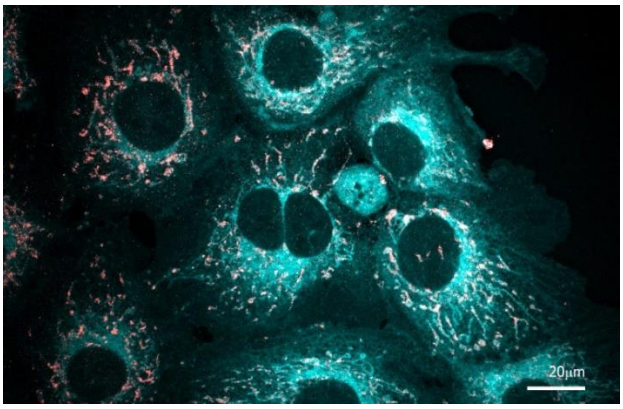
FLUOROPHORES FOR TARGETED INSIGHTS

IraZolve-ER Blue™

Cell Permeant Stain for labelling the Endoplasmic Reticulum

Product Name	**IraZolve-ER Blue™**
Product Code	**1101026**
CAS Number	**376367-93-0**
Ex/Em	**405 nm/ 500 nm**
Quantity	**1.0 mg**
Application	**Endoplasmic reticulum detection**



IraZolve-ER Blue™ localises to the endoplasmic reticulum in live and fixed cells. It is highly cell permeable, producing quick and reliable cell staining. IraZolve-ER Blue™ can detect the endoplasmic reticulum following antibody staining protocols and is compatible with a range of fluorescence applications.

Specifications

- Compatible with antibody staining
- Compatible with other dyes
- Simple and quick application
- Suitable for live or fixed cell imaging
- Low cytotoxicity
- Highly resistant to photobleaching
- Ideal for epi-fluorescence and confocal microscopy
- Stable 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

IraZolve-ER Blue™ should be stored as specified under proper storage condition at room temperature and protected from light. Product is stable for up to 6 months as a solid. Once reconstituted in DMSO will last at least 4 months.

Reagent Preparation

Reconstitute the vial containing ~1.0 mg of ReZolve-ER Blue™ with 144 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: IraZolve-ER Blue™ should not be reconstituted in aqueous solutions such as phosphate-buffered saline (PBS) or cell culture media. For use, IraZolve-ER Blue™ 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 Cells

For Adherent Cells:

1. Grow cells in live cell imaging chambers, with appropriate cell growth medium and under appropriate growth conditions, to desired confluency (70 – 80%)
2. Remove culture medium and add 10 – 50 µM of IraZolve-ER Blue™ in pre-warmed PBS or growth medium (1:1000 – 1:200 dilution of 10 mM stock solution). We recommend to begin with a working concentration of 20 µM.
3. Incubate cells for 30 minutes under appropriate growth conditions.
4. Wash cells 2 times in PBS for 5 minutes per wash.
5. Replace with fresh growth media for imaging. For best results use phenol red free growth media.

For Suspended Cells:

1. Take cells grown in suspension and centrifuge to obtain cell pellet. Remove the supernatant.
2. Resuspend cells 10 – 50 µM of IraZolve-ER Blue™ in pre-warmed PBS or growth medium (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 and allow to wash for 5 minutes.
5. Re-pellet the cells by centrifugation and resuspend in growth media for imaging.
6. Cells can be prepared as a wet mount or adhere cells to poly-L-lysine coated coverslips for imaging. For best results use phenol red free growth media.

For Co-Staining Experiment:

1. Prior to co-staining, make sure that the spectral profiles of counter-staining agent and IraZolve-ER Blue™ can be appropriately resolved.
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 replace PBS with phenol red free growth media for imaging.

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Staining Protocols for Fixed Cells

For Adherent and Non-Adherent Cells:

1. For adherent cells: grow cells on glass coverslips in appropriate growth media to desired confluence (70-80 %).
   For non-adherent cells: grow cells in suspension and adhere to poly-L-lysine treated coverslips following fixation and washing (steps 2 and 3).
2. Fix cells in pre-warmed 4% paraformaldehyde for 10 to 20 minutes at room temperature.
3. Wash cells 3 times in PBS for 5 minutes per wash.
4. Incubated fixed cells with 10 - 50 µM IraZolve-ER Blue™, prepared in PBS, for 30 minutes at room temperature.
5. Wash coverslips twice for 5 minutes in PBS.
6. Mount coverslips in an aqueous mounting media and image immediately for best results.

For Antibody Staining Experiment:

1. Grow and fix cells as above (steps 1-3).
2. Following fixation perform antibody staining according to standard protocols. Ensure the secondary antibody chosen is compatible with ReZolve-ER Blue™ (e.g. does not excite with 400 nm excitation).
3. Upon completion of antibody staining incubate cells with 10 - 50 µM prepared in PBS, for 30 minutes at room temperature. Protect coverslips from light to reduce damage to secondary antibody.
4. Wash coverslips twice for 5 minutes in PBS. Protect coverslips from light to reduce damage to secondary antibody.
5. Mount coverslips in an aqueous mounting media and image immediately for best results.

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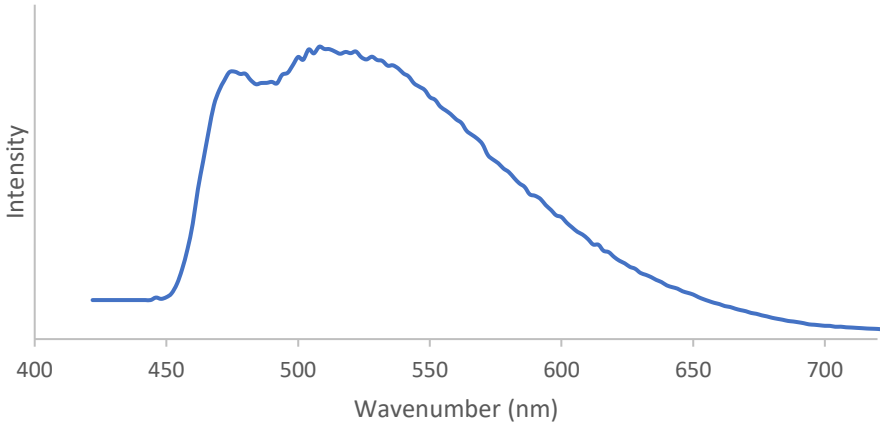
Imaging System Settings

Epi-fluorescent Microscopy: IraZolve-ER Blue™ 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 with an emission range 450-570 nm.

Confocal Microscopy: IraZolve-ER Blue™ can be excited by a 400 nm steady state laser, and emission should be collected using a detector suited to blue fluorophores such as DAPI. Alternatively, a spectral detector set for the emission of IraZolve-ER Blue™, 450-570 nm (Emmax = 500 nm) can be used.



Emission profile of IraZolve-ER Blue™ with 403 nm excitation.

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