Version 15-05/24

User's Manual

Nitrotyrosine ELISA Kit

REF **DEIANS071**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The CD Nitrotyrosine ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of 3-nitrotyrosine in protein sample. The quantity of 3-nitrotyrosine in protein sample is determined by comparing its absorbance with that of a known nitrated BSA standard curve. 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.

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

General Description

The modification of tyrosine residues in proteins to 3-nitrotyrosine by peroxynitrite 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 proinflammatory cytokines, as well as the proximity and compartmentation of these components.

Principles of Testing

The nitrotyrosine quantitation kit is a competitive ELISA. 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.

Reagents And Materials Provided

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. 10× Wash Buffer: One 100 mL bottle.

6. Substrate Solution: One 12 mL amber bottle.

7. Stop Solution: 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.

Materials Required But Not Supplied

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)

Storage

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.

Reagent Preparation

1. 1× Wash Buffer: Dilute the 10× Wash Buffer Concentrate to 1× with deionized water. Stir to homogeneity.

2. 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.

3. Preparation of Standard Curve

Prepare a dilution series of Nitrated BSA Standards in the nitrotyrosine concentration range of 0 nM-8000 nM by diluting the Nitrated BSA stock solution in Assay Diluent.

Standard Tubes	Nitrated BSA Standard (µL)	Assay Diluent (µL)	Nitrated BSA (µg/mL)	Nitrotyrosine (nM)
1	60	240	200	8000
2	100 of Tube #1	300	50	2000
3	100 of Tube #2	300	12.5	500
4	100 of Tube #3	300	3.125	125
5	100 of Tube #4	300	0.78	31.25
6	100 of Tube #5	300	0.195	7.81
7	100 of Tube #6	300	0.049	1.95
8	0	300	0	0

Assay Procedure

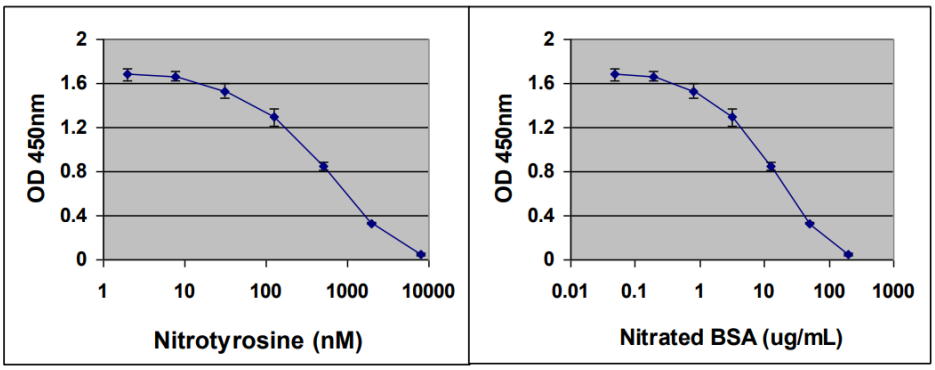
1. Prepare and mix all reagents thoroughly before use. Each protein sample including nitrated BSA and blank should be assayed in duplicate.
2. Add 50 µL of unknown protein sample or nitrated BSA standard to the wells of the EIA plate. Incubate at room temperature for 10 minutes on an orbital shaker.
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 1× 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 1× 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.**
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.

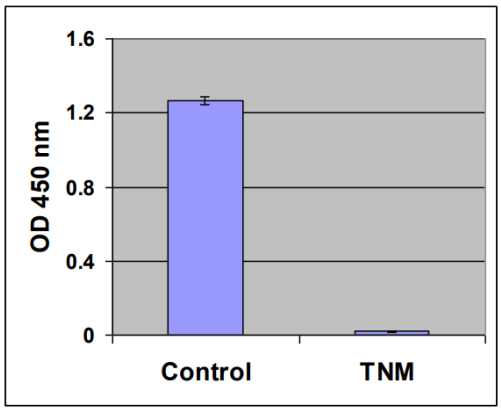
Typical Standard Curve

The following figures demonstrate typical Nitrotyrosine ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

Nitrotyrosine ELISA Standard Curve:



Protein Nitration by tetranitromethane. STO (MEF) cells were lysed in 25mM HEPES, pH 7.5, 150 mM NaCl, 1% NP-40, 10 mM $MgCl_2$, 1 mM EDTA, 2% Glycerol. Cell Lysate was nitrated with tetranitromethane (TNM). The protein 3-nitrotyrsone levels were determined as described in the assay instructions.



Limitations

These products are warranted to perform as described in their labeling and in CD literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS

EXPRESSED WARRANTY AND CD DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CD's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CD, to repair or replace the products. In no event shall CD be liable for any proximate, incidental or consequential damages in connection with the products.

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