Datasheet for ABIN2344944

Nitrotyrosine ELISA Kit

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Overview

Quantity:	96 tests
Target:	Nitrotyrosine
Reactivity:	Chemical
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	The nitrotyrosine quantitation kit is a competitive ELISA.
Brand:	OxiSelect™
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	20 nM
Characteristics:	The kit has a nitrotyrosine detection sensitivity range of 20 nM to 8.0 µM. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.
Components:	1. Nitrotyrosine Coated EIA Plate : one strip well 96-well plate. 2. Anti-Nitrotyrosine Antibody : One 20 µL vial of anti-nitrotyrosine Rabbit IgG. 3. Secondary Antibody, HRP Conjugate : One 20 µL vial. 4. Assay Diluent : One 50 mL bottle. 5. 10X Wash Buffer : One 100 mL bottle. 6. Substrate Solution : One 12 mL amber bottle.

Product Details

	7. Stop Solution (Part. No. 310808): One 12 mL bottle. 8. Nitrated BSA Standard : One 500 µL vial of 1 mg/mL Nitrated BSA in PBS with a nitrotyrosine content of 40 µM (2.7 mole of nitrotyrosine per mole of BSA). The protein nitrotyrosine level is predetermined by a spectrophotometric method as described by Ischiropoulos et al (See Ref. 3).
Material not included:	1. Protein samples such as purified protein, plasma, serum, cell lysate 2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 4. Multichannel micropipette reservoir 5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Target Details

Target:	Nitrotyrosine
Abstract:	Nitrotyrosine Products
Target Type:	Chemical
Background:	The modification of tyrosine residues in proteins to 3-nitrotyrosine by peroxynitrite (Figure 1) or other potential nitrating agents has been detected in biological systems that are subject to oxidative stress. Detection of nitrotyrosine-containing proteins has been reported in many human and animal diseases or cellular models of disease. While all tyrosine residues in proteins may theoretically be targets for nitration, presumably the efficiency of tyrosine nitration is dependent on various biological conditions such as the local production and concentration of the reactive species, the existence and availability of antioxidants and scavengers, the accumulation of inflammatory cell and the presence of pro- inflammatory cytokines, as well as the proximity and compartmentation of these components. The quantity of 3-nitrotyrosine in protein sample is determined by comparing its absorbance with that of a known nitrated BSA standard curve.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Sensitive detection of 3-Nitrotyrosine as low as 10 nM • Suitable for use with cell lysates, serum, plasma and purified proteins • Nitrated BSA provided as positive control
Plate:	Pre-coated

Application Details

Protocol:	The unknown protein nitrotyrosine sample or nitrated BSA standards are first added to a nitrated BSA preabsorbed EIA plate. After a brief incubation, an anti-nitrotyrosine antibody is added, followed by an HRP conjugated secondary antibody. The protein nitrotyrosine content in unknown sample is determined by comparing with a standard curve that is prepared from predetermined nitrated BSA standards.
Reagent Preparation:	• 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity. 3 • Anti-Nitrotyrosine Antibody and Secondary Antibody: Immediately before use dilute the Anti-Nitrotyrosine Antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.
Assay Procedure:	1. Prepare and mix all reagents thoroughly before use. Each protein sample including nitrated BSA and blank should be assayed in duplicate. 2. Add 50 µL of unknown protein sample or nitrated BSA standard to the wells of the EIA plate. Incubate at room temperature for 10 minutes on an orbital shaker. 3. Add 50 µL of the diluted anti-nitrotyrosine antibody to each well, incubate at room temperature for 1 hour on an orbital shaker. 4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer. 5. Add 100 µL of the diluted Secondary Antibody-Enzyme Conjugate to all wells. 6. Incubate at room temperature for 1 hour on an orbital shaker. 7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step. 8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes. Note: Watch plate carefully, if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation. 4 9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time). 10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, aliquot and store the Nitrated BSA Standard at -20°C to avoid multiple freeze/thaw cycles. Store all other kit components at 4°C until their expiration dates.

Product cited in:

Shafique, Torina, Reichert, Colantuono, Nur, Zeeshan, Ravichandran, Liu, Feng, Zeeshan, Benjamin, Irani, Harrington, Sellke, Abid: "Mitochondrial redox plays a critical role in the paradoxical effects of NAPDH oxidase-derived ROS on coronary endothelium." in: **Cardiovascular research**, Vol. 113, Issue 2, pp. 234-246, (2017) (PubMed).

Lambrechts, Balestra, Theron, Henckes, Galinat, Mignant, Belhomme, Pontier, Guerrero: " Venous gas emboli are involved in post-dive macro, but not microvascular dysfunction." in: **European journal of applied physiology**, Vol. 117, Issue 2, pp. 335-344, (2017) (PubMed).

Wu, Li, Zhu, Wang, Dai, Zhang, Zheng, Xu, Wang, Zhang, Zhou, Zhang, Shi: "Mdivi-1 Alleviates Early Brain Injury After Experimental Subarachnoid Hemorrhage in Rats, Possibly via Inhibition of Drp1-Activated Mitochondrial Fission and Oxidative Stress." in: **Neurochemical research**, Vol. 42, Issue 5, pp. 1449-1458, (2017) (PubMed).

Toth, Tarantini, Ashpole, Tucsek, Milne, Valcarcel-Ares, Menyhart, Farkas, Sonntag, Csiszar, Ungvari: "IGF-1 deficiency impairs neurovascular coupling in mice: implications for cerebromicrovascular aging." in: **Aging cell**, Vol. 14, Issue 6, pp. 1034-44, (2016) (PubMed).

Lux, Pokreisz, Swinnen, Caluwe, Gillijns, Szelid, Merkely, Janssens: "Concomitant Phosphodiesterase 5 Inhibition Enhances Myocardial Protection by Inhaled Nitric Oxide in Ischemia-Reperfusion Injury." in: **The Journal of pharmacology and experimental therapeutics**, Vol. 356, Issue 2, pp. 284-92, (2016) (PubMed).

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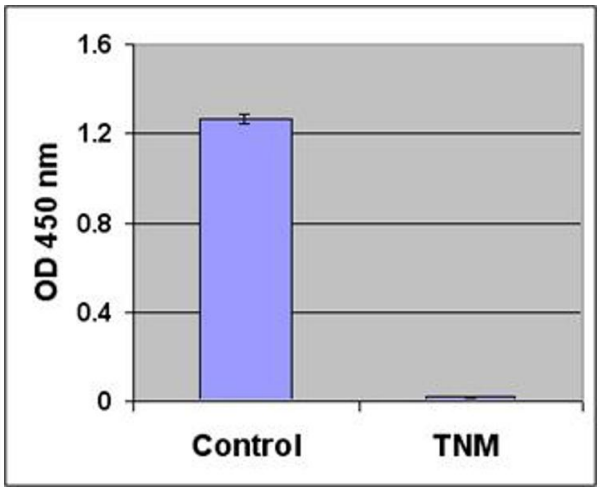

ELISA

Image 1. Protein Nitration by Tetranitromethane using the OxiSelect™ Nitrotyrosine ELISA Kit. STO (MEF) cells were lysed in 25 mM HEPES, pH 7.5, 150 mM NaCl, 1% NP-40, 10 mM MgCl2, 1 mM EDTA, 2% glycerol. The cell lysate was nitrated with tetranitromethane. The protein 3-nitrotyrosine levels were measured as described in the assay protocol.

