Product Manual

OxiSelect™ Nitrotyrosine Immunoblot Kit

Catalog Number

STA-303 10 blots

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Nitric oxide (NO) was initially identified as a muscle relaxation factor, mediating its effects on smooth muscle by coordinating the heme moiety of guanylate cyclase, leading to enhanced production of cGMP. Besides smooth muscle relaxation, NO also influences a variety of biological systems including cell proliferation, apoptosis, neurotoxicity, Parkinson disease, extracellular matrix remodeling. Many of its effects are mediated through protein nitration.

Peroxynitrite ($ONOO^-$) is formed in the chemical reaction between nitric oxide and superoxide. $ONOO^-$ nitrates free tyrosine and tyrosine residues in proteins; modifications that are used as markers of $ONOO^-$ action under conditions of cellular damage and in numerous diseases.

The OxiSelect™ Nitrotyrosine Immunoblot Kit offers a simple and complete system for the detection of nitrotyrosine in proteins. This kit also includes Nitrated BSA as positive control. Each kit provides sufficient quantities to perform at least 10 blots (7.5 cm x 8.5 cm).

Related Products

1. STA-304: Protein Tyrosine Nitration Control (Nitrotyrosine-BSA)
2. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
3. STA-308: OxiSelect™ Protein Carbonyl Immunoblot Kit
4. STA-309: Oxidized Protein Immunoblot Control (Carbonyl-BSA)
5. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
6. STA-318: OxiSelect™ AOPP Assay Kit
7. STA-319: AOPP-Human Serum Albumin (AOPP-HSA)
8. STA-816: OxiSelect™ N-epsilon-(Carboxymethyl) Lysine (CML) Competitive ELISA Kit
9. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA

Kit Components

1. Rabbit Anti-Nitrotyrosine Antibody (Part No. 230301): One tube – 100 µL
2. Secondary Antibody, HRP-conjugate (Part No. 230805): One tube – 100 µL
3. Nitrotyrosine Immunoblot Control (Part No. 230302): One tube – 100 µL (provided ready-to-use nitrotyrosine-BSA in 1X reducing SDS-PAGE Sample Buffer, pre-boiled)

Materials Not Supplied

1. Protein MW Standard
2. Polyacrylamide gels such as precast gels available from Invitrogen or BioRad
3. Electrophoresis Buffers
4. Electrophoresis and Western Blot Transfer Systems
5. Immunoblotting Buffers such as TBST (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween-20)

6. PVDF or Nitrocellulose Membrane (PVDF is recommended)
7. Methanol
8. Non-fat Dry Milk
9. ECL Reagents

Storage

Store all kit components at -20ºC. If the kit will not be used all at once, components should be aliquoted to avoid multiple freeze/thaw cycles.

Assay Protocol

I. Electrophoresis and Transblotting

1. Prepare samples for electrophoresis with reducing SDS Sample Buffer.
2. Load 20 µL of Nitrotyrosine Immunoblot Control (provided ready-to-use, pre-boiled) or sample to wells of a polyacrylamide gel. Also, it's recommended to include a pre-stained MW standard (as indicator of a successful transfer in step 3). Run the gel as per the manufacturer's instructions.
3. Transfer the gel proteins to a PVDF membrane as per the manufacturer's instructions.

   Note: We recommend using PVDF membrane instead of Nitrocellulose due to its low background signal after derivatization, resulting in stronger chemiluminescent signal.

II. Immunoblotting

1. Following the electroblotting step, immerse the PVDF membrane in 100% Methanol for 15 seconds, and then allow it to dry at room temperature for 5 minutes.

   Note: If Nitrocellulose is used instead of PVDF, this step should be skipped.
2. Block the membrane with 5% non-fat dry milk in TBST for 1 hr at room temperature with constant agitation.
3. Wash the blocked membrane three times with TBST, 5 minutes each time.
4. Incubate the membrane with Rabbit Anti-Nitrotyrosine Antibody, freshly diluted 1:1000 in 5% non-fat dry milk/TBST, for 1-2 hr at room temperature with constant agitation.
5. Wash the blotted membrane three times with TBST, 5 minutes each time.
6. Incubate the membrane with Secondary Antibody, HRP-conjugate, freshly diluted 1:1000 in 5% non-fat dry milk/TBST, for 1 hr at room temperature with constant agitation.
7. Wash the blotted membrane five times with TBST, 5 minutes each time.
8. Use the detection method of your choice. We recommend enhanced chemiluminescence reagents from Pierce.

Example of Results

The following figure demonstrates typical blot results of Nitrotyrosine-BSA immunoblot. One should use the data below for reference only. This data should not be used to interpret actual results.

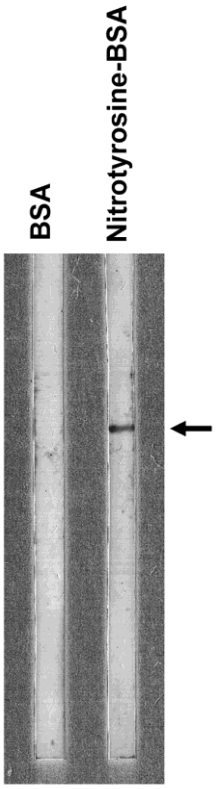

Figure 1: Immunoblotting of Nitrated BSA. Nitrotyrosine-BSA (Nitrotyrosine Immunoblot Control) was first electroblotted onto nitrocellulose membrane. Following the electroblotting procedure, the membrane was immunoblotting with anti-Nitrotyrosine antibody as described in the Assay Protocol.

References

1. Halliwell, B., Zhao, K., and Whiteman, M. (1999) *Free Radic. Res.* **31**, 651-669.
2. Greenacre, S. A. B., and Ischiropoulos, H. (2001) *Free Radic. Res.* **34**, 541-581.

Recent Product Citations

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4. Sureda, A. et al. (2016). Mediterranean diets supplemented with virgin olive oil and nuts enhance plasmatic antioxidant capabilities and decrease xanthine oxidase activity in people with metabolic syndrome: The PREDIMED study. *Mol Nutr Food Res*. doi:10.1002/mnfr.201600450.
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6. Capó, X. et al. (2015). Diet supplementation with DHA-enriched food in football players during training season enhances the mitochondrial antioxidant capabilities in blood mononuclear cells. *Eur J Nutr*. **54**:35-49.
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8. Sakellariou, G. K. et al. (2014). Neuron-specific expression of CuZnSOD prevents the loss of muscle mass and function that occurs in homozygous CuZnSOD-knockout mice. *FASEB J.* **28**:1666-1681.

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