**Gentaur Europe BVBA** Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

# EZCell™ Intracellular Nitric Oxide Synthase (NOS) Detection Kit



**(Catalog # K207-100; 100 assays; Store at -20°C)**

## I. Introduction:

Nitric oxide synthases (EC 1.14.13.39) (NOSs) are a family of enzymes that catalyze the production of nitric oxide (NO) from L-arginine. Nitric oxide (NO) plays an important role in neurotransmission, vascular regulation, immune response and apoptosis. There are three isoforms of NOS: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). nNOS accounts for the production of NO in central nervous system, where NO participates in cell communication and information storage. eNOS produces NO in blood vessels and is involved with the regulation of vascular function. In contrast to other isoforms, iNOS is expressed *de novo* under oxidative stress conditions and produces large amounts of NO as a part of body's defense mechanism. BioVision's Intracellular Nitric Oxide Synthase Detection kit uses a dye that reacts with intracellular NO produced by NOS to produce fluorescence (Ex/Em = 485/530 nm), which is proportional to the concentration of intracellular NOS and can be detected using a microplate reader or a fluorescence microscope. This kit provides a simple, non-radiometric method for detection of intracellular NOS in the cells.

## II. Application:

- Detection of NOS activity in adherent cells.
- Screening/studying/characterizing stimulators/inhibitors that affect intracellular levels of NOS.

## III. Sample Type:

- NOS producing cells or cell lines.

## IV. Kit Contents:

| Components | K207-100 | Cap Code | Part Number |
|---|---|---|---|
| NOS Assay Buffer<br>Staining Dye (In DMSO) | 100 ml<br>20 µl | NM<br>Amber | K207-100-1<br>K207-100-2 |

## V. User Supplied Reagents and Equipment:

- 96-well plate with clear flat bottom. Black plate is preferred.
- Multi-well spectrophotometer
- 37°C Incubator with 5% CO2
- Light and fluorescence microscope with Ex/Em = 485/530 nm

## VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. Read the entire protocol before performing the assay. Open all reagents under sterile conditions (e.g. cell culture hood).

- **Assay Buffer:** Store at 4°C or -20°C. Warm to 37°C before use.
- **Staining Dye:** Store at -20°C. Thaw to room temperature before use. Ready to use. Light sensitive, do not expose to intense light.

## VII. Intracellular Nitric Oxide Synthase Detection Protocol:

1. **Cell Culture:**
   a. In a 96 well plate, culture 5-10 x $10^4$ cells/well in 200 µl of desired media. Treat the cells with compounds of interest in 200 µl media. As a control, we recommend treating cells with vehicle alone. Grow cells overnight in a 37°C incubator containing 5% $CO_2$.
   b. **Adherent Cells**: Carefully remove the media using a micro-pipette without disturbing the cells. Gently wash the cells twice with 200 µl Assay Buffer each.

      **Suspension Cells**: Centrifuge the plate at 1000 *x g* for 5 min. In the absence of a plate centrifuge, carefully transfer the cells in a 1.5 ml conical tube and centrifuge the tube at 1000 *x g* for 5 min. Carefully remove the media using a micro-pipette without disturbing the cells. Gently resuspend the cells in 200 µl Assay Buffer. Repeat the wash step.

      **Note**: The wash steps are necessary to remove serum, BSA, or phenol red from the samples as these components affect the fluorescent signal.
2. **Staining:** Prepare a 1X working solution of the Staining Dye by diluting it 1:200 with the Assay Buffer, just before use. Add 20 µl of diluted Staining Dye per well with the cells and incubate for 1 h at 37°C in the incubator (in dark).
3. **Detection:**
   a. **Plate Reader**: Measure fluorescence at Ex/Em = 485/530 nm.
   b. **Fluorescence Microscope**: Wash the cells carefully to remove excess dye and add 200 µl assay buffer/well. Examine cells using light and fluorescence microscope (Ex/Em = 485 nm/530 nm). Acquire several images per well for analysis.

      **Note**: Since the Staining Dye photo-bleaches very rapidly, we recommend analyzing cells immediately.

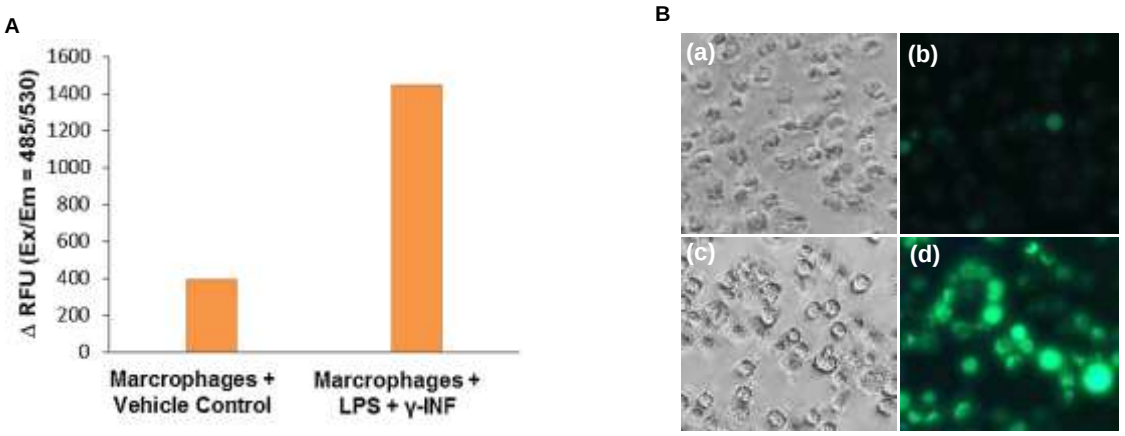


**Figure: NOS detection in Macrophages (J774A.1):** Macrophages were cultured overnight and treated the next day with either vehicle control (No treatment) or LPS (200 ng/ml) and γ-INF (100 ng/ml) for 24 hrs. After washing with Assay Buffer, the cells were stained with the Staining Dye for 1h at 37°C. **A) Detection with a plate reader:** The fluorescence signal was measured at Ex/Em = 485/530 nm. Treatment with LPS and γ-INF induced intracellular NOS in J774A.1 cells. **B) Detection with a fluorescent microscope:** The cells were washed with Assay buffer and imaged using Nikon TiE microscope. (a) and (b) control cells (vehicle treated) (c) and (d) cells treated with LPS (200 ng/ml) and γ-INF (100 ng/ml). Treatment with LPS and γ-INF induced intracellular NOS in J774A.1 cells.

## VIII. RELATED PRODUCTS:

Nitric Oxide Synthase Fluorometric Assay Kit (K206)
Nitric Oxide Synthase Inhibitor Screening Kit (K208)
Nitric Oxide Fluorometric Assay Kit (K252)
Diphenyleneiodonium chloride (2358)
Nitric Oxide Synthase Colorimetric Assay Kit (K205)
Nitric Oxide Colorimetric Assay Kit (K262)
eNOS Antibody (3426)
L-NMMA acetate (2348)

***FOR RESEARCH USE ONLY! Not to be used on humans***