

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Catalog No: E-BC-F020

Specification: 96T

Measuring instrument: Flow Cytometry

Elabscience® Nitric Oxide Synthase (NOS) Activity Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help:

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Intended use

The kit is suitable for detecting the activity of Nitric Oxide Synthase (NOS) in alive cell sample.

Detection principle

Nitric Oxide Synthase (NOS) exists in three distinct subtypes: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). nNOS produces nitric oxide (NO) in the nervous system, where it participates in neuronal signaling and memory formation. eNOS generates NO in blood vessels to regulate vascular function. iNOS is expressed de novo under oxidative stress conditions and produces high-output NO as part of host defense mechanisms. iNOS is de novo expressed under oxidative stress conditions and produces large amounts of NO as part of the body's defense mechanism.

NOS catalyzes the conversion of arginine and NADPH into citrulline and NO. The NO probe enters the cell and reacts with NO to produce fluorescence, measuring the fluorescence intensity can reflect the activity of NOS in the cell.

Kit components & storage

Item	Component	Size 1 (96 T)	Storage
Reagent 1	Buffer Solution	48 mL × 1 vial	-20°C, 12 months
Reagent 2	Coenzyme A	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Substrate	1.5 mL × 1 vial	-20°C, 12 months
Reagent 4	Coenzyme B	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 5	Probe	0.022 mL × 1 vial	-20°C, 12 months, shading light
	Black Clear-bottom Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Adherent cells: With a 200 μ L assay volume per well in a 96-well plate, 100 assays can be performed.

Instruments

Flow Cytometry, Incubator

Materials required but not provided

1×PBS (0.01 M, pH 7.4), 0.22 μ m filter membrane

Reagent preparation

- ① Equilibrate all the reagents to 25°C before use.
- ② Coenzyme A Working Solution preparation:
Dissolve one vial of Coenzyme A with 0.3 mL of Buffer Solution. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.
- ③ Coenzyme B Working Solution preparation:
Dissolve one vial of Coenzyme B with 1 mL of Buffer Solution. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.
- ④ Reaction Working Solution preparation:
Before testing, please prepare sufficient Reaction Working Solution. For example, prepare 1002 μL of Reaction Working Solution (mix well 860 μL of Buffer Solution, 50 μL of Cofactor A Working Solution, 80 μL of Substrate, 10 μL Coenzyme B Working Solution and 2 μL of Probe). After mixing, it is recommended to filter the Reaction Working Solution through a 0.22 μm filter membrane. The Reaction Working Solution should be freshly prepared before use. Stable for 4 h protected from light. (Reaction working solution contains NO probes, arginine, NADPH and other substrates.)
- ⑤ Blank Working Solution preparation:
Before testing, please prepare sufficient Blank Working Solution. For example, prepare 1000 μL of Blank Working Solution (mix well 860 μL of Buffer Solution, 50 μL of Cofactor A Working Solution, 80 μL of Substrate and 10 μL Coenzyme B Working Solution). After mixing, it is recommended to filter the Blank Working Solution through a 0.22 μm filter membrane. The Blank Working Solution should be freshly prepared before use. Stable for 4 h protected from light. (The Blank

Working Solution contains substrates such as arginine and NADPH, but does not contain NO probe.)

- ⑥ Recommended volumes of solution (Reagent 1 + Blank(Reaction Working Solution)) in different culture dishes:

Cell types	adherent cell				Suspended cell
Plate types	6-well plates	24-well plates	96-well plates	35 mm culture dish	2 mL centrifuge tube
Volume of solution required per well	1 mL/well	0.5 mL/well	0.2 mL/well	1.5 mL/well	0.3 mL/tube

The key points of the assay

- ① Wash away any residual probes after probe incubation. Otherwise it will lead to elevated background levels.
- ② DAF-FM DA fluorescent is easily quenched. Therefore, samples should be measured within 2 hours after incubation to prevent fluorescence decay and minimize potential errors.
- ③ Since DAF-FM DA tends to settle at low temperatures, equilibrate DAF-FM DA to 25°C before use and mix it thoroughly.
- ④ Phenol red and BSA interfere with DAF-FM DA detection and should be avoided.
- ⑤ Reaction Working solution and Blank Working solution must be sterilized by filtration through a 0.22 μm membrane to prevent adverse effects on cellular viability

Operating steps

Instrument parameter	
Flow Cytometry	FITC

Suspension cells

- ① Centrifuge at 1000×g for 5-10 min to collect cells. According to the experimental design groups, each tube contains 150 μL of cell suspension with a minimum cell count of 2–5×10⁴ cells. Set up blank tubes and experimental tubes. Add Blank Working Solution to the blank tube and add Reaction Working Solution to the experimental tube.
- ② Mix thoroughly and incubate at 37°C for 20-60 min. The optimal incubation time varies for different cell types and stimulus condition.
- ③ Centrifuge at 1000×g for 5-10 min to collect cells. Wash cells 1-3 times with PBS (0.01 M, pH 7.4) to thoroughly remove probe that did not enter the cells.
- ④ Resuspend the collected cell pellet in PBS (0.01 M, pH 7.4).
- ⑤ Detect by Flow Cytometry in the FITC channel.

Adherent cells

(The following procedure uses a 96-well plate as an example. For other plate or culture dishes, reagent volumes may be adjusted according to the recommended volumes of solution in the Reagent Preparation section, or modified based on your preferred solution volume. Note that the volume ratio of Buffer Solution to the Blank (Reaction) Working Solution is 1: 1.)

- ① Discard the culture medium from the well.
- ② Set up blank wells and experimental wells.

Blank well: add 100 μL of Buffer Solution, then add 100 μL of Blank Working Solution.

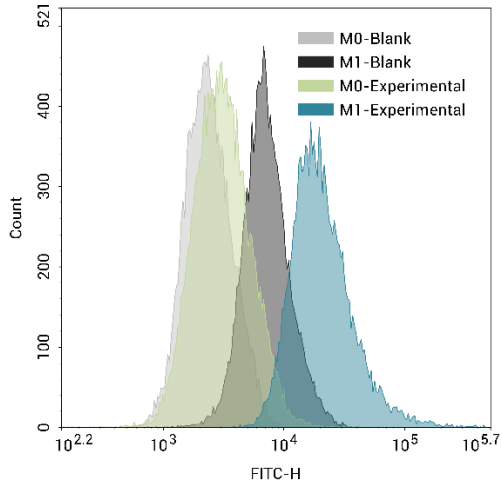
Experimental well: add 100 μ L of Buffer Solution, then add 100 μ L of Reaction Working Solution.

- ③ Mix thoroughly and incubate at 37°C for 20-60 min. The optimal incubation time varies for different cell types and stimulus condition.
- ④ Remove the solution in the well and wash 1–3 times with PBS (0.01 M, pH 7.4) to thoroughly remove probe that did not enter the cells.
- ⑤ Collect cells after digestion and centrifuge at 1000 \times g for 5-10 min to collect cell pellet.
- ⑥ Resuspend the collected cell pellet in PBS (0.01 M, pH 7.4).
- ⑦ Detect by Flow Cytometry in the FITC channel.

Note: If drugs are used to stimulate cells, the concentration of drug and stimulation duration needs to be determined based on the type of the cell type. For example, RAW 264.7 cells can be co-stimulated with LPS and IFN- γ for 12–48 hours. For stimulation durations of 2 hours or less, incubation can be performed simultaneously with the stimulation (i.e., Buffer Solution + Blank (Reaction) Working Solution, and the drug are incubated together). For longer stimulation durations (>2 hours), it is recommended to discard the culture medium after the drug stimulation is complete, then add Buffer Solution + Blank (Reaction) Working Solution for incubation.

Appendix I Case Analysis

After co-stimulating RAW 264.7 M0 cells with LPS and IFN- γ for 48 hours, the procedure was carried out as operating steps, and the results are:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.

