funakoshi
FRONTIERS IN LIFE SCIENCE

9-7 Hongo 2-Chome, Bunkyo-Ku
Tokyo 113-0033, Japan

PREX710-NHS <Super-Photostable Dye>

Catalog NO. FDV-0036

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

Product Background

Near infrared (NIR) dyes absorb and emit light in 700-900 nm, the out of visible light range. As the range of NIR shows low auto-fluorescence from biological samples, NIR dyes are one of the most suitable fluorophore for biological imaging, especially *in vivo* imaging. A lot of NIR dyes were previously developed and some dyes are commercially available. However, almost NIR dyes are based on the cyanine-type dye which contains hydrophobic long polymethine structures and show poor chemical and photo-stability. Generally, cyanine-type NIR dyes are tend to decompose in the water rapidly and considered as not enough dyes for bio-imaging. A novel NIR dye, **PREX710** (**P**hoto-**Re**sistant **X**anthene Dye **710** nm) is different from conventional cyanine-based NIR dyes because its backbone is a xanthene containing phosphine oxide. PREX710 could be excited by around 640-750 nm (max ~710 nm) and emit around 700-800 nm (max ~760 nm). PREX710 shows very highly chemical stability in both water and serum and also exhibits strong photostability. Combining two advantages of PREX710 enables us to monitor *in vivo* imaging for long-term, single molecule imaging, multiplex imaging with visible light fluorophores. PREX710 has been validated in *in vivo* imaging such as a deep blood vessel imaging in mouse brain, plant imaging omitting autofluorescence of chloroplasts, and stable single molecule imaging. PREX710-NHS (*N*-hydroxysuccinimide) ester is a reactive form of PREX710 dye suitable for the labeling of amino group-containing molecules such as proteins, antibodies, peptides, amino-nucleotides and any other small compounds. PREX710-conjugates can be applied in various NIR imaging with highly chemical stability and super-photostability.

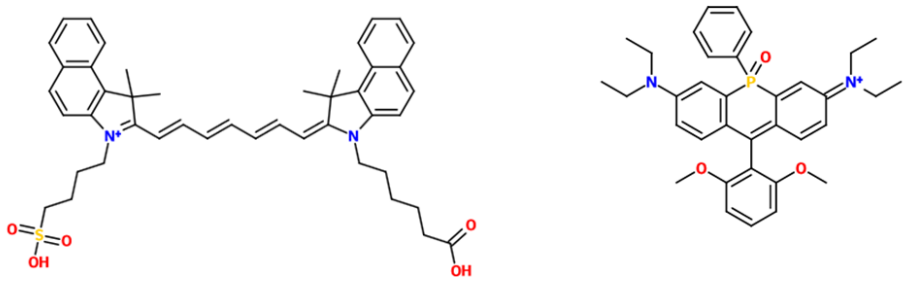
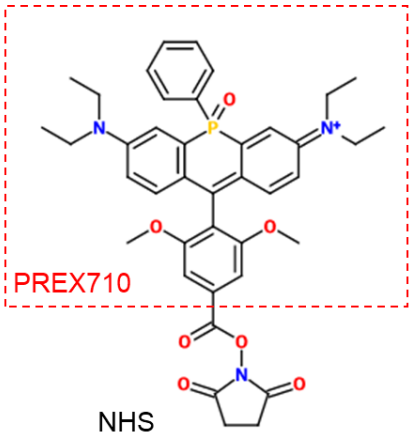


Figure 1 Cyanine-based NIR dyes and PREX710 NIR dye

Description

Catalog Number: FDV-0036
Size : 1 mg
Formulation : $C_{40}H_{43}ClN_3O_7P$ as chloride salt
Visual aspect : Dark green
Structure : See right
Molecular weight : 744.2 g/mol
Solubility : Stably soluble in DMSO, DMF
NOTE: Water solubility is good but NHS is rapidly hydrolyzed in water.
Please refer **"How to use"** later.
Ex/Em: 710 / 740 nm (Please find the spectrum in **"Reference data"**)



Application

- *in vivo* imaging
- single molecule imaging
- plant imaging omitting autofluorescence of chloroplasts
- multi color imaging with visible light fluorophore

Storage

Store powder at -20ºC.

How to use

General procedure of amino group-specific labeling

The vial contains 1 mg of PREX710-NHS with argon gas for protection of NHS ester group. As NHS ester may not be stable in atmosphere and water, once opening the vial please use it immediately. **Single-use is highly recommended. Do not guarantee the labeling activity of frozen stock solution.**

1. Just before use, dissolve 1 mg of PREX710-NHS in 100 µL of DMSO or DMF to prepare 10 mg/ml solution (hereafter called **Dye Soln.**).
   NOTE: NHS ester is easily hydrolyzed in the water. Dissolving PREX710 in water is **NOT recommended**. Anhydrous grade and amino-free of DMSO and DMF is highly recommended to keep the labeling activity of NHS ester.

2. Dissolve amine-containing biomolecules of interest in 0.1 M sodium bicarbonate buffer ($NaHCO_3/Na_2CO_3$, pH 8.2). The biomolecule concentration should be usually less than 20 mg/ml.
   NOTE:
   a) Alternative buffer is 0.1 M phosphate buffer (pH 8.2). Please avoid to use amino-group containing buffers such as Tris, glycine, and ammonium ion buffers.
   b) pH is the most important factor for NHS-amine conjugation. In acidic pH (<8.0) amino-groups are protected with protonation and in highly basic pH (>8.5) NHS ester tends to be hydrolyzed into the nonreactive free acid. Please be careful for pH of reaction buffer.

3. Add appropriate volume of **Dye Soln.** to the biomolecule solution, mix well and incubate for at least 1 hour at room temperature.
   NOTE: Amount of dye and biomolecules highly depends on biomolecular structure or labeling number on single biomolecules. Please optimize the amount of dye-NHS for each experiment.

4. Purify the conjugate by appropriate methods.
   NOTE:
   a) For macromolecules, gel filtration or ultrafiltration is commonly used to purify the conjugate.
   b) For small molecules or peptides, chromatography is suitable.

<Reference; Determination of labeling efficiency>
Degree of labeling (DOL) can be estimated by following equation.

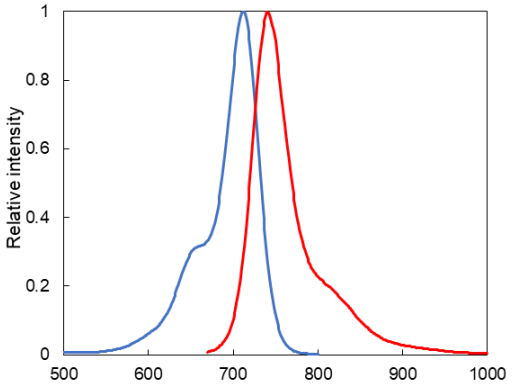
$$DOL = (A_{710} \times \varepsilon_{protein}) / \{(A_{280} - A_{710} \times CF) \times \varepsilon_{710}\}$$

$\varepsilon_{protein}$: molar absorption coefficient of protein (for example 210,000 $M^{-1}cm^{-1}$ for IgG)
ε_{710} : molar absorption coefficient of PREX710 (please use 108,400 $M^{-1}cm^{-1}$)
A_{280} : absorbance of labeled protein at 280 nm
A_{710} : absorbance of labeled protein at 710 nm
CF : correction factor of PREX710 dye. (please use 0.0594)

Reference data

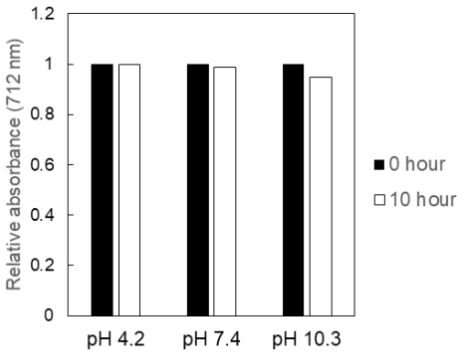
Fluorescent spectrum

UV-Vis and normalized fluorescence spectra of free-PREX710 dye in 10 mM PBS buffer (pH 7.4). Blue; absorption spectra. Red; excitation spectra.



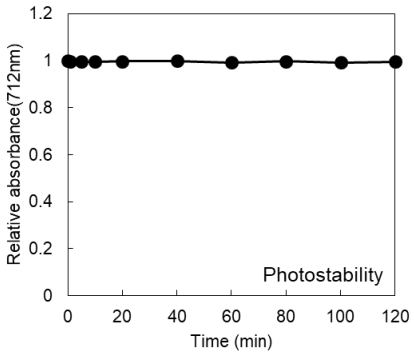
Effect of pH on the stability of PREX710 dye

Normalized absorbance of free-PREX710 dye measured at maximum 712 nm for 10 hours using three different pH buffers *in vitro*. PREX710 is highly stable from pH 4 to 10.



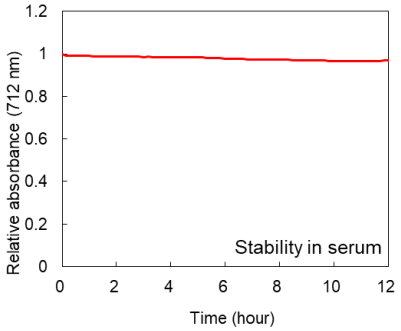
Photostability of PREX710 dye under Xe lamp irradiation

Free-PREX710 in PBS buffer (pH 7.4) were irradiated with high power (300 W) Xe lamp using continuous range of visible light 385-740 nm. The change of absorption spectra over time was monitored.



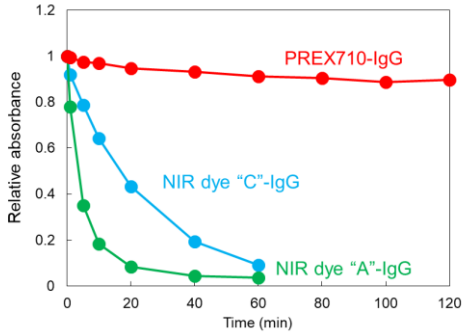
Chemical stability of PREX710 in blood serum

Normalized absorbance of free-PREX710 dye in 100% fetal bovine serum (FBS) was monitored at maximum 712 nm in 10 min intervals for 10 hours *in vitro*.



Photostability of PREX710-conjugated antibody under Xe lamp irradiation

Normalized absorbance of PREX710-, competitive NIR dye "A"- and "C"-conjugated antibodies under irradiation with high power (300 W) Xe lamp was monitored at each maximum wavelength.

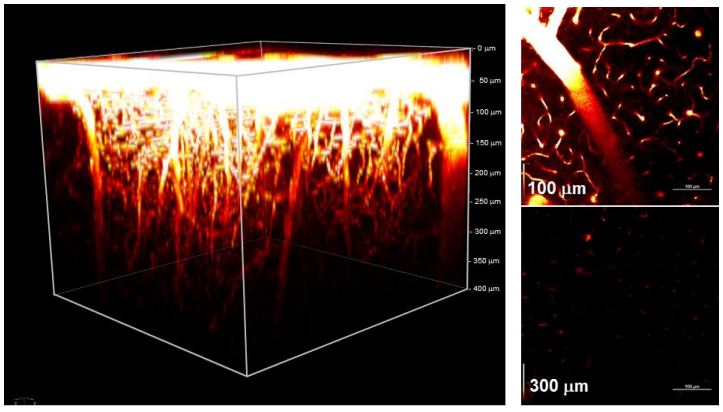


Application data

In vivo deep imaging of blood vessels in live mouse brain

PREX710-dextran conjugate was injected into bloodstream via a tail vein of young adult ICR mouse. The brain tissue was monitored by "openslull" method with confocal microscopy (Ex./Em. = 638 nm/ 667-733 nm). PREX710 was highly stable in blood and could be excited by near-infrared light which has little effect on hemoglobin.

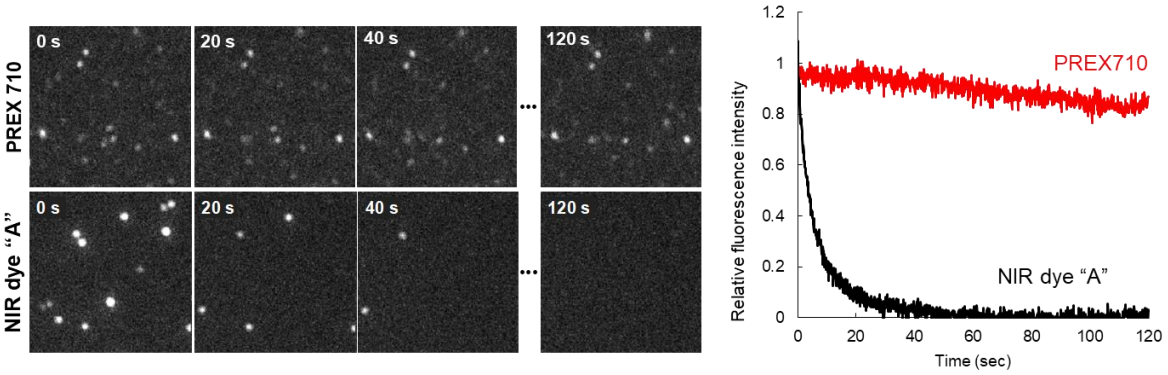
*This data was obtained by Dr. Imamura Lab, Ehime University.



Single molecule imaging

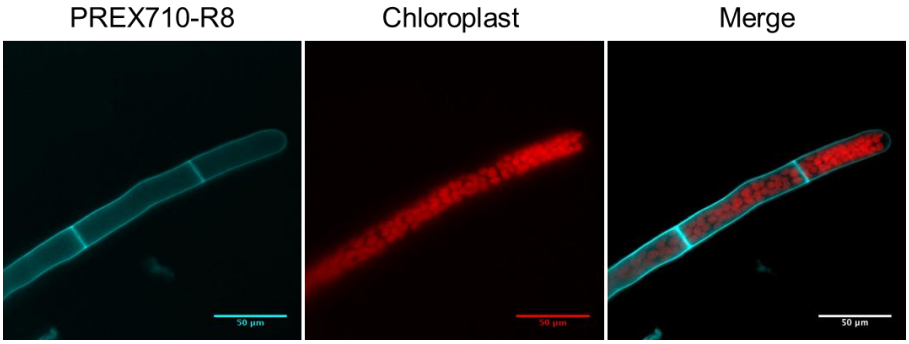
Avidin protein was labeled with PREX710-NHS or cyanine-type NIR dye “A” and subsequently immobilized on a glass slide via biotin-conjugated linker. Single-molecule images of PREX710 and NIR dye “A” were acquired continuously at 10 frames per second for 120 sec with excitation light at 640 nm (60 W/cm^2) in PBS (pH 7.4). PREX710 showed high photostability under the single molecule level compared with NIR dye “A”. PREX710 has a great potential to apply into single-molecular imaging.

*This data was obtained by Dr. Okada Lab, RIKEN.



Plant imaging

Physcomitrella patens was stained with PREX710-conjugated octaarginine (R8) and fluorescent signal was observed by fluorescent microscopy. PREX710 (Ex./Em. = 703-717 nm/754-816 nm), chloroplast (Ex./Em. = 300-400 nm/>420 nm). As fluorescent signal of PREX710 was clearly separated from chloroplast’s autofluorescence, PREX710 is a good fluorescent dye for plant imaging.



Reference

1. Grzybowski M., *et al*., *Angew. Chem. Int. Ed.*, **57**, 10137-10141 “A Highly Photostable Near-Infrared Labeling Agent Based on a Phospha-rhodamine for Long-Term and Deep Imaging”

Disclaimer/免責事項

This product has been commercialized by Funakoshi Co., Ltd. based on the results of academic research, and the advertisement text, figures and manuals (hereinafter “Product information”) have been prepared based on published research reports on March, 2020. The academic interpretation at the time of creation of the Product Information may change in accordance with future developments in the relevant research field and expansion of various scientific findings, and the latest version and certainty of the Product Information are not guaranteed. The specifications of this product and the Product Information are subject to change without notice. Please contact us for the latest information.

本商品は学術研究成果を基にフナコシ株式会社が商品化したもので、2020 年 3 月時点における公開研究報告を基に広告文章およびマニュアル（以下、商品資料）を作成しています。今後の当該研究分野の発展および各種学術知見の拡大にともない、商品資料作成時の学術的解釈が変更になる可能性があり、最新性・確実性を保証するものではありません。また、本商品の仕様および商品資料を予告なく変更する場合がございます。最新の情報に関しましては、弊社までご確認いただきますようお願い申し上げます。

funakoshi
FRONTIERS IN LIFE SCIENCE
URL: http://funakoshi.co.jp
9-7 Hongo 2-Chome, Bunkyo-ku, Tokyo 113-0033