

Protocol for ReZolve-ER™ Imaging of the ER

This is intended as a guide only - optimization may be need.

In Brief

ReZolve-ER™ labels the endoplasmic reticulum in live and fixed cells. ReZolve-ER™ passively diffuses across the plasma membrane into the cell, stains at low concentrations and has minimal cytotoxic effects. It can be used as a real-time imaging reagent, which can be imaged within minutes of addition and has minimal photobleaching. ReZolve-ER™ is easily washed from cells, and therefore is ideal for protocols that require intermittent monitoring of endoplasmic reticulum structures.

ReZolve-ER™ has been successfully imaged using epifluorescent microscopy, confocal microscopy and two-photon microscopy. Cell penetration and localisation of ReZolve-ER™ has been confirmed in a range of cell lines, including prostate cells (PNT2, PNT1a, LNCaP, 22RV1 and DU145), cardiomyocytes (H9c2) and neuronal cells (PC-12).

Specifications

- Simple and quick application
- Suitable for fixed or live cell imaging
- Low cytotoxicity
- Highly resistant to photobleaching
- Large stoke shift (Ex/Em 405 nm / 570 nm)
- Compatible with other fluorescent dyes and proteins
- Ideal for epi-fluorescence, confocal and multiphoton imaging
- Stable at room temperature

Precaution For Use

Please read the entire procedure before performing staining procedure for fixed or live cell imaging and consider the safety data sheet. For laboratory use only. Not fully tested. Not for drug, household, human or veterinary uses.

Storage Condition

ReZolve-ER™ will perform as specified if stored at room temperature and protected from light. Once reconstituted in DMSO use within 6 months.

Reagent Preparation

Reconstitute the vial containing ~1.8 mg of ReZolve-ER™ with 300 µL of DMSO to obtain a 10 mM stock solution, mix thoroughly before use. This stock solution can be stored at room temperature, protected from light. Note: ReZolve-ER™ should not be reconstituted in aqueous solutions such as phosphate-buffered saline (PBS) or cell culture media. For use ReZolve-ER™ should be diluted in a buffer or cell culture media to a concentration of 50 µM-100 µM immediately before use (this solution should not be stored for later use).

Staining Protocol For Live Cells

Staining

For adherent cells, remove the medium from the culture dish and replace it with media containing 50-100 μM of ReZolve-ER™. The optimal staining concentrations of ReZolve-ER™ may vary between cell lines.

Imaging

ReZolve-ER™ can be observed in cells within minutes following addition. For the brightest staining allow cells to incubate with ReZolve-ER™ for 15 minutes prior to imaging. Do not wash cells. Maintain ReZolve-ER™ in media for the duration of the imaging protocol.

Removal of ReZolve-ER™

To remove ReZolve-ER™ from cells, aspirate the ReZolve-ER™ containing media, briefly wash cells with PBS. Replace this with cell culture media that does not contain ReZolve-ER™. Some cells may require several wash steps.

Co-staining Experiments

Prior to co-staining experiments, make sure that the spectral profiles of counterstaining agent and ReZolve-ER™ can be appropriately resolved. Stain cells with counter-staining agent according to manufacturer's instructions. Following washes, add ReZolve-ER™ and stain cells as described above for image.

Staining Protocol For Fixed Cells

Unlike the conventional endoplasmic reticulum stains, cells fixed with 4% paraformaldehyde have been successfully stained with ReZolve-ER™. Other fixation methods have not been attempted to date.

Cell Fixation

Fix samples in 4% paraformaldehyde for 20 minutes at room temperature. Wash samples 3 x 10 minutes in PBS.

Staining

Incubate fixed cells with 50-100 μM ReZolve-ER™ prepared in PBS for 15 minutes at room temperature.

Imaging

Mount coverslips on cells in ReZolve-ER™ solution for imaging.

Fluorescent Imaging Settings

Epi-Fluorescent Microscopy

ReZolve-ER™ can be excited by UV (~ 365 nm) or blue light (405 nm) sources with emissions collected using a wideband pass filter, or narrowband pass filter within this emission range 550-650 nm.

Confocal or Two-Photon Microscopy

ReZolve-ER™ can be excited by a 400 nm steady state laser, or at 800-830 nm using a two-photon pulse laser. Ideally image with a spectral detector set for the emission of ReZolve-ER™ 500-600 nm ($E_{\max} = 570$ nm). Alternatively detected by using an emission filter suited to the detection of FITC based fluorophores. Note: Time gated imaging can be performed with these products and is ideal for samples with high level of endogenous fluorescence. Probe emission lifetime is ~30 microseconds.