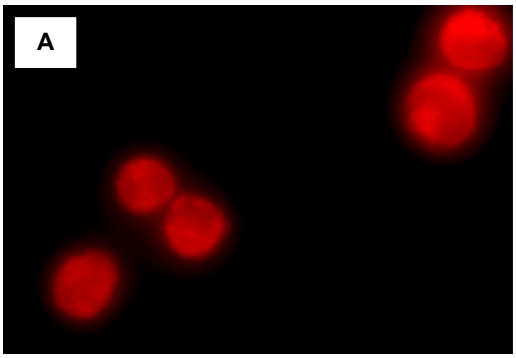
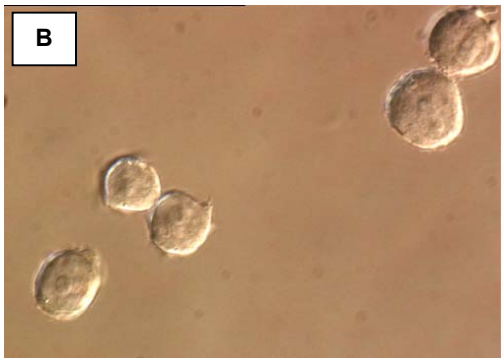
Magic Red™ Cathepsin Assays

Analyze cathepsins on a fluorescence microscope using ICT's Magic Red™ cathepsin detection kits.

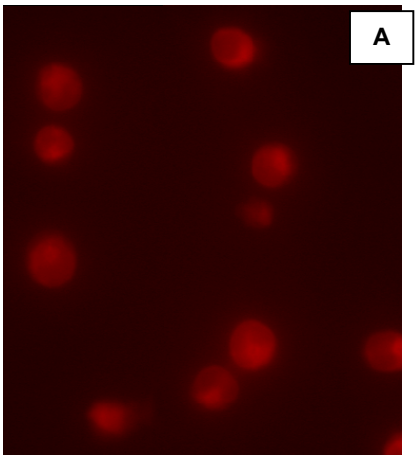
Microscope Data

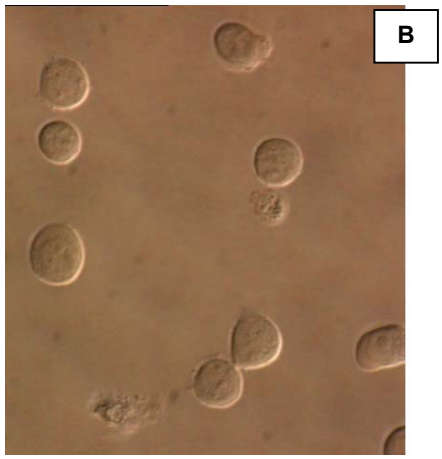




HL-60 cells were stained with 5 µM $MR(RR)_2$ for 60 minutes at 37°C. Intracellular localization of the hydrolyzed (fluorescent) $MR(RR)_2$ Cathepsin B substrate was detected on a Nikon Eclipse E 800 photomicroscope using a 510 – 560 nm excitation filter and a 570 – 620 nm emission/barrier filter set at approximately 700X magnification. Photo B shows the corresponding differential interference contrast image of the cells.

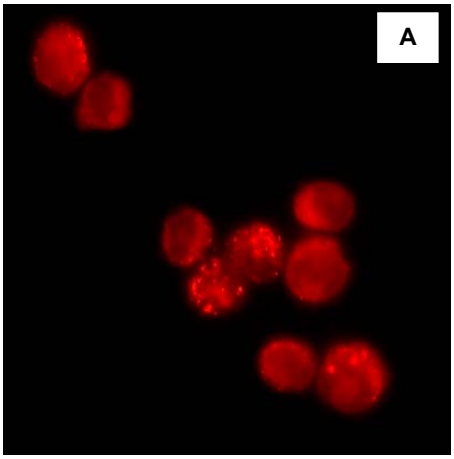
Microscope Data

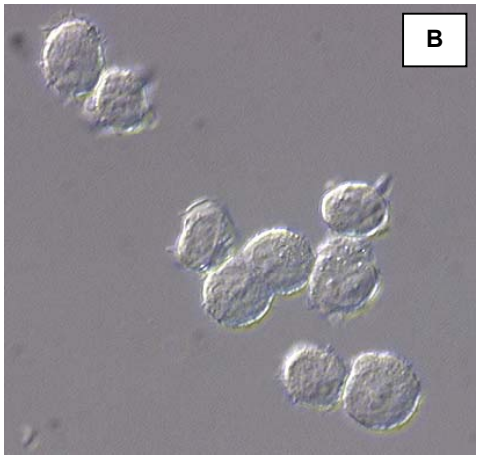




Jurkat cells were stained with 5 µM $MR(RR)_2$ for 60 minutes at 37°C. Intracellular localization of the hydrolyzed (fluorescent) $MR(RR)_2$ Cathepsin B substrate was detected on a Nikon Eclipse E 800 photomicroscope using a 510 – 560 nm excitation filter and a 570 – 620 nm emission/barrier filter set at approximately 700X magnification. Photo B shows the corresponding differential interference contrast image of the cells.

Microscope Data





THP-1 cells were stained with 5 µM $MR(RR)_2$ for 60 minutes at 37°C. Intracellular localization of the hydrolyzed (fluorescent) $MR(RR)_2$ Cathepsin B substrate was detected on a Nikon Eclipse E 800 photomicroscope using a 510 – 560 nm excitation filter and a 570 – 620 nm emission/barrier filter set at approximately 700X magnification. Photo B shows the corresponding differential interference contrast image of the cells.

Overview of the Protocol

Staining apoptotic cells with the Magic Red™ kit can be completed within a few minutes. However, the Magic Red™ kit 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 induction process. The Magic Red™ kit works with your current apoptosis protocols - induce apoptosis as you normally would and then label the cells with Magic Red™.

1. Culture cells to a density optimal for apoptosis induction according to your specific induction protocol, but not to exceed 10^6 cells/mL.
2. At the same time, culture a non-induced negative control cell population at the same density as the induced population for every labeling condition.
3. Induce apoptosis following your protocol (such as treating Jurkat cells with 2 μg/ml camptothecin for 3 hours).
4. Reconstitute the vial of lyophilized Magic Red™ with DMSO to form the 250X Magic Red™ stock concentrate.
5. Dilute the 25X Magic Red™ stock to the 30X working solution.
6. Stain cells by adding the 25X Magic Red™ solution.
7. Incubate for 1 hour.
8. Wash and spin cells.
9. If desired, label cells with Hoechst stain.
10. If desired, label cells with acridine orange.
11. Analyze data via fluorescence microscopy.

Contents of the Kit

- Magic Red™ lyophilized reagent (1 vial of 25-tests or 1 vial of 100-tests).
- Hoechst 33342 Stain (Prod. No. ALX-620-050)
- Acridine Orange

Recommended Materials and Reagents

- Cultured cells with media
- Reagents to induce apoptosis
- Hemocytometer
- Clinical centrifuge at <400 X g
- 37°C CO_2 incubator
- Ice or 4°C refrigerator to store cells
- dI H_2O, up to 2 mL
- PBS, pH 7.4, up to 100 mL
- Dimethyl Sulfoxide (DMSO), up to 50μL

Recommended Equipment

- Fluorescence microscope with appropriate filters: excitation 560 nm, emission >610 nm for Magic Red™; excitation at 480 nm and emission at >540 nm for AO; and excitation at 480 nm; and if Hoechst is used, a UV-filter with excitation at 365 nm, emission at 480 nm and slides.

Background on the Magic Red™ Cathepsin Detection Kits

Our Magic Red™ products are substrate-based assays designed to detect protease activity within whole living cells, using a fluroescence microscope. ICT's Magic Red™ assays are based on a cresyl violet leaving group that fluoresces once the enzyme specific peptides are cleaved.

No wash steps are required, making these kits very user friendly.

In our kits, Magic Red™ is bound to specific peptides that when cleaved, cause the Magic Red™ to fluoresce. By conjugating the Magic Red™ to different peptide sequences, different enzymes can be detected.

ICT is initially offering 3 different Magic Red™ cathepsin assays and 1 Magic Red™ caspase assay (see previous pages). All assay kits are available in 25-test and 100-test sizes.

Price List for Magic Red™ Cathepsin Detection Kits

Catalog Number	Magic Red™ Peptide	Target Cathepsin	Product Name	# Tests
ICT-937-T025	MR-$(RR)_2$	B	Magic Red™-$(RR)_2$ Cathepsin B Assay Kit	25
ICT-938-T100	MR-$(RR)_2$	B	Magic Red™-$(RR)_2$ Cathepsin B Assay Kit	100
ICT-939-T025	MR-$(LR)_2$	K	Magic Red™-$(LR)_2$ Cathepsin K Assay Kit	25
ICT-940-T100	MR-$(LR)_2$	K	Magic Red™-$(LR)_2$ Cathepsin K Assay Kit	100
ICT-941-T025	MR-$(FR)_2$	L	Magic Red™-$(FR)_2$ Cathepsin L Assay Kit	25
ICT-942-T100	MR-$(FR)_2$	L	Magic Red™-$(FR)_2$ Cathepsin L Assay Kit	100