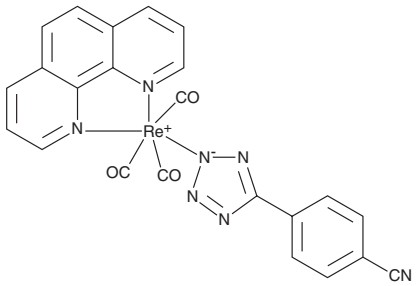
PRODUCT INFORMATION

ReZolve-L1™

Item No. 25907



CAS Registry No.: 1369583-75-4
Formal Name: (OC-6-33)-tricarbonyl(1,10-phenanthroline-κN^1,κN^{10})[4-(2H-tetrazol-5-yl-κN^2)benzonitrilato]-rhenium
MF: $C_{23}H_{12}N_7O_3Re$
FW: 620.6
Ex./Em. Max: 405/550 nm
Supplied as: A solid
Storage: -20°C
Stability: ≥2 years

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

ReZolve-L1™ is a fluorogenic probe that localizes to polar lipids and can be used to label lipid droplets and other high lipid-content compartments in live and fixed cells as well as frozen tissue samples. It selectively co-localizes with the lipid stain Oil Red over the mitochondrial and lysosomal dyes MitoTracker® Red and LysoTracker® Green, respectively, in *Drosophila* adipose fat body cells.[1] ReZolve-L1™ is compatible with epifluorescent, confocal, and two-photon microscopy applications as well as infrared and Raman spectroscopy applications. ReZolve-L1™ displays excitation/emission maxima of 405/550 nm, respectively, and can be used for live and fixed cell and frozen tissue applications.

Assay Protocol (microscopy applications)

The amount of ReZolve-L1™ provided is sufficient to label 150-300 slides or 1,440-2,880 individual wells of cells when utilized in a 96-well plate format, depending on the protocol and application used.

1. **Prepare lipid droplet-staining solution**
   a. Reconstitute ReZolve-L1™ with 300 µL of DMSO and mix thoroughly to obtain a 10 mM ReZolve-L1™ stock solution.
   b. Store ReZolve-L1™ stock solution at room temperature protected from light.

2. **Prepare and stain the cells**
   a. Live adherent cells:
      i. Grow cells in 6-well plate on coverslips to desired confluency (~70-80%).
      ii. Remove culture medium and add pre-warmed PBS or serum-free medium containing ReZolve-L1™ at a final concentration of 10-20 µM.*
      iii. Incubate cells at 37°C, 5% CO_2 for 30 minutes.
      iv. Observe cells using fluorescence technique of choice.
   b. Live suspended cells:
      i. Pellet cell suspension and remove supernatant.
      ii. Resuspend cells in pre-warmed PBS (37 °C) or serum-free medium containing ReZolve-L1™ at a final concentration of 10-20 µM.*
      iii. Incubate cells at 37°C, 5% CO_2 for 30 minutes.
      iv. Re-pellet the cells and resuspend in PBS or serum-free medium.
      v. Pipette cells onto a coverslip for imaging in PBS or serum-free medium OR adhere cells to a poly-L-lysine (or similar) coated coverslip by pipetting cells onto the coverslip, allow cells to settle for 2-5 minutes, and wet mount coverslip.
      vi. Observe using fluorescence technique of choice.

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c. Fixed cells:
   i. Fix cells in 4% paraformaldehyde for 20 minutes at room temperature.
   ii. Wash samples 3 x 10 minutes in PBS.
   iii. Incubate samples in PBS containing ReZolve-L1™ at a final concentration of 10-20 µM for 30 minutes at room temperature with gentle agitation by platform rocker (or similar) at low rpm.
   iv. Wash coverslips 2 x 1 minute in PBS.
   v. Wet mount coverslip.
   vi. Observe using fluorescence technique of choice.

**Note 1: ReZolve-L1™ should not be diluted into buffers containing detergents or supplements with high lipid content, such as Tween20 or fetal bovine serum (FBS). Dye may precipitate out of solution if used at concentrations higher than recommended.*

3. **Prepare and stain frozen tissue samples:**
   a. Sample preparation:
      i. Prepare and mount tissue sections on slides using standard protocols for frozen tissue.
      ii. Samples should be kept in the dark at room temperature for approximately 20 to 30 minutes until thawed.
      iii. Incubate cells at 37°C, 5% CO_2 for 30 minutes to 2 hours.
      iv. Wash samples 3 x 5 minutes in PBS.

Note 2: To quench endogenous fluorescence, incubate samples in PBS (pH 7.4) with 100 mM glycine for 20 minutes at room temperature. Other methods to quench fluorescence may be used, such as UV irradiation, however, harsh treatments may induce lipid leaching and/or interfere with lipid binding and should be avoided

   b. Staining tissue sections:
      i. Incubate samples in PBS containing ReZolve-L1™ at a final concentration of 10-20 µM for 2 hours at room temperature with gentle agitation by platform rocker (or similar) at low rpm.
      ii. Wash samples 3 x 5 minutes in PBS.
      iii. Mount coverslips using aqueous mounting media.
      iv. Observe using fluorescence technique of choice.

Assay Protocol (other spectroscopy applications)

1. **Sample preparation**
   a. Grow adherent cells on a silicon nitride substrate or calcium fluoride slides for infrared or Raman spectroscopy, respectively.
   b. Fix cells with cold methanol for preservation of lipids.
   c. Incubate samples in PBS containing ReZolve-L1™ at a final concentration of 10-20 µM for 30 minutes at room temperature with gentle agitation by platform rocker (or similar) at low rpm.
   d. Wash coverslips 2 x 1 minute in PBS.
   e. Image using spectroscopy technique of choice.*

Note 1: Dehydration may be required following staining as these techniques can be hindered by water content.

Reference

1. Bader, C.A., Brooks, R.D., Ng, Y.S., *et al.* Modulation of the organelle specificity in Re(I) tetrazolato complexes leads to labeling of lipid droplets. *RSC Adv.* **31(4)**, 16345-16351 (2014).

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