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FRONTIERS IN LIFE SCIENCE

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NucleoSeeing™ <Live Nucleus Green>

Catalog NO. FDV-0029

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

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②QRコードより

Product Background

Cell nucleus is one of the most important organelle which stores DNA and chromatin complex in eukaryotic cells and play important roles in various biological functions such as gene expression and epigenetic modulation. To monitor dynamics of cell nucleus in live cells, many cell nucleus-specific dyes have been developed. Hoechst33342 dye is one of the most famous dyes and is used for various applications because of its membrane permeable property. However, Hoechst33342 requires UV excitation (~350 nm) and has blue fluorescent property (~450 nm). As UV excitation shows strong photo-toxicity for cells, Hoechst33342 is not suitable for monitoring nucleus dynamics on live cells. Several visible-light excitable dyes for nucleus imaging on live cells had been commercially available, however, they have some disadvantages such as DNA/RNA specificity, or cell toxicity.

NucleoSeeing™ is a novel type of nucleus imaging probe in live cells (Ref.1). This probe has a unique chemical structure containing diacetyl-fluorescein and Hoechst33342 as DNA-binding tag linked by the optimal PEG linker. Although NucleoSeeing™ has two fluorophores, fluorescence of this probe is strongly quenched under no DNA conditions. Only in the presence of double stranded DNAs, it binds to DNA and emit fluorescence excited at 488 nm and detected at 520 nm (Figure 1). Based on this photoproperty, NucleoSeeing™ allows to monitor cell nucleus with highly S/N value against cytosol or other organelles. Furthermore, this probe specifically interacts with DNA same as Hoechst dyes. Compared with Hoechst33342, NucleoSeeing™ has advantages which are not only lower photo-toxicity, but also lower compound's cellular toxicity. Staining of various mammalian cultured cell lines and cultured mouse slice brain tissue were validated. Owing to its low photo- and cytotoxicity, long-term time lapse imaging (~20 hours) of nucleus and chromatin dynamics is well succeeded. Not only mammalian cells, but also some plant cells were tested, such as *Arabidopsis thaliana* Guard cells and epidermal cells (Ref.2). Although plant cells show autofluorescence derived from chloroplasts, NucleoSeeing™ could separate nucleus signal from autofluorescence signal from chloroplasts.

In addition to nucleus-specific imaging, NucleoSeeing™ can be applied for nucleus-specific pH sensing. A principle is based on pH-dependent fluorescent properties of fluorescein and Hoechst33342, components of NucleoSeeing™. When this probe is excited at 405 nm, values of fluorescent ratio ($F_{Fluorescein}/F_{Hoechst}$) depend on pH during pH5.5-8.5. This product is world-first commercially available nucleus-specific pH sensing reagent. Please find the "Appendix" for pH sensing.

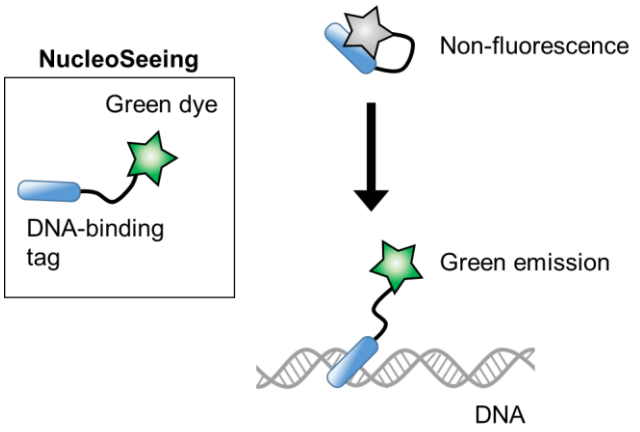


Figure 1. Overview of NucleoSeeing™

Description

Catalog Number : FDV-0029
Size : 0.1 mg
Molecular weight : 1425 g/mol as tri-TFA salt
Solubility : Soluble in DMSO
Fluorophore : Fluorescein (green fluorescent dye)
Ex/Em: 488 nm/520 nm
*FITC filter sets are available.

Application

- Live cell nucleus imaging of cultured cells, cultured tissues
- Live cell nucleus imaging of plant cells
- Cell nucleus staining of fixed cells
- Cell nucleus-specific pH sensing (See "Appendix")

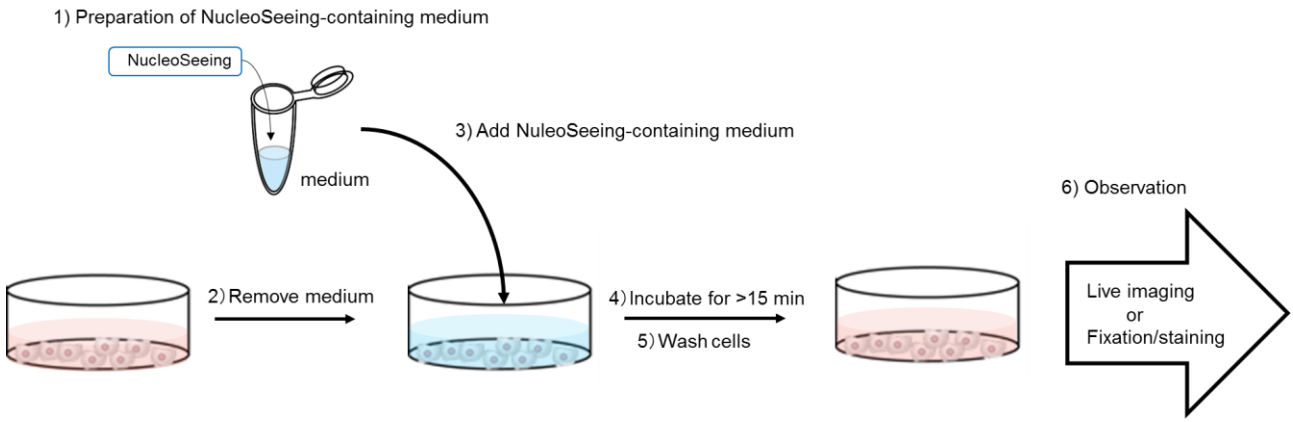
Reconstitution and Storage

Reconstitution : stock solution in 100% DMSO.
Storage (solution) :
Store powder at -20ºC.
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

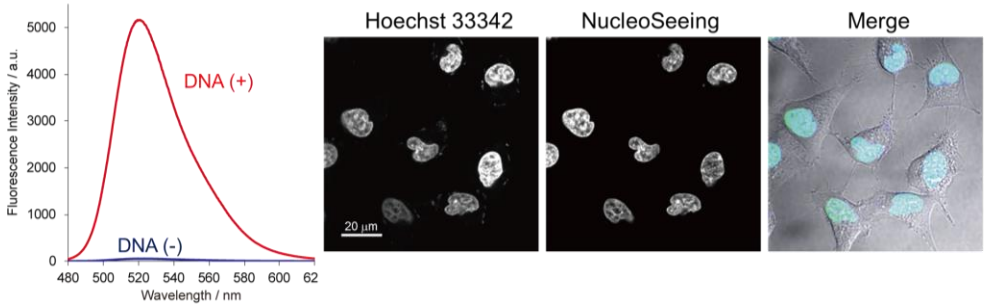
1. Prepare 1-20 μM NucleoSeeing™ in fresh medium
   NOTE: Please optimize concentration of reagent for your experiments. NucleoSeeing™ is compatible with FBS-containing medium. When the user NucleoSeeing™ in FBS-containing medium, relatively higher concentration (>5 μM) of NucleoSeeing™ is recommended. Detail is described in "**Application data**".
2. Remove culture medium
3. Add NucleoSeeing™ -containing medium to cells
4. Incubate cells at 37ºC for over 15 min
5. Wash cells by PBS or medium (Option)
   NOTE: For short-term observation, reversible staining or high sensitive detection, wash out is recommended. On the other hand, for long-term observation, no wash out/medium change is highly recommended.
6. Observe cells under live condition or after fixation by 4% PFA and methanol



Application data

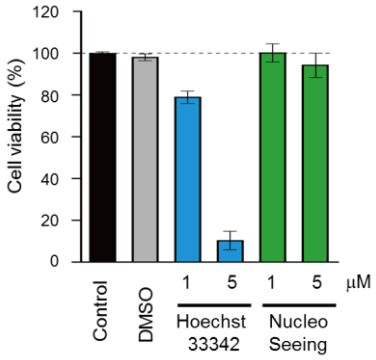
Basic information of NucleoSeeing™

Left: Fluorescent spectrum excited at 460 nm in the presence and absence of synthetic DNA. NucleoSeeing™ shows strong green emission only in the presence of DNA.
Light : Fluorescent imaging by conventional Hoechst33342 and NucleoSeeing™. NucleoSeeing™ is well overlapped with DNA-specific dye Hoechst33342.



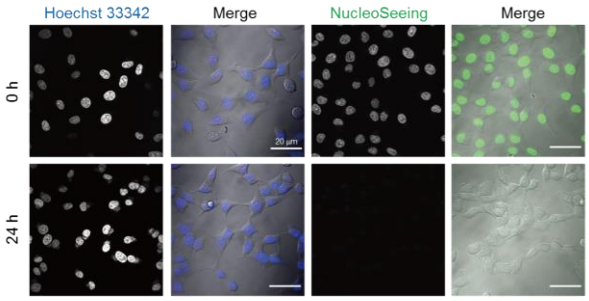
Cell toxicity

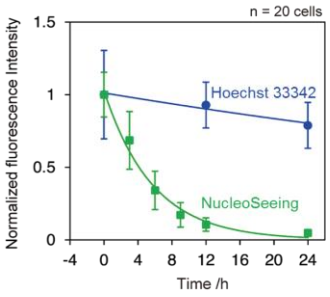
Cell toxicities of Hoechst33342 and NucleoSeeing™ were tested by the MTT assay. While Hoechst shows potent cell toxicity dose-dependent manner, NucleoSeeing™ keeps cell viability at least 5 μM.



Reversible staining of NucleoSeeing™

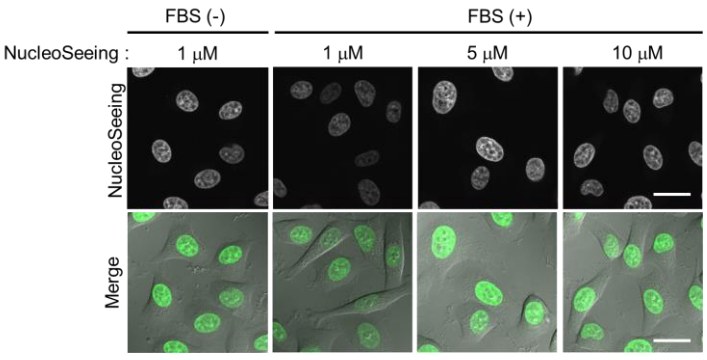
After HeLa cells were treated 1 μM Hoechst33342 and NucleoSeeing™ for 15 min, cells were washed by PBS and cultured for 24 hours. While over 80% of blue signal of Hoechst33342 was still remained, green signals of NucleoSeeing™ were dramatically reduced within 12 hours. NucleoSeeing™ can be used for reversible nucleus staining.





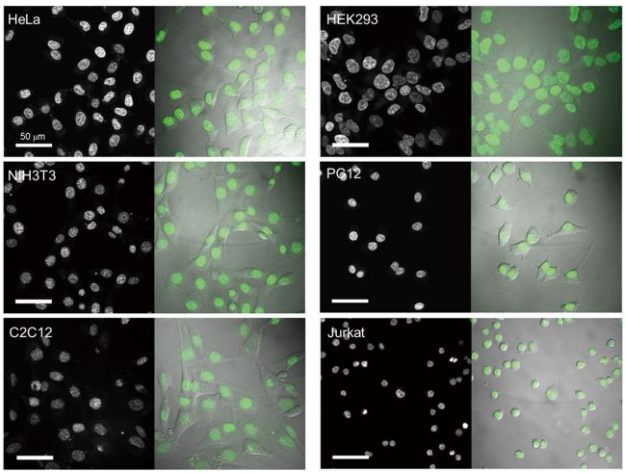
Influence of FBS on NucleoSeeing™ staining

To check any influence of FBS on nucleus staining by NucleoSeeing™, HeLa cells were treated 1 μM NucleoSeeing™ in serum-free medium or 1, 5, 10 μM NucleoSeeing™ in 10% FBS-containing media for 15 min. Images were acquired by confocal microscopy (Ex. 488/ Em. 500-600 nm) in the same condition (laser power and detection sensitivity). Compared with 1 μM in serum-free condition, nucleus fluorescent intensity stained by 1 μM with 10% FBS was partially reduced. Higher concentration such as 5 μM or 10 μM of NucleoSeeing™ in FBS condition recovered fluorescent intensity.



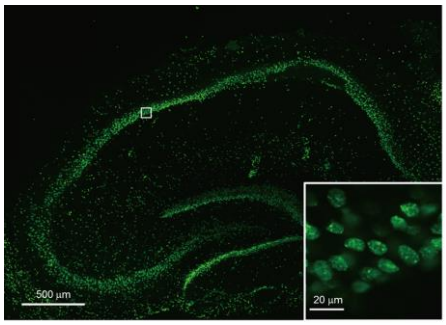
Staining of various mammalian cells

Six types of mammalian cell lines were treated with 1 µM NucleoSeeing™ for 15 min and observed in live cell condition.



Staining of cultured slice tissue

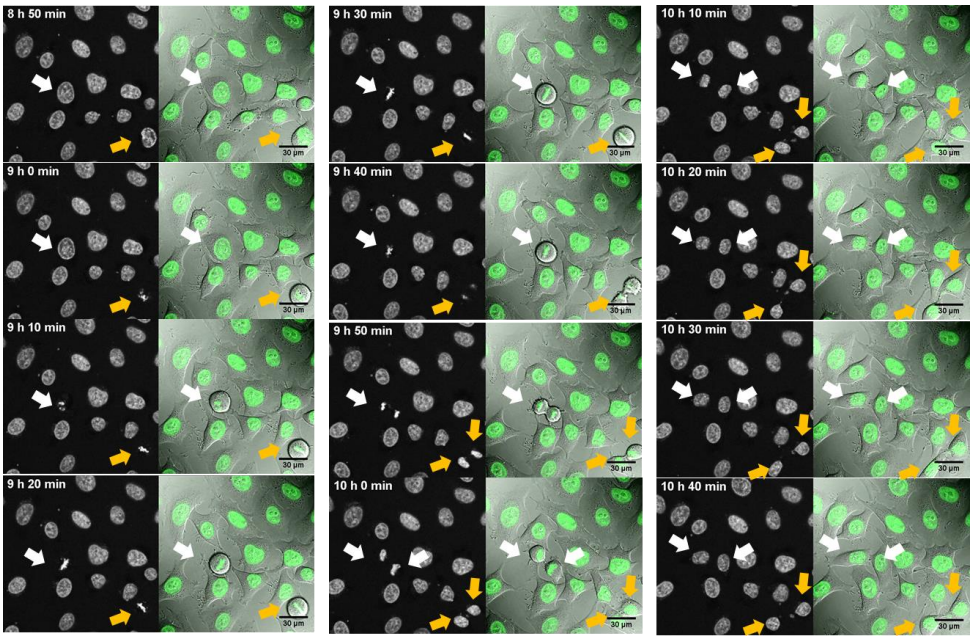
Cultured mouse hippocampal brain tissue was treated with 20 µM NucleoSeeing™ for 15 min and observed in non-fixed condition.



Long-term time lapse imaging of mitotic cells

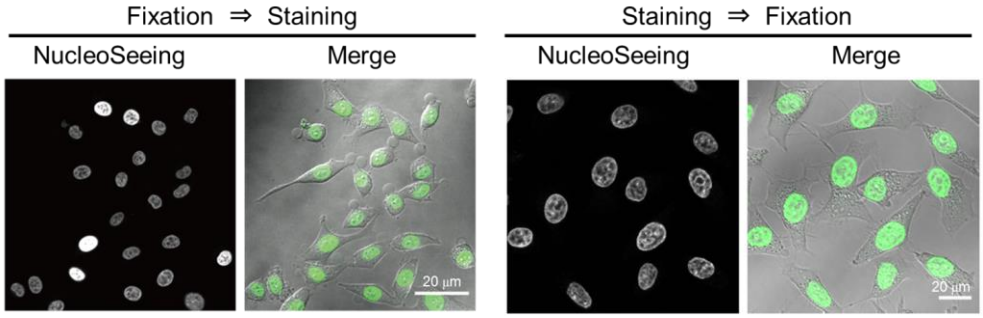
HeLa cells on the glass bottom dish were treated with 0.5 µM NucleoSeeing™ in 10% FBS-containing DMEM for 1.5 hours. After treatment of NucleoSeeing™, cells were observed for 20 hours by confocal microscopy without any washout or medium change (Time lapse condition: Ex 488/Em 500-600 nm, 60x oil lens, 10 min interval). Several mitotic cells were observed (white and orange arrow indicated cells in the mitotic process).

*Movie data is available on our website.



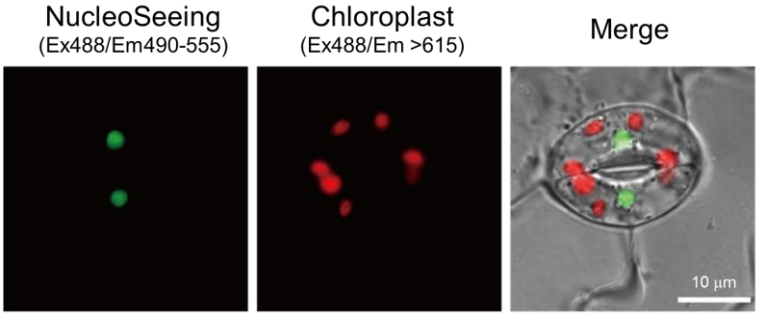
Staining of fixed cells

Left: Staining after fixation. PFA-fixed HeLa cells were treated by 1 μM NucleoSeeing™ for 15 min, washed by PBS and observed (Ex. 488 nm/ Em 520 nm).
Right: Fixation after staining. HeLa cells were treated by 5 μM NucleoSeeing™ for 15 min in live cell condition. After wash the cells, the cells were fixed with 4% PFA. The cells were observed (Ex. 488 nm/ Em 520 nm).
NOTE: MeOH fixation is also validated but signal may be decreased before MeOH fixation.



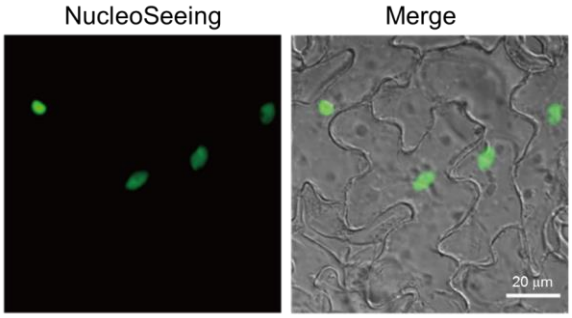
Staining of *Arabidopsis thaliana* Guard cells

Arabidopsis thaliana leaves were treated with 20 μM NucleoSeeing™ in 10 mM MES-KOH, 50 mM KCl (pH 6.2) buffer for 60 min and observed in living condition (Ex. 488 nm and Em. 490-555 nm for NucleoSeeing™, Em. >615 nm for chloroplast as autofluorescence). Green signals from NucleoSeeing™ were clearly separated from plant autofluorescence derived from chloroplast.



Staining of *Arabidopsis thaliana* epidermal cells

Arabidopsis thaliana leaves were treated with 20 μM NucleoSeeing™ in 10 mM MES-KOH, 50 mM KCl (pH 6.2) buffer for 60 min and observed in living condition. Ex. 488 nm and Em. 490-555 nm for NucleoSeeing™.



Appendix : Nucleus-specific pH sensing

Ref.3 showed NucleoSeeing™ can be used in nucleus-specific pH sensing in live cells. As cell nucleus is separated from cytosol by nuclear membrane with unique double lipid-bilayers, it is considered that the physical environment of nucleus is different from cytosol. Some evidences suggested that nuclear-specific proton pumps (H^+-ATPases) regulate intra-nuclear pH condition and may play important roles in nuclear physiology. However, research tools for monitoring nucleus pH sensing are limited.

Nucleus imaging by NucleoSeeing™, Ex. 488 nm/ Em. 520 nm is comfortable. In the case of nucleus-specific pH sensing, excitation at 405 nm and double detection at 520 nm and at 460 nm should be used. The ratio of fluorescent intensity (F_{520}/F_{460}) depends on pH (Figure A1). To monitor nucleus pH, cell-based control experiments with different pH buffer containing nigericin, a representative K^+/H^+-ionophore, required (Figure A2). Calibration curves should be obtained every experiments.

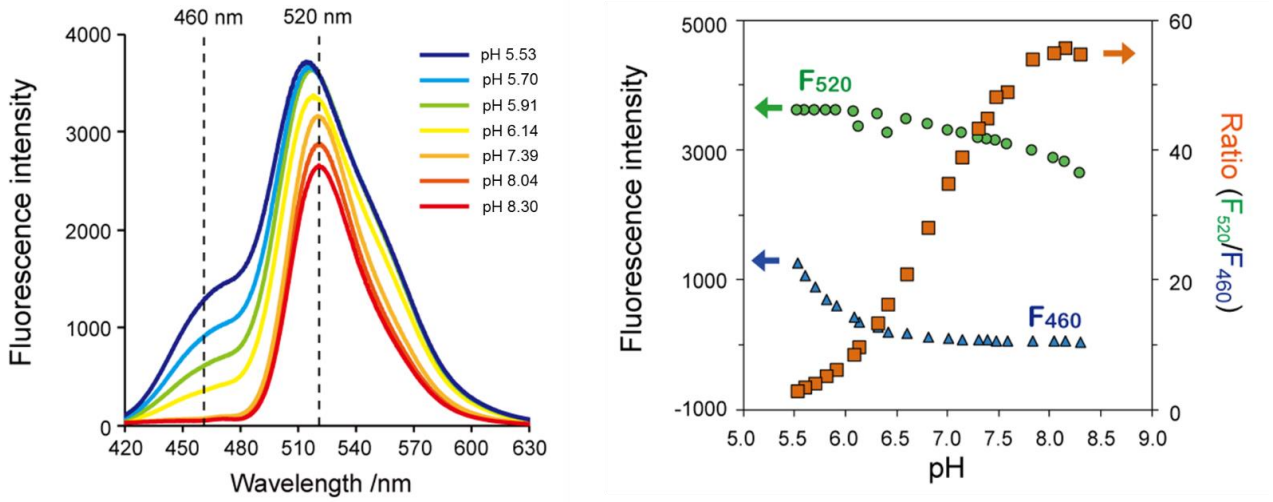


Figure A1 pH-dependent fluorescent profile

Left: Fluorescence spectral changes of synthetic intracellular form of NucleoSeeing™ (see "NOTE" below) with synthetic DNA upon pH change measured in 50 mM MOPS, 100 mM NaCl. Excitation at 405 nm.

Right: pH-dependent changes in the fluorescence intensity at 460 nm (F_{460}) and 520 nm (F_{520}), and the ratio (F_{520}/F_{460})

NOTE : NucleoSeeing™ containing hoechst33342-diacetylfluorescein is highly optimized for live cell imaging. It enters into cells and is rapidly deacetylated to hoechst33342-fluorescein by intracellular esterases. So, the deacetyl-form of NucleoSeeing™ is active probe for DNA staining. Figure A1 was obtained from "chemically synthesized deacetyl-form of NucleoSeeing™". Please note this data is not reproduced by using our NucleoSeeing™ itself *in vitro*.

Additionally required reagents

- nigericin (a representative K^+/H^+ ionophore)
- pH standard buffers (ex. 10 mM MOPS, 130 mM KCl, 10 mM NaCl, 1 mM $MgSO_4$) with pH range 5.5-8.5

How to use

1. Prepare pH-standard buffers for calibration curve (10 mM MOPS, 130 mM KCl, 10 mM NaCl, 1 mM $MgSO_4$) at pH 5.5-8.5 and 10 μg/ml nigericin -containing pH-standard buffers.
2. Cells were incubated with NucleoSeeing™ (1-10 μM) for 15 min
3. After wash cells twice with pH-standard buffers of the indicated pH, the cells were incubated with 10 μg/ml nigericin -containing pH-standard buffers at 37°C for 30 min
4. The cells were observed at Ex 405nm and Em 520 nm and 460 nm.

Data example

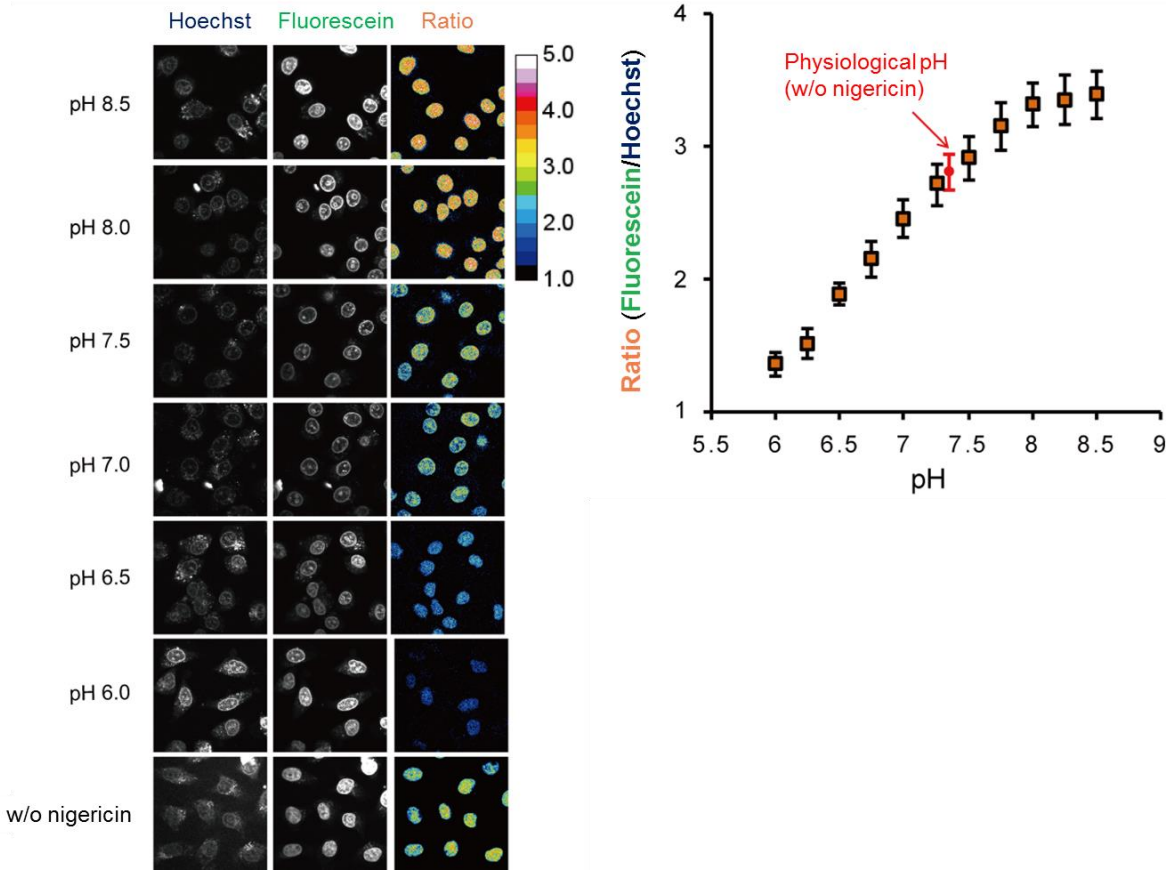


Figure A2 *in cellulo* ratiometric imaging

Cells were incubated with 5 μM NucleoSeeing™ for 15 min, washed and observed after nigericin-treatment at the indicated pH or directly as intact cells. Excitation at 405 nm and detection at 430-510 nm as Hoechst filter and 520-620 nm as Fluorescein filter.

Reference

1. Nakamura *et al., Chem. Commun.*, **50**, 6149-6152 (2014) Hoechst tagging: a modular strategy to design synthetic fluorescent probes for live-cell nucleus imaging.
2. Ueda *et al., ACS Cent. Sci.*, **3**, 462-472 (2017) Noncanonical function of a small-molecular virulence factor coronatine against plant immunity: an in vivo raman imaging approach.
3. Nakamura and Tsukiji, *Bioorg. Med. Chem. Lett.*, **27**, 3127-3130 (2017) Ratiometric fluorescence imaging of nuclear pH in living cells using Hoechst-tagged fluorescein.

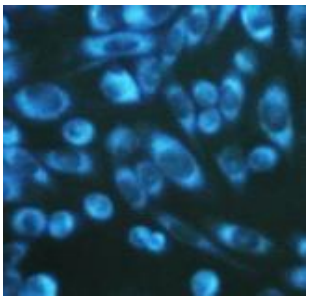
Related products

CytoSeeing™ <Reversible Cytoplasm Blue>

CytoSeeing™ is a blue and reversible cytoplasm-staining dye for monitoring cell morphology. It allow 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 (commercial DAPI filters are available)

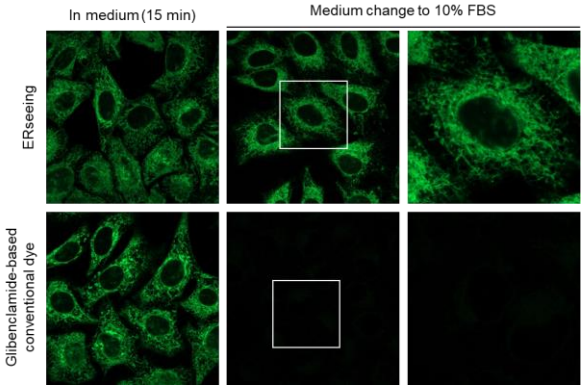


ERseeing™ <Endoplasmic reticulum Green>

ERseeing™ is a novel type of ER-staining dye and shows little pharmacological effects compared with conventional glibenclamide-based ER dyes. ERseeing™ is irreversible staining and is compatible with medium change for long-term imaging.

Catalog No. FDV-0038
Size 10 nmol
Features

- Recommended Ex/Em: 509 nm/524 nm
- Lless pharmacological effect on ER proteins
- Suitable for long-term live cell imaging



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