay kit

PRODUCT SPECIFICATIONS

Reagents	MR-(DEVD)2
Suspension Cells	Culture cells up to 1×10^6 cells/mL. Induce apoptosis following your protocol, and create positive and negative controls. Reconstitute the MR-(DEVD)2 reagent with DMSO to form the stock concentrate (which can be frozen for future use). Dilute the MR-(DEVD)2 stock concentrate 1:5 with diH ₂ O to form the working solution. Add ~10 - 15 uL of the working solution directly to a 300-500 uL aliquot of your cell culture for labeling. The working solution is used at approximately 1:30 to label suspension cells. Incubate 30 minutes - 4 hours at 37 degree C in the dark. If desired label DNA with Hoechst stain, and/or fix cells. Unstained cells may be labeled with Acridine Orange to reveal the lysosomes. Analyze data using a fluorescence microscope, fluorescence plate reader, or flow cytometer.
Adherent Cells	Culture cell monolayers to 80-90% confluency. Induce apoptosis following your protocol, and create positive and negative controls. Gently remove cell culture media from flask and rinse cell culture surface once with sterile PBS. Save any loose cells for combination with trypsinized cells in Magic Red MR-(DEVD)2 staining steps. Add trypsin to cell monolayer and collect disassociated cells for analysis. Dilute the newly trypsinized cells in cell culture media containing 10-20% FBS and pellet down cells in this media to concentrate cells. Reconstitute the MR-(DEVD)2 reagent with DMSO to form the stock concentrate (which can be frozen for future use). Dilute the MR-(DEVD)2 stock concentrate 1:5 with diH ₂ O to form the working solution. Add ~ 15 uL of the working solution directly to a 500 uL aliquot of your adherent cell culture for labeling. The working solution is used at approximately 1:30 to label adherent cells. Incubate 30 minutes - 4 hours at 37 degree C in the dark. If desired label DNA with Hoechst stain, and/or fix cells. Unstained cells may be labeled with Acridine Orange to reveal the lysosomes. Analyze data using a fluorescence microscope or fluorescence plate reader.
Target	Caspase 3, Caspase 7
Excitation / Emission	590 nm / 620 nm
Method of Analysis	Fluorescence Microscope, Fluorescence Plate Reader
Types of Samples	Cell Culture, Tissue
Preparation and Storage	Store at 2-8 degree C

IMAGES



Application Data

Rat fibroblasts were seeded in a 12-well plate at 1×10^4 cells/mL and irradiated the following day. 23.1 uL of MR-(DEVD)2 solution was mixed with media and added. 1 hour later, 300 uL media was added and cells MR-DEVD data were photographed over 16 hours. Bright field images show increasing red intensity as caspase activity and apoptosis progressed (Dr. Martin Purschke, Massachusetts General Hospital).



Application Data

View a time-lapse movie of caspase-3/7 activation in cultured rat fibroblasts. Data courtesy of Dr. Martin Purschke, MA General Hospital. Windows Media Player or similar required to play the .avi file.

ADDITIONAL INFORMATION

Related Product Information for Magic Red Caspase 3 & 7 assay kit	Description: Magic Red Caspase 3 and 7 Assay Kits measure apoptosis-associated DEVDase enzyme activity in living, intact cells. The presence of the active forms of caspases 3 and 7 in apoptotic cells causes the hydrolysis of the two DEVD target sequences from the cresyl violet fluorophore (Magic Red), converting it to the fluorescent form. When the cresyl violet dye is di-substituted with two DEVD caspase-3 /7 target sequences, the reagent is essentially non- fluorescent. Because the Magic Red probe is cell membrane permeant, a cell lysis step is not required to perform the assay. As apoptosis progresses and caspase activity increases, the red fluorescent signal increases. Magic Red (cresyl violet) excites at 540-590 nm (590 nm optimal) and emits at >610nm (630 nm optimal). Detection of DEVD target sequence hydrolysis via the use of the Magic Red fluorogenic substrate assay is a reliable method for monitoring apoptosis induction events and caspase 3&7 activity over time in whole, living cells. Each assay kit includes the Magic Red-(DEVD)2 substrate reagent, Hoechst 33342 stain, and acridine orange stain. This allows cells to be simultaneously evaluated for both the presence of apoptotic nuclear morphology and caspase-3/7 DEVDase activity in fluorescence microscopy analysis techniques. Acridine Orange dye is included to visualize lysosomal organelle structure. Analysis by fluorescence plate reader or flow cytometer may also be performed if the instrument is equipped with a green laser excitation option. Background: Magic Red Caspase 3 Assay for Apoptosis Detection via Activated Caspase 3 and 7. Caspase-3 is a member of the apoptosis executioner group that also includes caspases -6 and -7 [1]. The inactive zymogen form of caspase-3 is efficiently processed by any of the initiator caspases (caspase-8, caspase-9, and caspase-10). All executioner caspases will target the various cytoskeletal structural proteins as well as PARP and ICAD leading to the morphological and biochemical changes that drive the apoptotic process [2]. Detect Caspase 3 and 7 Activity in Real Time with Magic Red Detection of DEVDase enzyme activity (caspase 3 activity) is a reliable method for assessing apoptotic activity in experimental cell populations [3]. Magic Red Caspase 3 and 7 Assay Kits measure apoptosis-associated DEVDase enzyme activity in living, intact cells via the red fluorescent Magic Red caspase 3 and 7 fluorogenic substrate, MR-(DEVD)2, which is primarily targeted by caspases 3 and 7 of the apoptotic protease cascade. The presence of the active form of caspases 3 and 7 in apoptotic cells causes the hydrolysis of the two DEVD target sequences from the cresyl violet fluorophore (Magic Red), converting it to the fluorescent form. When the cresyl violet dye is di-substituted with two DEVD caspase-3/7 target sequences, the reagent is essentially non-fluorescent. As apoptosis progresses and caspase activity increases, the red fluorescent signal increases. Magic Red (cresyl violet) excites at 540-590 nm (590 nm optimal) and emits at >610nm (630 nm optimal). Because the Magic Red substrate probe is cell membrane permeant, a cell lysis step is not required to perform the assay.
Product Categories/Family for Magic Red Caspase 3 & 7 assay kit	Apoptosis Assays/Apoptosis Detection
References	Elmore, S. 2007. Apoptosis: A review of programmed cell death. Toxicol. Pathol. 35: 495-516. McStay, G.P., G.S. Salvesen, and D.R. Green. 2008. Overlapping cleavage motif selectivity of caspases: implications for analysis of apoptotic pathways. Cell Death Differ. 15: 322-331. Lee, B.W., G.L. Johnson, S.A. Hed, Z. Darzynkiewicz, J.W. Talhouk, and S. Mehrotra. 2003. DEVDase detection in intact apoptotic cells using the cell permeant fluorogenic substrate, (z-DEVD)2-cresyl violet. BioTechniques 35: 1080-1085.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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 Online datasheet: <https://stage.aaabiotech.com/assay-kits/magic-red-caspase-3-7/52705>