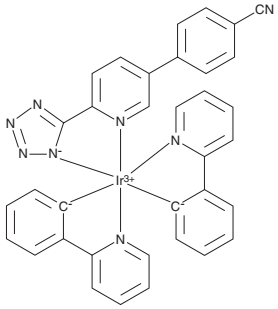
PRODUCT INFORMATION

IraZolve-L1™

Item No. 25911

CAS Registry No.: 2169684-98-2
Formal Name: (OC-6-44)-*bis*[2-(2-pyridinyl-κN) phenyl-κC][6-(1H-tetrazol-5-yl-κN^1)-3-pyridinecarbonitrilato-κN^1]-iridium
MF: $C_{29}H_{19}IrN_8$
FW: 671.7
Ex./Em. Max: 405/600 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

IraZolve-L1™ is a cell-permeable fluorogenic probe that can be used to label intracellular lipid droplets and the endoplasmic reticulum in live and fixed cells. It is highly photostable and has low cytotoxicity making it suitable for time-lapse imaging of live cells. IraZolve-L1™ is compatible with epifluorescent, confocal, and two-photon microscopy applications. It displays excitation/emission maxima of 405/600 nm, respectively, and can be used for live and fixed cell applications.

Assay Protocol

The amount of IraZolve-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 IraZolve-L1™ with 149 µL of DMSO and mix thoroughly to prepare a 10 mM IraZolve-L1™ stock solution.
   b. Store IraZolve-L1™ stock solution at room temperature protected from light.

Note 1: IraZolve-L1™ should not be reconstituted in aqueous solutions such as PBS or cell culture media.

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 (37°C) or serum-free medium containing IraZolve-L1™ to a final concentration of 10-20 µM (1:1,000-1:500 dilution of 10 mM stock solution).
      iii. Incubate cells at 37°C, 5% CO_2 for 30 minutes.
      iv. Remove staining media and wash cells 3 x 1 minute in PBS.
      v. Mount coverslips in an aqueous mounting media for immediate imaging.
      vi. 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 to a final concentration of 10-20 µM (1:1,000-1:500 dilution of 10 mM stock solution).
      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.

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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.

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.
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 IraZolve-L1™ at a final concentration of 10-20 µM for 1.5 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.

Note 3: For epifluorescence applications, IraZolve-L1™ can be excited at approximately 365 nm (UV) or 405 nm. For confocal and two-photon applications, it can be excited at 400 nm and 800-830 nm, respectively. Time-gated imaging is ideal for samples with a high level of endogenous fluorescence and IraZolve-L1™ has an emission lifetime of approximately 30 microseconds.

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