funakoshi
FRONTIERS IN LIFE SCIENCE

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

Controllable NOdonor <NO-Rosa5>

Catalog NO. FDV-0032

Research use only, not for human or animal therapeutic or diagnostic use.
This product has been commercialized with the support of Nagoya City University.

Product Background

Nitric oxide (NO), a gaseous free radical, is one of the signaling molecules produced by NO synthases physiologically. NO plays a key role in a wide range of biological processes, such as vasodilation, neurotransmission, platelet adhesion, inflammation. NO is extremely unstable in physiological pH and temperature and easily oxidized to nitrite and nitrate. Based on its instability in cells, NO only works very limited space (~100 μm) from its origin and half time ($t_{1/2}$) is estimated as only a few seconds. Although its biological importance, it is difficult to handle NO molecule in biological experiments. To investigate the effects of NO on various biological phenomena, synthetic "NO donors", which are compounds releasing NO molecules, have been used. Many kind of NO donors including organic nitrates/nitrites, NO-coordinated metal ions, *N*-diazeniumdiolates (also called NONOates) and *S*-nitrosothiols, have been discovered and contributed to elucidating biological effects of NO molecule. However, these NO donors generate NO molecules continuously over a period of time and homogenously in the experimental buffer. It is hard to control NO releasing spatio-temporally and to reproduce physiological role of NO molecules which act as signaling molecule in very limited space (~100 μm) and period (a few seconds). To overcome this point, recently photo-controllable NO donors, are expected as valuable tools for spatial-temporal control of NO releasing. Although several UV-controllable NO donors have been developed, these compounds are not suitable to biological experiments due to its UV photo-toxicity. Visible light-controllable NO donors are desired for biological applications.

Controllable NOdonor (original name; NO-Rosa5) is a novel yellowish-green light-controllable NO donor originally developed by Drs. Hidehiko Nakagawa and Naoya Ieda, Nagoya City University. Controllable NOdonor has two moieties, rosamine fluorescent dye as light-harvesting group and *N*-nitrosoaminophenol structure as an NO-releasing moiety, and releases NO molecules triggered by photoinduced electron transfer (PeT). It validated low photo-toxicity, low cellular toxicity, and efficient NO releasing by yellowish-green light irradiation. Ref. 2 reported its biological applications including *in cellullo* NO-releasing assay and *ex vivo* vasodilation assay.

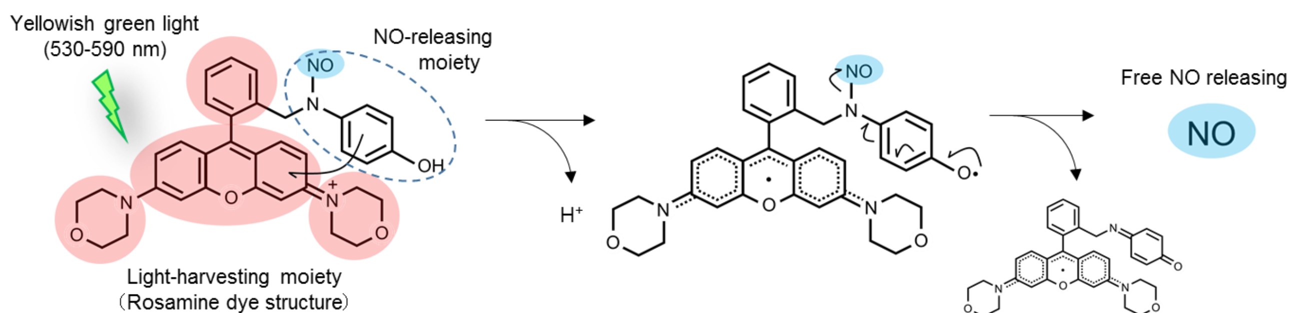


Figure 1. Overview of light-induced NO releasing of Controllable NOdonor

Description

Catalog Number: FDV-0032
Size : 0.25 mg
Formulation : $C_{34}H_{33}N_4O_5Cl$
Molecular weight : 613.11 g/mol as a mono chloride salt
Solubility : Soluble in DMSO

Application

- Spatio-temporal control of NO molecules in cultured cells and tissues
  Light sources: Xenon light source with equipped with a 530-590 nm band-pass filter
  Validated equipment MAX303 (Asahi Spectra)
  He-Ne 543 nm laser in confocal microscopy

Reconstitution and Storage

Reconstitution : Add 100% DMSO into vial to prepare 1-10 mM stock solution.
Storage :
Powder : Store at -20°C.
Stock solution :
- Make aliquots and store at -20 °C with protecting from light.
- Avoid repeated freeze-thaw cycles.
- To avoid photo-induced decomposition, one-time use of each aliquot is highly recommended.

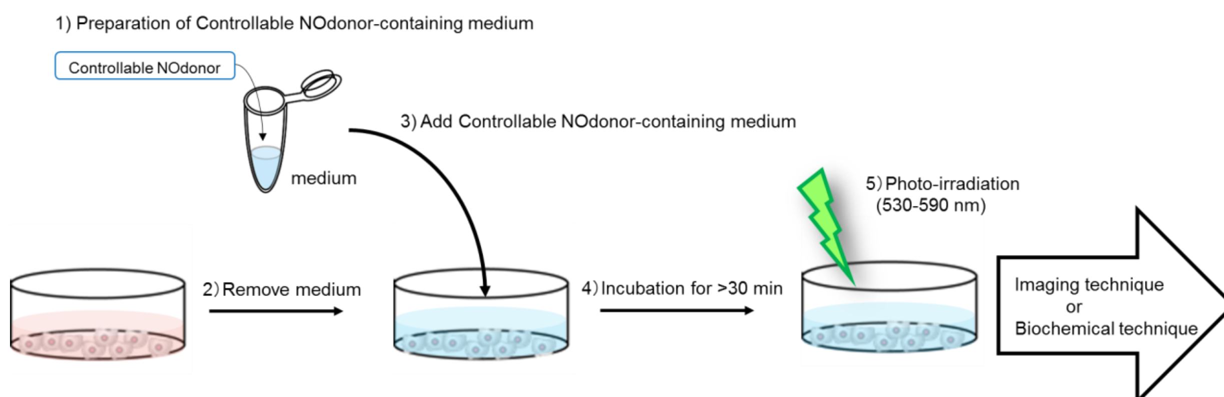
<NOTE> Controllable NOdonor may be decomposed by environmental light such as room light or sunlight. When making and storing aliquots, please carefully check light condition around the experimental space.

How to use

NO release in cultured cells

1. Add Controllable NOdonor (final. 1-10 μM) in fresh media
2. Replace the cultured medium to ControllableNOdonor containing medium
3. Culture cells for at least 30 min
4. Photo-irradiate culture dish by any light sources such as 543 nm laser of confocal microscopy or xenon lamp

*NOTE(1): Photo-irradiation condition should be optimized by users for each experiments.
*NOTE(2): All procedures are recommended in the dark condition to avoid non-specific NO release from Controllable NOdonor by environmental light source.

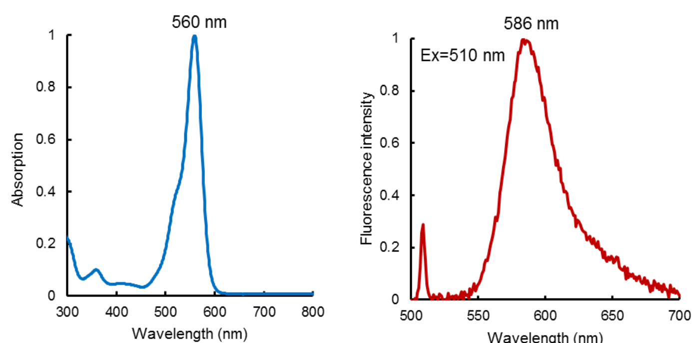


Experimental guide

Absorption and fluorescent spectrum

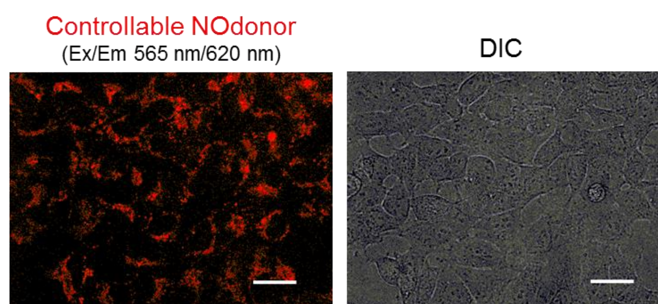
Left: Absorption spectra. 500-600 nm wavelength is available for NO-releasing. (λ_{max}~560 nm)

Right : Fluorescence spectra exited at 510 nm. Controllable NOdonor emits red fluorescence.



Fluorescent imaging of Controllable NOdonor

Red fluorescence (Ex.565 nm/ Em. 620 nm) of NO-Rasa5 was detected by confocal microscopy.



Selection guide of compatible fluorophores

Due to Controllable NOdonor releasing NO molecule upon 500-600 nm photo-irradiation, fluorophores, including fluorescent dyes, fluorescent reagents, and fluorescent proteins which are excitable by 500-600 nm wavelength are not available.

Furthermore, Controllable NOdonor itself shows red fluorescent emission. When customers use any fluorophores with Controllable NOdonor, please refer to following information.

(1) Not recommended fluorophores

- Excitation with 500-600 nm such as red fluorophores (for example Cy3, TAMRA, RFP protein etc.)

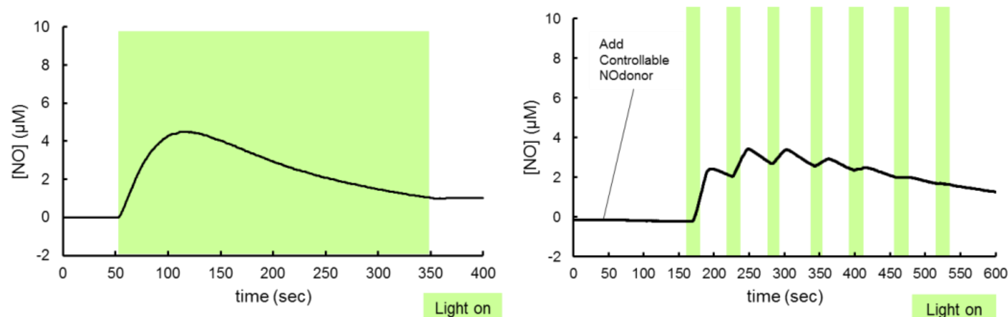
(2) Available fluorophores

- Excitation with <500 nm such as blue fluorophores (for example coumarin dye, BFP protein etc.) or green fluorophores (for example fluorescein, GFP protein etc.)
- Excitation with >600 nm such as near infrared red or far red fluorophores (for example Cy5, Cy7 etc.)

Reference data

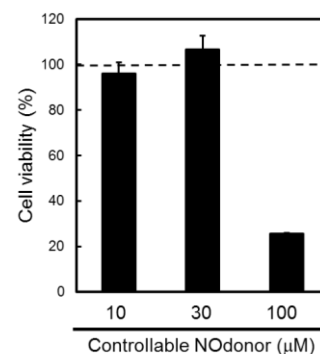
Visible light-induced NO releasing *in vitro*

Quantitative analysis of NO release from Controllable NOdonor with NO electrode. Controllable NOdonor (final 10 μM) in 100 mM HEPES buffer was irradiated with a MAX-303 (Asahi Spectra) equipped with a 530-590 nm band-pass filter (light intensity: 70 mW/cm^2) . Left: Continuous irradiation. Right: Short pulse irradiation.



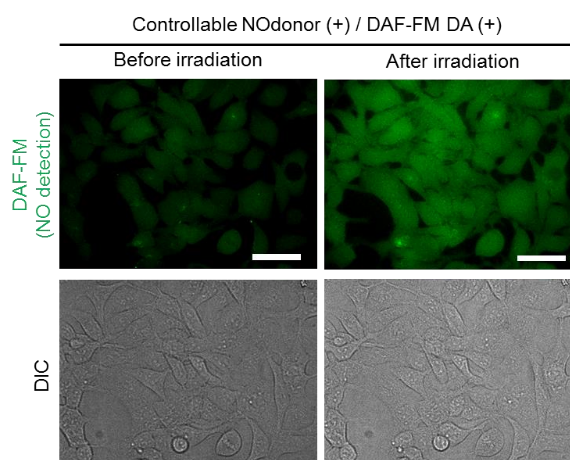
Cytotoxicity of Controllable NOdonor

HEK293T cells in 96 well plate were treated with 0, 10, 30 and 100 μM of Controllable NOdonor for 48 hours. After incubation, cell viability was assessed by MTT assay. Little toxicity of 10 and 30 μM of Controllable NOdonor was observed. Please note high concentration (100 μM) impaired cell viability.



Light-induced NO releasing in cultured cells

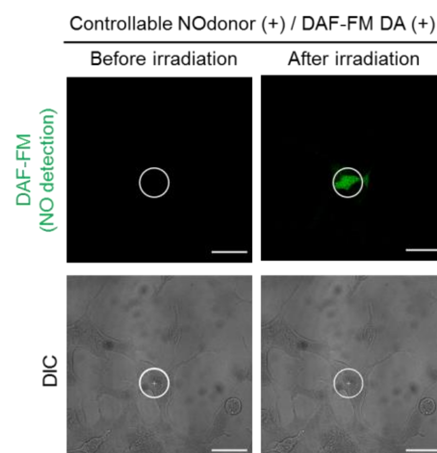
HEK293T cells were firstly treated DAF-FM DA (10 μM), a NO detection reagent, for 30 min. After wash cells with PBS two times, the cells were subsequently treated with 10 μM Controllable NOdonor for 30 min. The cells were irradiated with a MAX-303 (Asahi Spectra) equipped with a 530-590 nm band-pass filter (light intensity: 146 mW/cm^2) for 15 min. Observed the cells under a confocal fluorescence microscopy (Ex. 500 nm /Em. 515 nm for DAF-FM reagent). (scale bar = 40 μm)



Application data

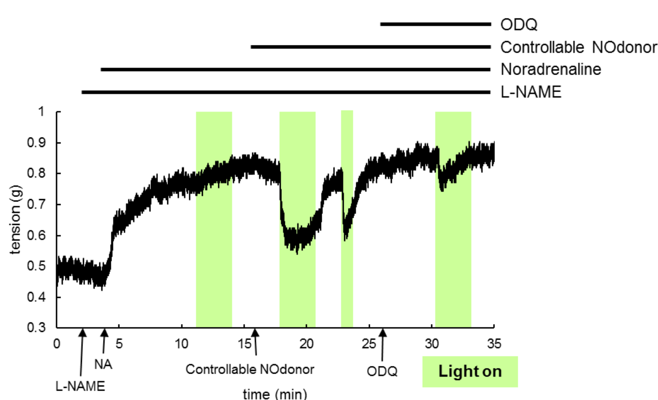
In cellullo region-specific NO generation with Controllable NOdonor

HEK293T cells were firstly treated with DAF-FM DA (10 μM) for 30 min. After wash cells with PBS, the cells were subsequently treated with 10 μM Controllable NOdonor for 60 min. The dish was photoirradiated inside the indicated white circle (*r*=31 μm) using a 543 nm laser of confocal microscopy. Left; before irradiation, Right; after irradiation. Only inside the white circle, NO was detected. (sale bar = 40 μm)



ex vivo aorta vasodilation with Controllable NOdonor

A rat aortic strip was placed in a Magnus tube filled with Krebs buffer and incubated at 37°C. The strip was pre-treated with L-NAME (100 μM), an inhibitor for endogenous eNOS enzyme, and noradrenaline (10 μM), a compound for vascular contraction. Before Controllable NOdonor addition, an irradiation of yellowish green light had no effect on vasodilation. Subsequently, Controllable NOdonor (10 μM) was added to tube and the strip was irradiated light. Tension of aorta strip was dramatically reduced, indicating vascular relaxing. Finally, ODQ, an inhibitor of soluble guanylyl cyclase (sGC), was added and canceled the effect of light-induced NO-releasing. All light irradiation was performed by MAX-303 (Asahi Spectra) equipped with 530-590 nm band-pass filter (light intensity: 70 mW/cm^2).



Reference

1. Ieda *et al., Sci. Rep.*, **9**, 1430 (2019) Structure-efficiency relationship of photoinduced electron transfer-triggered nitric oxide releasers.
2. Ieda *et al., Chem. Pharm. Bull.*, **67**, 576-579 (2019) , *In cellullo* and *ex vivo* availability of yellowish-green-light-controllable NO releaser.
3. Okuno *et al., Org. Biomol. Chem.*, **15**, 2791-2796 (2017) A yellowish-green-light-controllable nitric oxide donor based on N-nitrosoaminophenol applicable for photocontrolled vasodilation.

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 May, 2019. 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.

本製品は学術研究成果を基にフナコシ株式会社が製品化したもので、2019 年 5 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、製品資料）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、製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、本製品の仕様および製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。

E-mail Newsletter Sign Up

Japanese

English

Related products: Oxidative Stress

AcroleinRED <Cell-based Acrolein Detection Reagent>

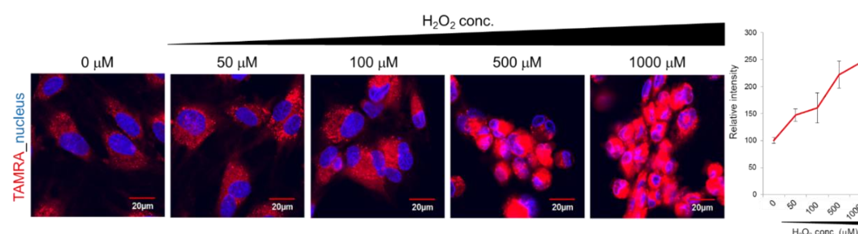
Acrolein is one of the most toxic oxidative stress marker and AcroleinRED is the world first cell-based acrolein detection reagent. Only addition of AcroleinRED to medium of cultured cells enable to visualized cellular acrolein. As

Catalog No. FDV-0022
Size 0.5 mg

Data example

Observation of oxidative stress-induced acrolein production

HUVECs were pretreated with 0-1000 μM H_2O_2 for 2 hours and subsequently treated with 10 μM AcroleinRED for 30 min. Right after labeling, cells were washed, stained with hoechest and observed under live cell condition. In the absence of H_2O_2, the acrolein endogenously produced by HUVECs could be observed. Intracellular TAMRA signals were increased in the H_2O_2 dose-dependent manner compared with the endogenous acrolein level.



PolyamineRED <Intracellular Polyamine Detection Reagent>

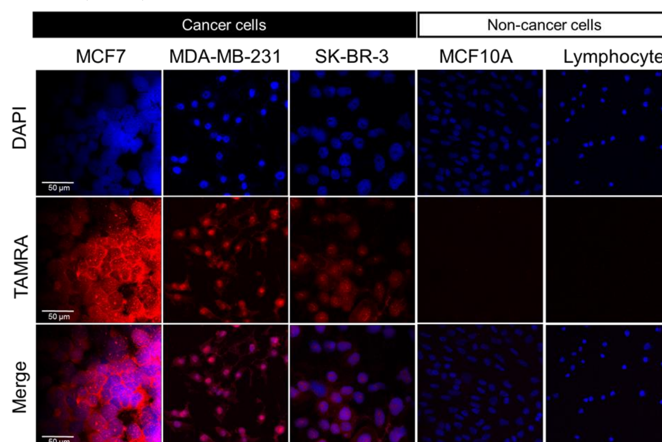
PolyamineRED is the world first intracellular polyamine detection reagent. This product specifically reacts with polyamine species such as putrescine, spermidine and spermine and transfers red fluorescent dye TAMRA to intracellular polyamines.

Catalog No. FDV-0020
Size 0.5 mg

Data example

Polyamine imaging in both cancer and non-cancer cells by PolyamineRED

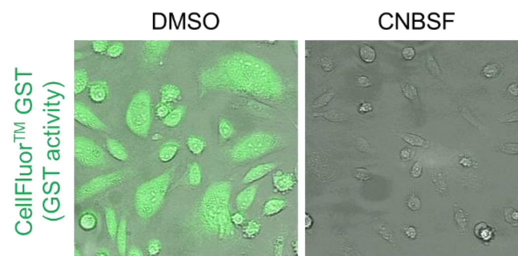
Three cancer cell lines (MCF7, MDA-MB-231 and SK-BR-3) and two non-cancer cells (MCF10A and human lymphocyte) were treated with 30 μM of PolyamineRED for 10 min. After incubation, cells were washed three times by PBS, followed by DAPI staining and formalin fixation.



CellFluor™ GST <Cell-based GST Activity Assay Reagent >

CellFluor™ GST is a novel fluorescent probe for monitoring wide GST members' activity both *in celluo* or *in vitro*. CellFluor™ GST releases green fluorophore rhodamine 110 upon GST activities. This probe has cell-permeability and can detect intracellular GST activity.

Catalog No. FDV-0030
Size 0.1 µmol
Features
- Easy and quick protocol
- Broad specificity for various GST family members
- Ex/Em: 496 nm/520 nm
  (Compatible with commercial FITC filters)



CNBSF <Irreversible GST Inhibitor >

CNBSF is a novel GST inhibitor which irreversibly blocks GST enzymes. CNBSF has membrane-permeability and can be applied into live cell experiments.

Catalog No. FDV-0031
Size 10 mg
Features
- Membrane-permeable and irreversible inhibitor
- Broad specificity for various GST family members
- Covalent inhibition of GSTP1 was experimentally confirmed by MS analysis