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FRONTIERS IN LIFE SCIENCE

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ERseeing <Endoplasmic Reticulum Green>

Catalog NO. FDV-0038

Research use only, not for human or animal therapeutic or diagnostic use.

Product Background

Endoplasmic reticulum (ER) is the largest organelle in the cell and has unique and dynamic tubular or sheet structures. ER plays essential roles in biosynthesis, precise folding and quality control of proteins and is a traffic origin of secreted pathway proteins including the Golgi apparatus, exocytosis, plasma membrane, and extracellular proteins. The major functions of ER are not only protein synthesis, but also carbohydrate metabolism, calcium storage, lipid metabolism, and lipid droplet synthesis. Visualization of ER structure in live cells is very important for the understanding of ER function and physiological significance of ER-resident proteins.

The most conventional ER-staining dye is based on glibenclamide-fluorophore conjugate. Glibenclamide is known as a potent and specific inhibitor of the sulphonylurea receptors of ATP-sensitive K^+ channels which are selectively localized on ER, glibenclamide-based ER dyes can visualize ER structures. However, its pharmacological activity negatively affects K^+ channel functions in ER. In addition to the harmful influence of glibenclamide-based dyes for the cells, glibenclamide is a reversible inhibitor and can be washed out by wash step and medium change. Consequently, glibenclamide-based ER dyes can visualize only pharmacologically affected cells and not suitable for long-term imaging experiments.

To overcome these problems, our ERseeing exhibit little effect on the ER functions pharmacologically and can visualize ER after washout or medium change. ERseeing has two units, a rhodol-type green fluorescent dye, and a thioester-type protein labeling group with rhodol-derivative having a high affinity to ER membrane. Right after addition of ERseeing to culture media, it can be accumulated into ER membranes. Protein labeling occurs with ERseeing non-specifically conjugates the rhodol fluorescent dyes onto ER-proteins by nucleophilic attack forming a covalent bond between ERseeing and ER-proteins resulting in a stable ER-rhodol label. ERseeing enables visualization of the ER structure even after washout or medium changes. This reagent is a powerful tool to monitor ER structures in live cells with little pharmacological effects.

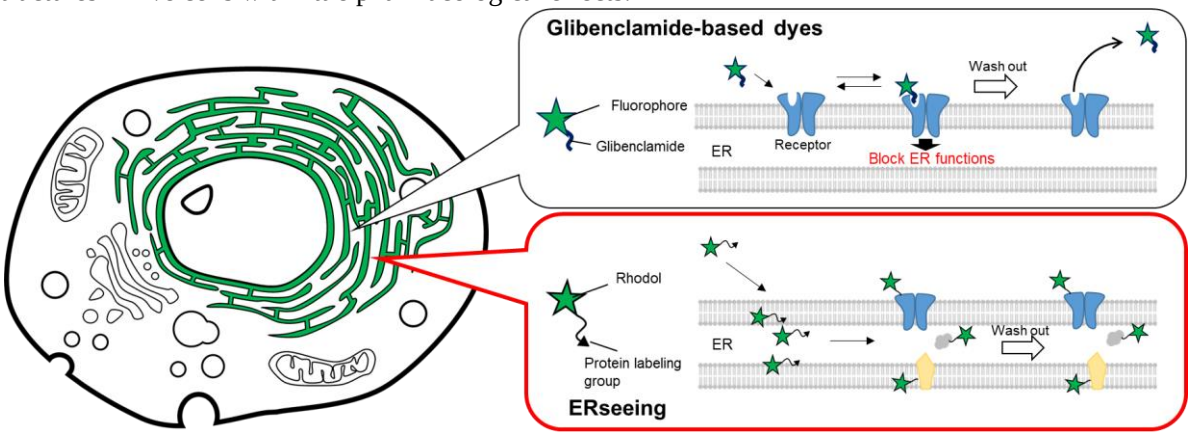


Figure 1. Overview of ERseeing and conventional glibenclamide-based dyes

Description

Catalog Number: FDV-0038
Size: 10 nmol
Formulation: $C_{37}H_{28}F_2N_2O_4S$
Molecular weight: 634.6g/mol
Solubility: Soluble in DMSO
Ex/Em: 509 nm/524 nm
*FITC filter sets are available.

Application

- Live cell ER imaging of cultured cells

NOTE: After staining live cells, cell fixation is compatible. However, this reagent does not stain ER specifically in fixed cells, staining step should be under live cell condition.

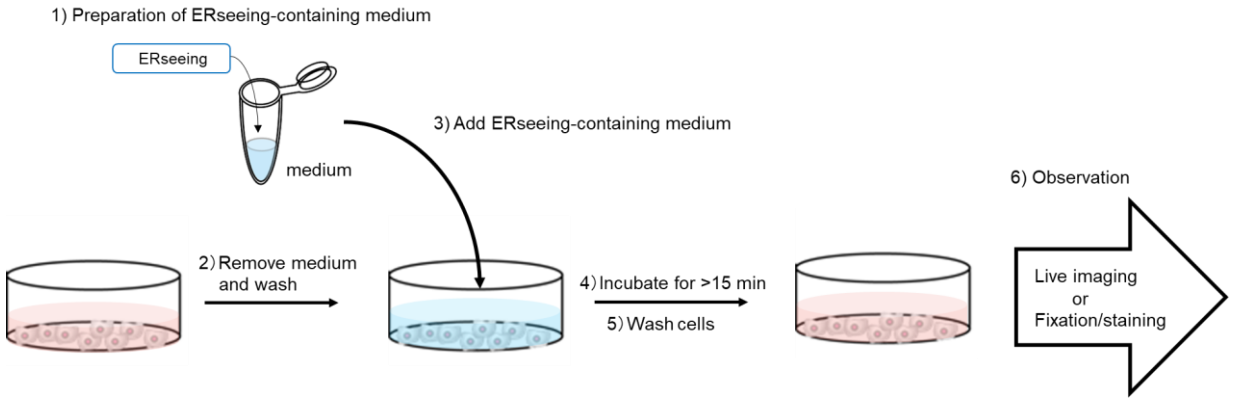
Reconstitution and Storage

Reconstitution: stock solution recommended concentration 0.1 mM to 1 mM in 100% DMSO.
Storage :
Store powder at -20°C.
After reconstitution in DMSO, aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles.
Protect from light.

How to use

General procedure of nucleus imaging

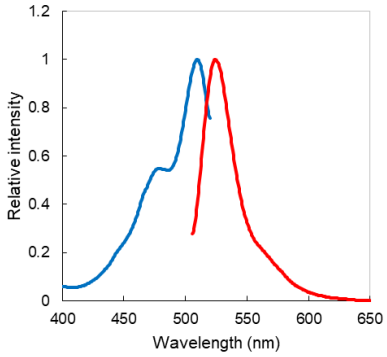
1. Prepare 0.1-1 μM ERseeing in serum-free medium.
   NOTE: Highly recommend starting with 0.1 μM ERseeing, higher concentrations such as 1 μM reagent may show non-specific staining. Empirically optimize and determine the concentration of ERseeing for your experiments.
2. Remove culture medium and wash cells PBS several times
3. Add ERseeing-containing medium to cells.
4. Incubate cells at 37°C for over 15 min.
   NOTE: Staining efficiency depends on incubation time. If you need to observe cells without washout step, 15 min staining is recommended. If you would like to observe stained cells after washout, 1-hour staining recommended.
5. Wash cells with PBS or medium (Optional).
6. Observe cells under live condition or after fixation by 4% PFA and methanol.



Reference data

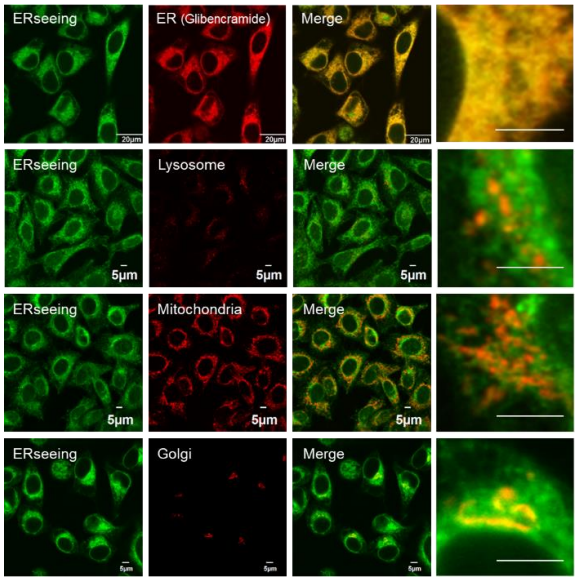
Absorption and fluorescent spectrum of ERseeing

Excitation (blue) and fluorescent (red) spectrum. Ex_{max}/Em_{max} = 509/524 nm. Commercial FITC filter sets are compatible.



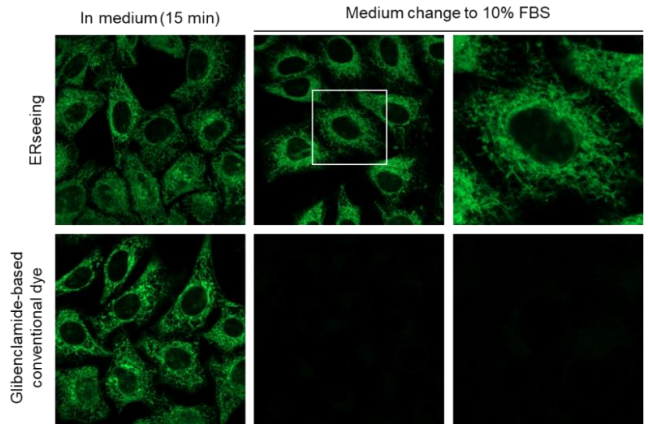
ER specificity

HeLa cells were stained with ERseeing (100 nM) and organelle markers, Glibencramide-type ER staining, lysosomal staining, mitochondrial staining, and Golgi apparatus staining. ERseeing was highly overlapped with conventional Glibencramide-type ER staining (Piason coefficiency >0.9) but not correlated with lysosome marker or mitochondria marker. Only a small portion of staining by ERseeing was overlapped with Golgi apparatus staining. It was considered that this is attributed to the vesicle transport of ERseeing or ERseeing labeling proteins from ER to Golgi apparatus. The ER-to-Golgi trafficking inhibitor decreased overlap between ERseeing-staining and the Golgi apparatus-staining (Detail information is described in Ref. 1).



Comparison ERseeing between conventional dye

HeLa cells were treated with ERseeing or Glibenclamide-based dye for 15 min and observed without washout (Left). Both reagents show ER staining. After that, cells were washed by PBS, added fresh media containing 10% FBS and observed again. While the glibenclamide-based dye showed a very weak signal from the cells, ERseeing maintains a good signal from ER. ERseeing is suitable for long-term imaging after medium changes.



Reference

1. Fujisawa *et al., J. Am. Chem. Soc.*, **140**, 17060-17070 (2018) Chemical Profiling of the Endoplasmic Reticulum Proteome Using Designer Labeling Reagents.

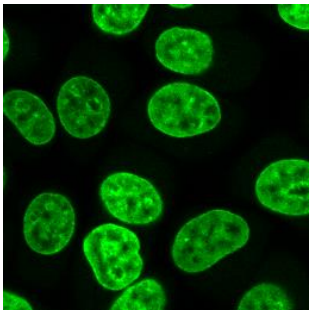
Related products

NucleoSeeing <Live Nucleus Green>

NucleoSeeing is DNA-responsive green dye for monitoring cell nucleus in live cells. As it shows low cytotoxicity and phototoxicity, it is very suitable for long-term live imaging of cell nucleus.

Catalog No. FDV-0029
Size 0.1 mg
Features

- Easy and quick procedure
- Compatible with 10% FBS
- Validated for both adherent cells and floating cells
- Little influence on cellular functions
- Ex/Em: 488 nm/520 nm (commercial FITC filters are available)

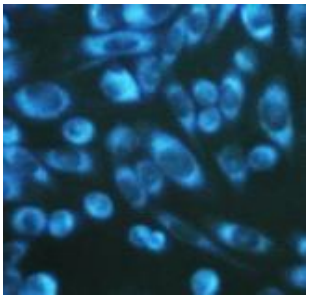


CytoSeeing <Reversible Cytoplasm Blue>

CytoSeeing is a reversible blue cytoplasm-staining dye for monitoring cell morphology. It allows to observe cell structure and to reuse the cells after removing dyes.

Catalog No. FDV-0017
Size 1 mg
Features

- Easy and quick staining less than 10 min
- Washable, reversible staining
- Validated for both adherent cells and floating cells
- Little influence on cellular functions
- Ex/Em: 345 nm/456 nm



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