

PathScan® Total iNOS Sandwich ELISA Kit

✓ 1 Kit
(96 assays)

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New 12/09

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #18126
Swiss-Prot Acc. #P29477

Species Cross-Reactivity: M

Description: The PathScan® Total iNOS Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of iNOS. An iNOS rabbit antibody has been coated onto the microwells. After incubation with cell lysates, iNOS protein is captured by the coated antibody. Following extensive washing, an iNOS mouse detection antibody is added to detect the captured iNOS protein. Anti-mouse IgG, HRP-linked Antibody #7076 is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for the developed color is proportional to the quantity of iNOS.

Specificity/Sensitivity: Cell Signaling Technology's PathScan® Total iNOS Sandwich ELISA Kit #7097 detects endogenous levels of total iNOS protein.

Background: Nitric Oxide Synthase (NOS) catalyses the formation of nitric oxide (NO) and citrulline from L-arginine, oxygen and cofactors. Three family members have been characterized: neuronal NOS (nNOS), which is found primarily in neuronal tissue; inducible NOS (iNOS), which is induced by interferon gamma and lipopolysaccharides in the kidney and cardiovascular system; and endothelial NOS (eNOS), which is expressed in blood vessels (1). NO is a messenger molecule with diverse functions throughout the body including the maintenance of vascular integrity, homeostasis, synaptic plasticity, long-term potentiation, learning, and memory (2,3).

Background References:

- (1) Tsutsui, M. (2004) *J Atheroscler Thromb* 11, 41-8.
- (2) Son, H. et al. (1996) *Cell* 87, 1015-23.
- (3) Hawkins, R.D. (1996) *Neuron* 16, 465-7.

| Products Included | Volume | Solution Color |
|-------------------------------------|----------|----------------|
| iNOS Antibody Coated Microwells* | 96 tests | |
| iNOS Detection Antibody | 11 ml | Green |
| Anti-mouse IgG, HRP-linked Antibody | 11 ml | Red |
| TMB Substrate | 11 ml | Colorless |
| STOP Solution | 11 ml | Colorless |
| Sealing Tape | 2 sheets | |
| 20X Wash Buffer | 25 ml | Colorless |
| Sample Diluent | 25 ml | Blue |
| 10X Cell Lysis Buffer #9803** | 15 ml | Yellowish |

* 12 8-well modules -Each module is designed to break apart for 8 tests.

**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

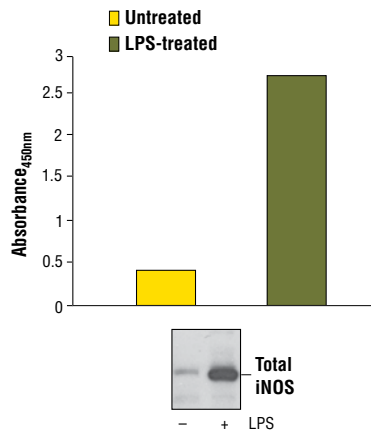


Figure 1. Treatment of Raw264.7 cells with LPS stimulates expression of iNOS, detected by the PathScan® Total iNOS Sandwich ELISA Kit. Raw264.7 cells (80-90% confluent) were treated with 1 µg/ml LPS for 6 hours at 37°C. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using iNOS Antibody (Mouse Specific) #2982 is shown in the bottom figure.

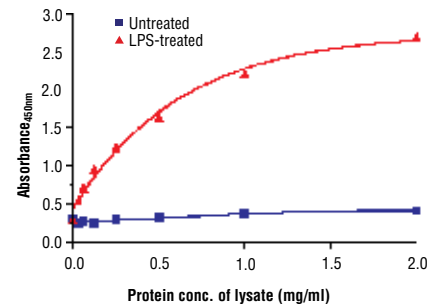


Figure 2. The relationship between the protein concentration of lysates from untreated and LPS-treated Raw264.7 cells and the absorbance at 450 nm using the PathScan® Total iNOS Sandwich ELISA Kit is shown.

Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na₃VO₄, 1 μg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Add 100 μl of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 μl of cell lysate into the tube and vortex for a few seconds. Generally, sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction. Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results. However, dilution factors need to be titrated when specific cell lysates are used.

3. Add 100 μl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.
4. Gently remove the tape and wash wells:
 - a. Discard plate contents into a receptacle.
 - b. Wash 4 times with 1X Wash Buffer, 200 μl each time for each well.
 - c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 - d. Clean the underside of all wells with a lint-free tissue.
5. Add 100 μl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step 4.
7. Add 100 μl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step 4.
9. Add 100 μl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.
10. Add 100 μl of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
 - a. Visual Determination — Read within 30 minutes after adding STOP Solution.
 - b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.