

Magic Red Cathepsin B Assay Kit

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CATALOG #
AAA52722

PRODUCT SYNONYMS

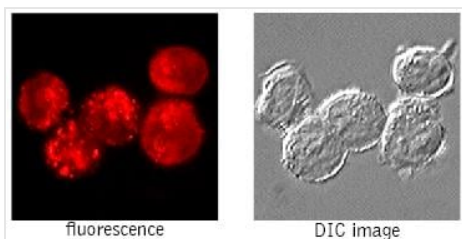
Magic Red Cathepsin B; Magic Red Cathepsin B Assay Kit; Magic Red Cathepsin B assay kit

PRODUCT SPECIFICATIONS

Preparation and Storage

Store at 2-8 degree C

IMAGES



Application Data

Cathepsin B-positive THP-1 cells fluoresced after staining with MR-(RR)2.
Red fluorophore concentrates inside lysosomes (Dr. Brian W. Lee, ICT).

ADDITIONAL INFORMATION

Related Product Information for Magic Red Cathepsin B assay kit	Background/Introduction: Magic Red® Cathepsin assay kits enable researchers to quantitate and monitor intracellular cathepsin B, K, or L activity over time in vitro. The Magic Red (MR) reagent is a non-cytotoxic substrate that fluoresces red upon cleavage by active cathepsin enzymes. Elevated cathepsin enzyme activity in serum or the extracellular matrix often signifies a number of gross pathological conditions. Cathepsin-mediated diseases include: Alzheimer's; numerous types of cancer; autoimmune related diseases like arthritis; and the accelerated breakdown of bone structure seen with osteoporosis^{1,2}. Up-regulated cathepsin B and L activity has been linked to several types of cancer. These include cancer of the colon, pancreas, ovaries, breast, lung, and skin (melanoma)³⁻⁶. Upregulation of cathepsin K has been shown in lung tumors⁷. Increased cathepsin K activity has also been linked to degenerative bone diseases including osteopetrosis and post-menopausal osteoporosis^{1, 8}. Cathepsins are usually characterized as members of the lysosomal cysteine protease (active site) family⁹ and the cathepsin family name has been synonymous with lysosomal proteolytic enzymes¹. In actuality, the cathepsin family also contains members of the serine protease (cathepsin A and G) and aspartic protease (cathepsin D and E) families as well. These enzymes exist in their processed form as disulfide-linked heavy and light chain subunits with molecular weights ranging from 20-35 kDa¹⁰. Cathepsin C is the noted exception, existing as an oligomeric enzyme with a MW ~200 kDa¹¹. Initially synthesized as inactive zymogens, cathepsins are post-translationally processed into their active configurations after passing through the endoplasmic reticulum and subsequent incorporation into the acidic environment of the lysosomes^{1, 11}. Magic Red detection substrates utilize the photostable red fluorophore, cresyl violet. When bi-substituted via amide linkage to two cathepsin target peptide sequences, such as (leucine-arginine)², the bi-substituted cresyl violet is nonfluorescent¹⁰. Following enzymatic cleavage at one or both arginine (R) amide linkage sites, the mono and non-substituted cresyl violet fluorophores generate red fluorescence when excited at 550-590 nm. Magic Red cathepsin B substrate, MR-(RR)², is comprised of cresyl violet coupled to two pairs of the amino acid sequence, arginine-arginine (RR), which is the preferential target sequence for cathepsin B. In cathepsin K substrate, MR-(LR)², cresyl violet is coupled to two pairs of leucine-arginine (LR). MR cathepsin L substrate, MR-(FR)², contains two pairs of phenylalanine-arginine (FR) coupled to cresyl violet. Cathepsins, like most other crucial cell survival enzymes, are somewhat permissive in the target amino acid sequence they will recognize and cleave. Although Magic Red substrates contain the amino acid target sequence preferred by a particular cathepsin enzyme, they can also recognize other active cathepsins or proteases when they are present. MBS encourages validation of cathepsin activity by an orthogonal technique. To use Magic Red, add the substrate directly to the cell culture media, incubate, and analyze. Because MR is cell-permeant, it easily penetrates the cell membrane and the membranes of the internal cellular organelles - no lysis or permeabilization steps are required. If cathepsin enzymes are active, they will cleave off the two dipeptide cathepsin targeting sequences and allow the cresyl violet fluorophore to become fluorescent upon excitation. The red fluorescent product will stay inside the cell and will often aggregate inside lysosomes (cathepsins are lysosomal) and other areas of low pH, such as inside the mitochondria. As protease activity progresses and more MR substrate is cleaved, the signal will intensify as the red fluorescent product accumulates within various organelles, enabling researchers to watch the color develop over time and quantify cathepsin B, K, or L activity. By varying the duration and concentration of exposure to the MR substrate, a picture can be obtained of the relative abundance of cathepsin enzymatic activity. Positive cells will fluoresce red and have pronounced red lysosomes and mitochondria. Negative cells will exhibit very low levels of background red fluorescence evenly distributed throughout the cell. This background level of substrate activity could be the result of constitutively synthesized serine proteases that target analogous amino acid sequences for hydrolysis. Please note that Magic Red substrates can undergo spontaneous hydrolysis over time, resulting in increased background fluorescence. Appropriate controls are necessary for accurate interpretation of the results. There is no interference from pro-cathepsins forms of the enzymes. If the treatment or experimental condition stimulates cathepsin activity, cells containing elevated levels of cathepsin activity will appear brighter red than cells with lower levels of cathepsin activity. The MR fluorophore, cresyl violet, fluoresces red when excited at 550-590 nm¹⁰. The red fluorescent signal can be monitored with a fluorescence microscope or plate reader. It has an optimal excitation of 592 nm and emission of 628 nm¹². Hoechst 33342 is included with the kit to concurrently label nuclei after labeling with MR. It is revealed under a microscope using a UV-filter with excitation at 365 nm and emission at 480 nm. Acridine orange (AO) is also included in the kit to identify lysosomes and other intracellular organelles (Figures 3 and 4). It is revealed under a microscope using excitation at 480 nm and emission at >540 nm, or alternatively with excitation at 550 nm and emission at >610 nm.
Product Categories/Family for Magic Red Cathepsin B assay kit	Apoptosis Assays/Cathepsin Assays

References

Takayuki, T., S. Yonezawa, A.H. Dehdarani, and J. Tang. 1986. Comparative studies of two cathepsin B isozymes from porcine spleen. *J. Biol. Chem.* 261: 9368-9374. Hashimoto, Y., H. Kakegawa, Y. Narita, Y. Hachiya, T. Hayakawa, J. Kos, V. Turk, and N. Katunuma. 2001. Significance of cathepsin B accumulation in synovial fluid of rheumatoid arthritis. *Biochem. Bioph. Res. Co.* 283: 334-339. Szpaderska, A.M., and A. Frankfater. 2001. An intracellular form of cathepsin B contributes to invasiveness in cancer. *Cancer Res.* 6: 3493-3500. Kos, J., H. J. Nielsen, M. Krasovec, I.J. Christensen, N. Cimerman, R. W. Stephens, and N. Brunner. 1998. Prognostic values of cathepsin B and carcinoembryonic antigen in sera of patients with colorectal cancer. *Clin. Cancer Res.* 4: 1511-1516.

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.

AAA Biotech | Tel: +1 (619) 604-9440 | Fax: +1 (619) 604-9441 | contact@aaabiotech.com

Online datasheet: <https://stage.aaabiotech.com/assay-kits/magic-red-cathepsin-b/52722>