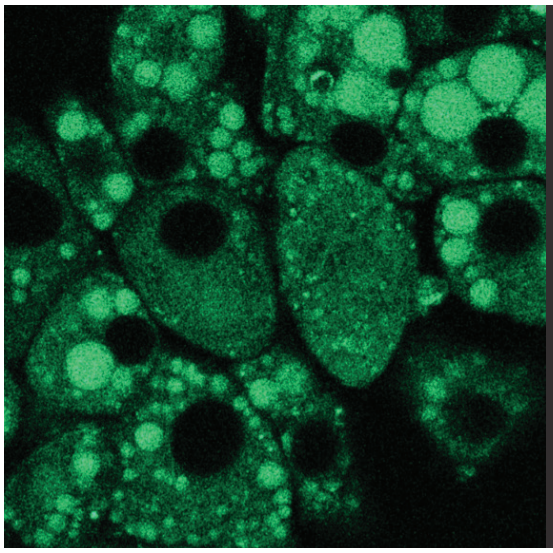


ReZolve-L1™

Data Sheet

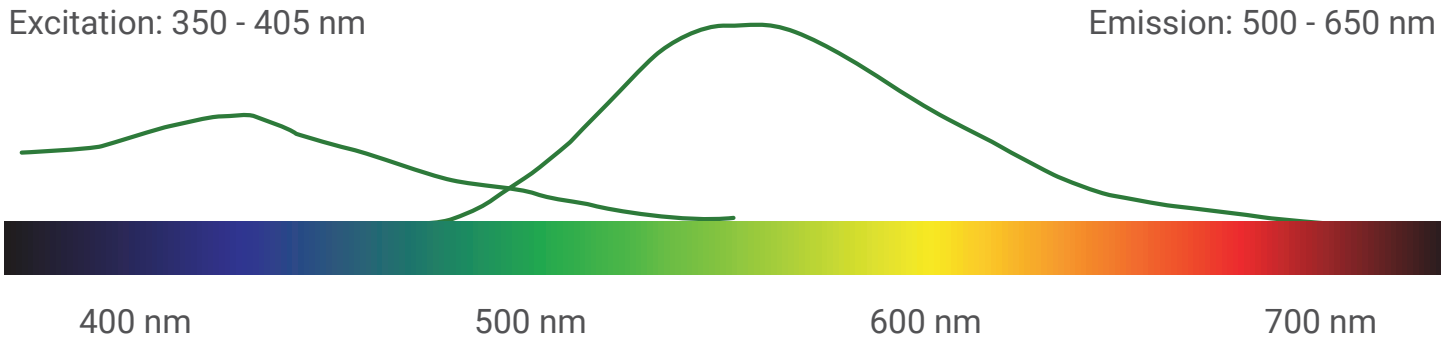
Product code: 1101021

CAS: 1369583-75-4

Cellular imaging of polar lipids for live and fixed cell use.

Compatible with other dyes.

Spectral properties:



ReZolve-L1™ is a cell-permeant stain that has an affinity for polar lipids and can be used in a wide variety of live and fixed cells.

Specs

- Resistant to photobleaching > 30min
- Suitable for fixed and live cells
- Compatible with other dyes
- Simple and quick application
- Low cytotoxicity
- Ideal for epifluorescence, confocal and multiphoton imaging
- Infrared and Raman active
- Stable at room temperature

References

- Bader, C. A. et. al. 'A molecular probe for the detec-tion of polar lipids in live cells" accepted 2016 to ***PlosOne***
- Bader, C. A. et. al. 'Unprecedented staining of polar lipids by a luminescent rhenium species revealed by FTIR microspectroscopy in adipocytes' ***Molecular BioSystems, 12,*** 2064-2068, 2016
- Bader, C. A. et. al. 'Modulation of the cellular orga-nelle specificity in Re(I) tetrazolato complexes leads to unprecedented phosphorescent labelling of lipid droplets'; ***RSC Adv.***, 4, 16345-16351, 2014

Stratech REZOLVE SCIENTIFIC stratech.co.uk/rezolve

ReZolve-L1™
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-L1™

ReZolve-L1™ localises with polar lipids allowing the labelling of lipid droplets and other high lipid content compartments in a range of cell and tissue types. ReZolve-L1™ is compatible with live and fixed sampled and can be imaged by single photon or multi photon techniques. ReZolve-L1™ is also compatible with vibrational spectroscopy as this complex is infrared and Raman active.

Cell penetration and localisation of ReZolve-L1™ has been comfirmed in a range of cell lines; in-cluding adipocytes (3T3-L1), protate cells (PNT2, PNT1a, LNCaP, 22RV1 and DU145), candiomyocytes (H9c2) and neuronal cells (PC-12), and tissues; including adipose tissue (sheep and *Drosophila*), muscle tissue (sheep cardiac and skeletal) and brain tissue (murine).

Reagent preparation

Reconstitute the vial containing ~1.8 mg of ReZolve-L1™ 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-L1™ should not be reconstituted in aqueous solutions such as phosphate-buffered saline (PBS) or cell culture media. For use ReZolve-L1™ should be diluted in an appropriate buffer or cell culture media to a concentration of 10µM-20µM immediately before use (this solution should not be stored for later use). Note: It is not recommended that detergents such as Tween20 or supplements with high lipid content such as foetal calf serum are used in preparation of these reagents. ReZolve-L1™ has low solubility in aqueous solutions so may precipitate at concentrations higher than those recommended.

Storage and stability

ReZolve-L1M 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

1. For adherent cells. Grow cells in 6 well-plates on coverslips with appropriate culture medium and under appropriate growth conditions. When cells have reached the desired confluence, remove the culture medium and add 10-20 µM of reagent (1:1000-1:500 dilution of 10 mM stock solution) in pre-warmed PBS or serum-free culture medium. Incubate cells for 30 minutes under appropriate growth conditions. Note: Serum-free culture medium is recommended for staining, as the lipids in the serum can sequester the dye. Wash coverslips twice for one minute in PBS. Mount coverslips in an aqueous mounting media for imaging. Note: Glycerol based mounting media can be used but may reduced the fluorescence intensity observed.

2. For cell suspension. For suspension cells, centrifuge to obtain a cell pellet and remove the supernatant. Resuspend the cells in pre-warmed PBS or serum-free culture medium containing 10-20 µM of ReZolve-L1™. Incubate cells for 30 minutes under appropriate growth conditions. Re-pellet the cells by centrifugation and resuspend in PBS or serum-free culture medium for immediate imaging. Cells can be prepared as a wet mounted or adhere to poly-L-lysine coated coverslips and mounted in an aqueous mounting media for imaging.

3. Co-staining experiments. Prior to co-staining experiments, make sure that the spectral profiles of counter-staining agent and ReZolve-L1™ can be appropriately resolved. Stain cells as described above with a reduced washing step to 30 seconds following incubation. Stain cells with counter-staining agent according to manufacturer's instructions. Following washes, mount in an aqueous mounting media for imaging.

Staining protocol for fixed cells

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

2. Staining fixed cells. Incubated fixed cells with 10-20 µM ReZolve-L1™ prepared in PBS for 30 minutes at room temperature. For best results provide gentle agitation by a platform rocker (or similar) at low rpm. Wash coverslips twice for one minute in PBS with agitation.

3. Imaging. Mount coverslips in an aqueous mounting media and image immediately for best results.

Staining protocol for frozen tissue sections

1. Sample preparation. Prepare and mount tissue sections on slides using standard protocols for frozen tissue. Once sectioned keep in the dark at room temperature until thawed (~20- 30 minutes). Wash samples 3 times for five minutes in PBS. Note: For quenching endogenous fluorescence, we recommend to incubate samples in 100 mM glycine in PBS (pH to 7.4 with 1M tris base, if required) for 20 minutes at room temperature. Other treatments such as UV irradiation may also be useful for quenching endogenous fluorescence, however avoid harsh treatments which may leach lipids from samples or interfere with lipid binding.

2. Staining sections. Incubate samples with 10-20 µM ReZolve-L1™ in PBS (1:1000-1:500 dilution of 10mM stock solution) for 2 hours at room temperature with gentle agitation provided by a platform rocker (or similar) at low rpm. Wash samples three times for five minutes in PBS at room temperature with agitation.

3. Imaging. Mount coverslips in an aqueous mounting media and image immediately for best results.

Staining protocol for *Drosophila* tissue

1. Sample preparation. Dissect *Drosophila* tissues in PBS with clean tools. Gently adhere tissues to the Poly-L-Lysine or Poly-L-ornithine covered coverslips. For *fixation* of fat body tissues, 2% paraformaldehyde in PBS for 30 minutes on ice followed by 3 by 10 minute washes in PBS at room temperature, is recommended.

2. Tissue staining. Incubate tissue with 10-20 µM ReZolve-L1™ in PBS (1:1000-1:500 dilution of 10 mM stock solution) for 30 minutes at room temperature. Wash tissue twice for one minute in PBS.

3. Imaging. Mount coverslips in an aqueous mounting media and image immediately for best results. Note: Image coupling gels such as cabomer-940 are ideal for tissue mounting as they support tissue integrate (see Rothstein EC et al. (2006) J Microsc 222: 58-64. for protocol).

Imaging settings

Epi-fluorescence Microscopy
ReZolve-L1™ can be excited by UV (≈365 nm) or blue light (405 nm) sources. Collect images using a wide band pass filter, or narrowband pass filter within this emission range within the range of 500-650 nm.

Confocal and Two-Photon Microscopy
ReZolve-L1™ 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-L1™, 490-670 nm (Em_{max} = 570 nm). Alternatively detected by using an emission filter suited to the detection of FITC based fluorophores.
Note: Time gated image can be performed with these products and is ideal for samples with high level of endogenous fluorescence. Probe emission lifetime is ~30 microseconds.

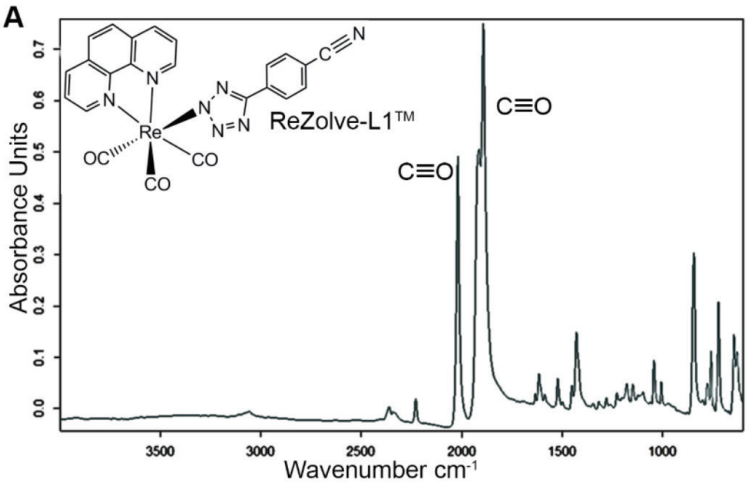
Vibrational spectroscopy

ReZolve-L1™ can detected by infrared and Raman spectroscopy methods. Sample preparation will need to be adapted appropriately for these techniques.

Sample preparation. Adherent cell culture can be grown on silicon nitride substrate which are compatible with infrared, or calcium fluoride slides which is compatible with Raman spectroscopy. Fixation with cold methanol is recommended for best preservation of lipids. Following staining dehydration maybe required as these techniques can be hindered by water content. (see Bader CA, et al. Mol Biosyst 2016; 12:2064-8. for example of application).

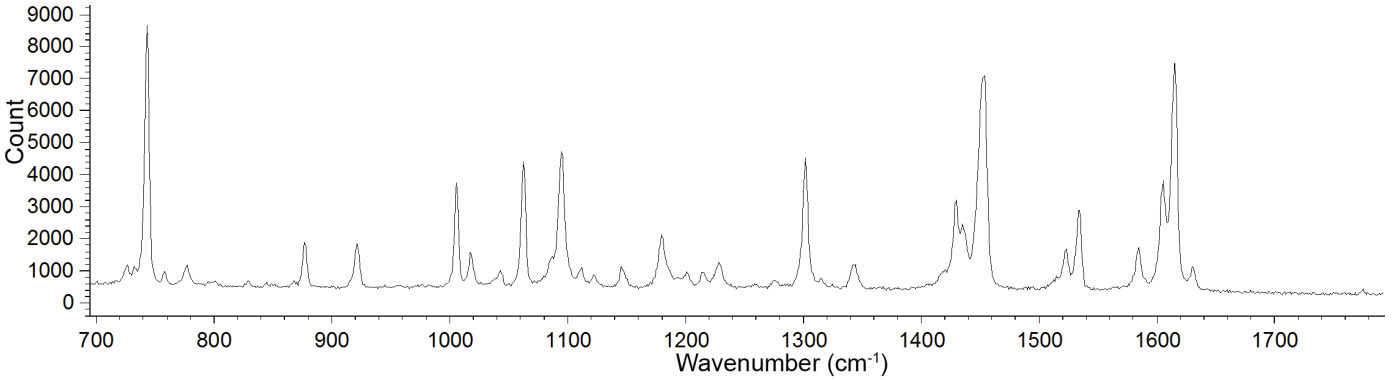
Infrared spectroscopy

The FTIR spectrum of solid ReZolve-L1™ is domi-nated by the strong Re carbonyl stretching bands centred at ~2027 cm-1 and the doublet 1915/1893 cm-1, characteristic of facial tricabonyl complexes. This is a spectral region where vibrational modes due to biochemical components are conveniently negligible.



Raman spectroscopy

ReZolve-L1™ excitation can be achieved at 785 nm with a spectra as shown below.



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REZOLVE SCIENTIFIC

stratech.co.uk/rezolve
technical@stratech.co.uk
+44 (0) 1638 782600