



ReZolve-ER™

Product code: 1101022

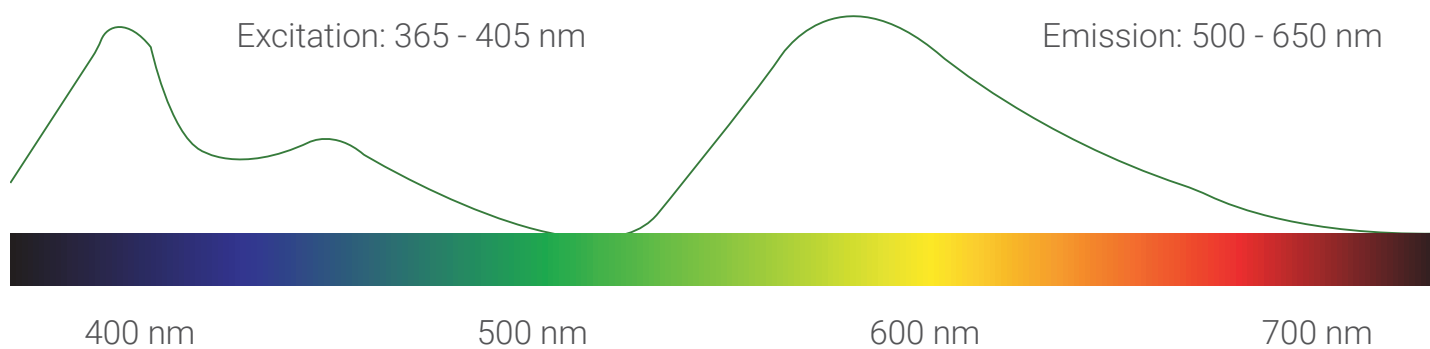
CAS: 1404104-40-0

ReZolve-ER™ is a live cell imaging agent with rapid cell uptake for imaging the endoplasmic reticulum (ER).

Spectral properties

✓ Excitation: 365 - 405 nm

✓ Emission: 500 - 650 nm



ReZolve-ER™ is a cell permeable reagent which localises to the endoplasmic reticulum within minutes.

Specs

- Highly resistant to photobleaching
- Suitable for live and fixed cells
- Compatible with other dyes
- Simple and quick application
- Low cytotoxicity
- Ideal for epifluorescence, confocal and multiphoton imaging
- Stable at room temperature

References

Bader, C. A., Sorvina, A., Simpson, P. V., Wright, P. J., Stagni, S., Plush, S. E., Massi, M. and Brooks, D. A. (2016), Imaging nuclear, endoplasmic reticulum and plasma membrane events in real time. *FEBS Lett*, 590: 3051–3060.

ReZolve-ER™

Preparation and Staining Procedures

Precautions for use

Please read entire procedure before staining samples and consider the safety data sheet. For laboratory use only. Not for drug, household or other uses

Cell Staining with ReZolve-ER™

ReZolve-ER™ labels the endoplasmic reticulum in live and fixed cells. ReZolve-ER™ passively diffuse across the plasma membrane into the cell and staining at low concentrations 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™ imaging reagent is easily washed from cells, and therefore, is ideal for protocols which require intermittent monitoring of endoplasmic reticulum structures.

ReZolve-ER™ has been successfully imaged using two-photon microscopy, confocal microscopy and epifluorescent 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).

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 50µM-100µM immediately before use (this solution should not be stored for later use).

Storage and stability

ReZolve-ER™ will perform as specified if stored at room temperature and protected from light once in DMSO, and used within 6 months of reconstitution in DMSO.

Staining protocol for live cells

- Staining cultured cells** - For adherent cells, remove the medium from the culture dish and replace it with 50-100 μM solution of ReZolve-ERTM prepared in appropriate cell culture media. The optimal staining concentrations of ReZolve-ERTM may vary between cell lines.
Note: It is recommended that cell culture media used for the staining does not contain foetal calf serum (FCS), or other high lipid content ingredients. If FCS is required, an increased incubation time may be necessary to obtain optimal staining.
- Imaging ReZolve-ERTM** - ReZolve-ERTM can be observed in cells within minutes following addition. For the brightest staining allow cells to incubate with ReZolve-ERTM for 15 minutes prior to imaging. Do not wash cells and maintain ReZolve-ERTM in media for the duration of the imaging protocol.
- Removal of ReZolve-ERTM** - To remove ReZolve-ERTM from cells, aspirate the ReZolve-ERTM containing media, briefly wash cells with PBS. Replace this with cell culture media which does not contain ReZolve-ERTM
- Co-staining experiments** - Prior to co-staining experiments, make sure that the spectral profiles of counter-staining agent and ReZolve-ERTM can be appropriately resolved. Stain cells with counter-staining agent according to manufacturer's instructions. Following washes, add ReZolve-ERTM 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-ERTM. 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 fixed cells** - Incubate cells with a 50-100 μM solution of ReZolve-ERTM prepared in PBS for 15 minutes at room temperature. Note: Although ReZolve-ERTM is detectable in cells within minutes, we suggest to incubate cells for 15 minutes to allow consistent results.
- Imaging** - Mount cells in ReZolve-ERTM solution for imaging.

Imaging settings

Epi-fluorescence Microscopy

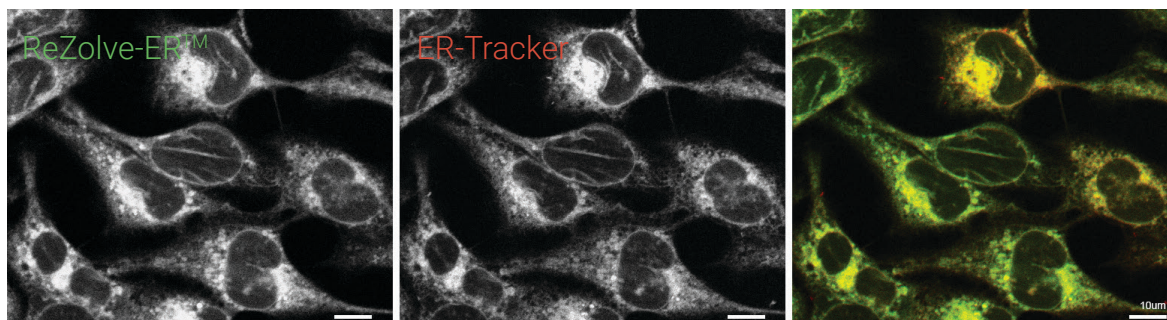
ReZolve-ERTM can be excited by UV (≈ 365 nm) or blue light (405 nm) sources. Collect images using a wideband pass filter, or narrowband pass filter within the range of 500-650 nm.

Confocal and Two-Photon Microscopy

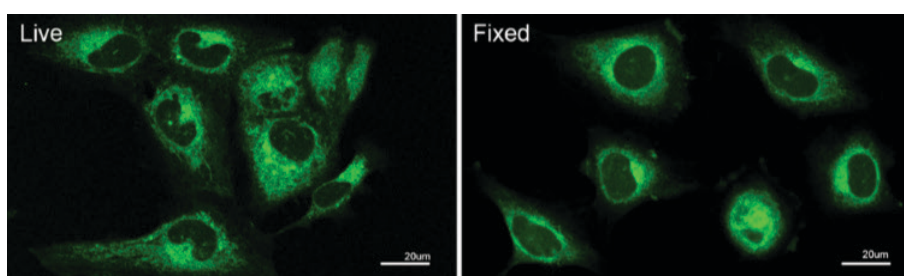
ReZolve-ERTM can be excited by a 400 nm steady state laser, or 800-830 nm using a two-photon pulse laser. Ideally image with a spectral detector set for the emission of ReZolve-ERTM, 490-670 nm ($E_{m_{max}} = 570$ nm). Alternatively detected by using an emission filter suited to the detection of FITC based fluorophores.

Images

Prostate cells stained with ReZolve-ER™ and counter stained with ER-Tracker™ Red



ReZolve-ER™ in live and paraformaldehyde fixed prostate cells



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Precautions and disclaimer

For laboratory use only.
Not for drug, household or other uses.
Consider the safety data sheet prior to use.

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