Check videos of protocol, examples
of results and much more on:
idylle-labs.com/brighter

A protocol designed in February 2023.

BrightER

PROTOCOL

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1. The material you need

REAGENTS

- BrightER 5 mM in DMSO/PEG solution

CONSUMABLES

- Coverslips or imaging chambers (depending on your experiment)
- Cell culture medium

LABWARE

- Water-bath heating at 70°C

2. Storing the BrightER stock solution

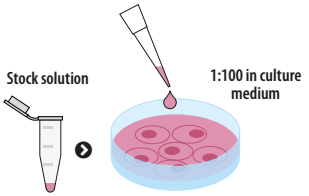
- BrightER can be stored at 4°C for up to one month. For a long-term storage, aliquot and store at -20°C.
- Always keep the vial protected from light.

3. Using BrightER

- Seed your cells on coverslips or imaging chambers and culture as desired.

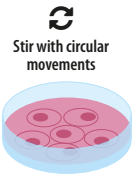
We recommend imaging cells at a low density for an optimal visualization of the Endoplasmic Reticulum structure.

- Take your BrightER stock solution out 20-30 minutes before use to bring it to room temperature.
- Wash cells with PBS to get rid of cellular debris.
- Add pre-warmed culture medium.
- Vortex your BrightER stock solution.
- Add the BrightER stock solution, respecting a 1:100 dilution factor in your cell culture medium. The BrightER final concentration is 50µM.

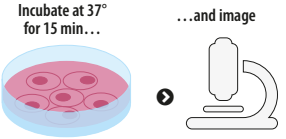


This concentration should guarantee a bright signal but might need to be adjusted depending on the cell type used.

- Stir manually the dish with circular movements so that the BrightER is dispersed evenly on the cell layer.



- Incubate at 37°C for 15 min and proceed immediately to imaging.



BrightER is conjugated to a rhodamine dye with an excitation peak at 557 nm and an emission peak at 576 nm. It can therefore be visualized using any green excitation filter block in fluorescence microscopy (TRITC, DsRed, etc).

- The BrightER fluorescence signal is observable for at least 3 hours. If the fluorescence signal is fading, the cell culture medium can be supplemented with additional BrightER.

4. Trouble shooting

- Aggregates may form in the BrightER solution upon storage. If necessary, vortex thoroughly for 30 seconds and heat at 70°C for 90 seconds. Doing this twice should help dissolving aggregates.

You may transfer the BrightER stock solution into an Eppendorf vial to facilitate the heating process when using a water-bath.