BIOLUMINOR

NO-550

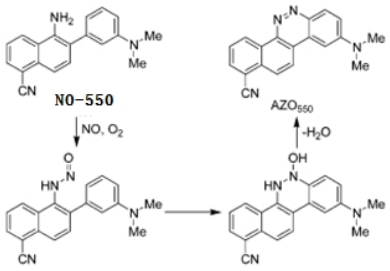
Table 1 Contents and storage

Material	Amount	Concentration	Storage
NO-550 ($C_{19}H_{17}N_3$; MW:287.36)	200 μL	10 mM stock solution in anhydrous DMSO	♦ ≤-20℃ ♦ Desiccate ♦ Protect from light ♦ Avoid freeze-thaw cycles ♦ Store in single-use aliquots, if possible
If refreezing after use, seal the vial tightly.			

Introduction

Nitric oxide (NO) play a myriad roles in physiology and diseases, such as vasodilation and neurotransmission [1,2].

NO-550 is designed for highly specific for cellular imaging of NO while being inert to other ROS/RNS species. NO-550 displays a rapid and linear response to NO with a red-shifted 1500-fold turn-on signal from a dark background. Excellent selectivity was observed against other reactive oxygen/nitrogen species, pH, and various substances. NO-550 crosses cell membranes and is suitable for both intra- and extracellular NO quantifications. The high specificity, dark background, and low pH dependence make NO-550 a superior probe for NO detection when used as an imaging agent.



Scheme 1 Fluorogenic detection of NO with NO-550[3].

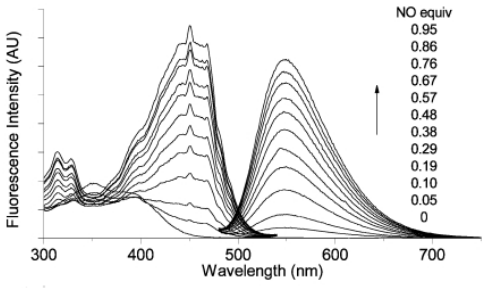


Fig. 1 Spectroscopic properties of NO sensing using NO-550 in vitro. Excitation and emission spectra of NO-550 (50 μM) in PBS spiked with various levels of NO (λ_{ex} = 470 nm, λ_{em} = 550 nm). Spectra were collected 2 min after NO addition.

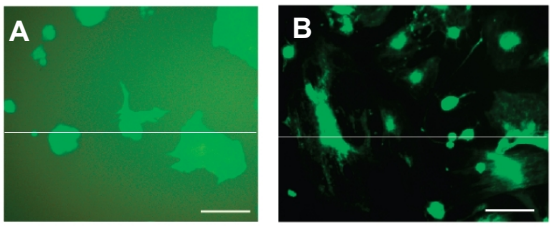


Fig. 2 Superior imaging of intracellular NO with NO-550 (B) than DAF-2DA which is widely used for imaging of NO (A) by fluorescence confocal microscopy (λex@460 nm; λem@560 nm).

Guidelines for Use

Before opening, allow the vial to warm to room temperature and then briefly centrifuge the vial in a micro centrifuge to deposit the DMSO solution at the bottom of the vial.

The concentration of probe for optimal staining will vary depending on the particular cell type and the permeability of the cells or tissues to the probe. Here we suggest some initial conditions to use as a guideline. Dilute the stock solution in the growth media to the final working concentration (we recommend at least 10 μM, 500-1000 fold dilution）.

Suggested Assay Procedure

Cell Preparation: Astrocytes were cultured in DMEM with 10% fetal bovine serum and 1% penicillin/ streptomycin/ amphotericin B; PC-12 cells were cultured in F12K/DMEM with 7.5% heat-inactivated house serum, 2.5% fetal bovine serum, and 1% penicillin/ streptomycin/amphotericin B.

Induce NO production: 1. Astrocytes were stimulated with 200 U/mL interferon-γ (IFN-γ) and 10 ng/mL interleukin-1ß (IL-1ß) for 24–48 h; (2) PC-12 cells were stimulated with 50 ng/ml nerve growth factor (NGF) for 8–10 days.

Addition of NO-550: The treated cells were rinsed in Dulbecco's phosphate-buffered saline, pH 7.2 (PBS) and kept in PBS during the imaging procedure. 10 μM of NO-550, diluted from stock solution, was incubated with cells for approximately 15 min at 37° C before imaging.

Phase contrasts and fluorescence images could be acquired using a fluorescence microscope equipped with a standard rinse and imaging.

For control experiment: cells preloaded with NO-550 were incubated with 1 mM sodium nitroprusside for 15 min before analysis

References

(1) *Nitric Oxide*; Mayer, B., Ed.; Handbook of Experimental Pharmacology, Vol. 143; Springer: Berlin, 2000.

(2) *Nitric Oxide Biology and Pathobiology*; Ignarro, L. J., Ed.; Academic Press: San Diego, CA, 2000.

(3) *A highly selective low-background fluorescent imaging agent for nitric oxide*; Y. Yang, S.K. Seidlits, M.M. Adams, et al.; Journal of the American Chemical Society, 2010. 132(38): p. 13114-13116.

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