Jan 11, 2026

Magic Red® Cathepsin B Assay Protocol

DOI
https://dx.doi.org/10.17504/protocols.io.kqdg32mb1v25/v1

Ali Ghoochani[1,2,3,4], Monther Abu-Remaileh[1,2,3,4]

[1]Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA;
[2]Department of Genetics, Stanford University, Stanford, CA 94305, USA;
[3]The Institute for Chemistry, Engineering and Medicine for Human Health (Sarafan ChEM-H), Stanford University, Stanford, CA 94305, USA;
[4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA

asap

Ali Ghoochani
Stanford University

DOI: https://dx.doi.org/10.17504/protocols.io.kqdg32mb1v25/v1

Protocol Citation: Ali Ghoochani, Monther Abu-Remaileh 2026. Magic Red® Cathepsin B Assay Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg32mb1v25/v1

Protocol status: Working
We use this protocol and it's working

Created: October 13, 2024

Last Modified: January 11, 2026

Protocol Integer ID: 109768

Keywords: red fluorescent signal, magic red substrate, based assay, fluorophore cresyl violet, fluorescence microscope, fluorescence, cresyl violet fluorophore, immunochemistry technology, assay

Abstract

The Magic Red Cathepsin B Assay Kit from ImmunoChemistry Technologies is a fluorescence-based assay designed to detect and measure intracellular cathepsin B activity in live cells. This kit utilizes the Magic Red substrate (MR-RR2), which contains the fluorophore cresyl violet conjugated to a cathepsin B-specific dipeptide sequence (arginine-arginine). Upon cleavage by active cathepsin B, the substrate releases the cresyl violet fluorophore, resulting in a red fluorescent signal that can be detected using a fluorescence microscope or plate reader.

Materials

Magic Red Cathepsin B Assay Kit (ImmunoChemistry Technologies, Cat# 937.

Prepare Magic Red Staining Solution

1 Reconstitute Magic Red Substrate (MR-RR2) with 50 µL DMSO.

Add Staining Solution to Cells

2 Once cells are ready for the experiment:

2.1 Dilute the stock 1:10 with diH2O to form the staining solution.

2.2 Dilute the Magic Red staining solution 1:25. Add 20 µL staining solution to 480 µL media.

2.3 Incubate the cells with the Magic Red staining solution for 00:30:00 at 37 °C 30m

Imaging

3 Immediately image the cells using a fluorescent or confocal microscope.

3.1 Use filters appropriate for Magic Red detection (excitation: 550-590 nm, emission: >610 nm)

Note: If the background fluorescence appears high or if there is excess staining solution, a gentle PBS wash can be considered to reduce background.