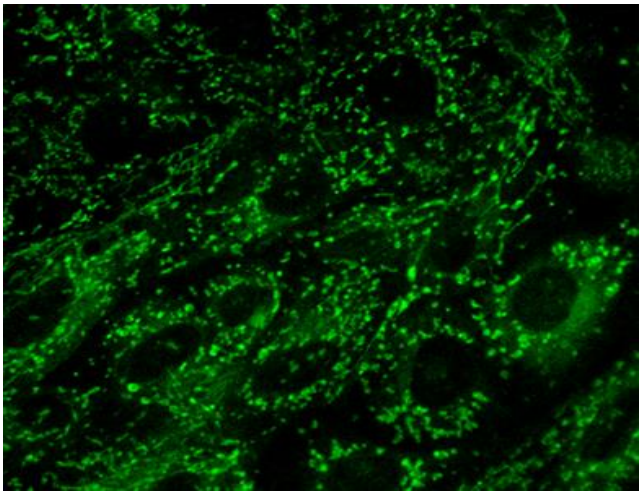
FLUOROPHORES FOR TARGETED INSIGHTS

IraZolve-Mito

MONITOR MITOCHONDRIA IN LIVE AND FIXED SAMPLES

Product Name	IraZolve-Mito™
Product Code	**1101024**
CAS Number	**2172800-69-8**
Ex/Em	**405 nm/ 600 nm**
Quantity	**0.5 mg**
Application	**Mitochondrial stain**



IraZolve-Mito™ localises to mitochondria in live cells and tissue. IraZolve-Mito™ is also suitable for some the detection of mitochondria in fixed and frozen tissue samples. This agent is suitable for a range of fluorescence applications including imaging by confocal microscopy and multi photon microscopy.

Cell penetration and localisation of IraZolve-Mito™ has been confirmed in prostate cells (PNT2 and 22RV1), cardiomyocytes (H9c2) and cancer cell lines (HeLa). Mitochondrial localisation has been shown in live tissues, including live adipose tissue (sheep) and muscle tissue (sheep cardiac and skeletal) and frozen or PFA fixed muscle tissue (sheep skeletal).

Specifications

- Simple and quick application
- Suitable for live cell imaging
- Suitable for live and fixed tissue
- Low cytotoxicity
- Highly resistant to photobleaching
- Large stoke shift (Ex/Em 405 nm / 600 nm)
- Compatible with other fluorescent dyes
- Compatible with epi-fluorescence, confocal and multiphoton imaging
- Delivery at room temperature

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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

As a solid IraZolve-Mito™ is suitable for storage at room temperature protected from light. Product is stable for up to 6 months if stored as specified. **Once reconstituted in DMSO IraZolve-Mito™ should be stored at 4°C**. Reconstituted stock solution should be used within 2 months of reconstituting for best staining results.

Reagent Preparation

Reconstitute IraZolve-Mito™ (~0.5 mg) with 55 µL of DMSO to prepare a 10 mM stock solution. Mix thoroughly with a vortex before use.

Note: IraZolve-Mito™ should not be reconstituted in aqueous solutions such as phosphate-buffered saline (PBS) or cell culture media. IraZolve-Mito™ should be diluted in an appropriate buffer or cell culture media to a concentration of 10µM - 50µM immediately before use (this solution should not be stored for later use).

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Staining Protocol For Live Cell

For Adherent Cells:

1) Grow cells in 6 well-plate on coverslips with appropriate culture medium and under appropriate growth conditions
2) Grow cells to desired confluence (70 – 80%)
3) Remove culture medium and add pre-warmed PBS or cell culture media containing 10 – 50 µM of IraZolve-Mito™ (1:1000 – 1:200 dilution of 10 mM stock solution)
4) Incubate cells for 30 minutes under appropriate growth conditions
5) Wash coverslips twice for one minute in PBS
6) Mount coverslips in aqueous mounting media for imaging
   Note: Glycerol based mounting media may reduce the fluorescence intensity of IraZolve-Mito™.

For Suspended Cells:

1) Centrifuge cell suspension to obtain cell pellet and remove the supernatant
2) Resuspend cells in pre-warmed PBS (37°C) or serum-free medium containing 10 – 50 µM of IraZolve-Mito™ (1:1000 – 1:200 dilution of 10 mM stock solution)
3) Incubate cells for 30 minutes under appropriate growth conditions
4) Re-pellet the cells by centrifugation and resuspend in PBS or cell culture medium
5) Cells can be prepared as a wet mounted or adhere to poly-L-lysine coated coverslips and mounted in an aqueous mounting media for immediate imaging

For Co-staining Experiment:

1) Prior to co-staining, make sure that the spectral profiles of counter-staining agent and IraZolve-Mito™ can be appropriately resolved. (In general dyes which do not excite with 405 nm excitation can be imaged alongside IraZolve-Mito™, blue dyes such as DAPI are also compatible as they emit at a lower wavelength than IraZolve-Mito™)
2) Stain cells as described above with a reduced washing step to 30 seconds following incubation
3) Stain cells with counter-staining agent according to manufacturer's instructions
4) Following washes, mount in an aqueous mounting media for imaging
   Note: IraZolve-Mito™ is not suitable for fixed cell staining of mitochondria

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Staining Protocol For Tissue Sections

Unlike the conventional mitochondrial stains, paraformaldehyde fixed and live tissue sections have been successfully stained with IraZolve-Mito™. Other fixation methods have not been attempted to date.

If **endogenous fluorescence** is an issue in your tissue sample, quenching can assist in imaging. For quenching endogenous fluorescence, we recommend incubating 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.

Sample Preparation: Tissues can be stained immediately upon collection or stored for later staining. We recommend 4% paraformaldehyde fixation or flash freezing for tissue storage. Sample preparation will depend on the tissue type and imaging platform. In general, IraZolve-Mito™ can stain tissue sections of up to 5mm thick. Live samples can be sectioned using a sharp scalpel or knife. Fixed and frozen can also be prepared in this manner or in OCT sectioned by microtone to your desired thickness.

Staining Sections: Incubate samples with 10-50 µM IraZolve-Mito™ in PBS or appropriate media (1:1000 - 1:200 dilution of 10mM stock solution) for 30 minutes 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. Mount tissue in aqueous mounting media and image immediately for best results.

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Fluorescent Imaging Settings

Epi-fluorescent Microscopy:

IraZolve-Mito™ 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:

IraZolve-Mito™ 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 IraZolve-Mito™, 500-650 nm (E_{max} = 600 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.

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