Product Manual

OxiSelect™ Protein Carbonyl Immunoblot Kit

Catalog Number

STA-308 **10 blots**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

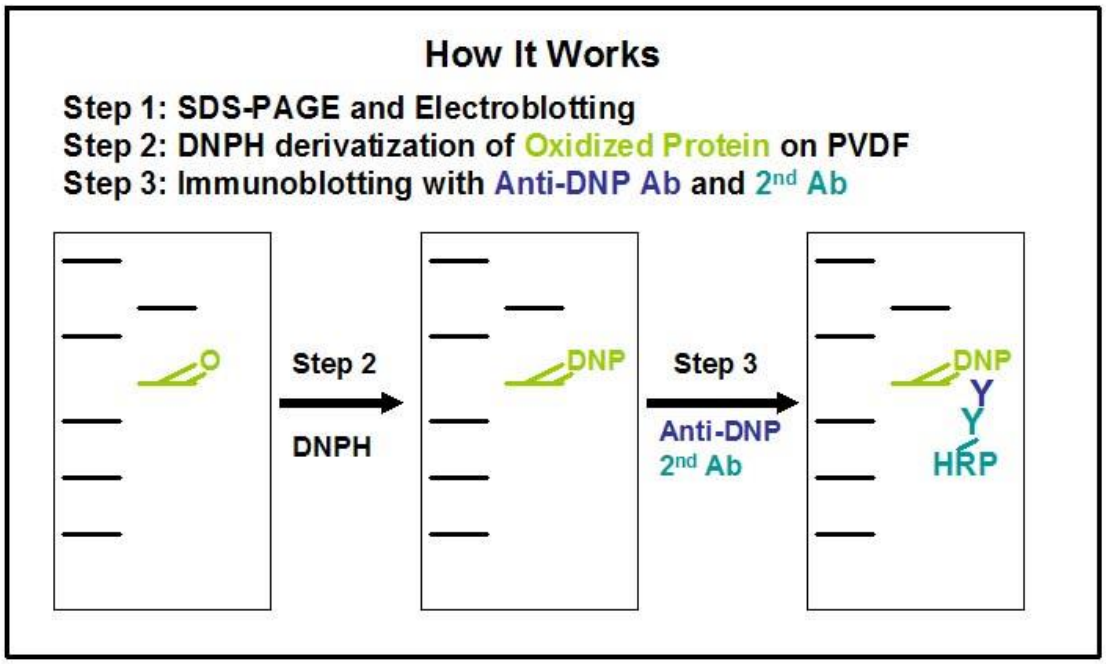
Protein oxidation is defined as the covalent modification of a protein induced either directly by reactive oxygen species or indirectly by reaction with secondary by-products of oxidative stress. Oxidative modification of proteins can be induced in vitro by a wide array of pro-oxidant agents and occurs in vivo during aging and in certain disease conditions.

There are numerous types of protein oxidative modification. The most common products of protein oxidation in biological samples are the protein carbonyl derivatives of Pro, Arg, Lys, and Thr. These derivatives are chemically stable and serve as markers of oxidative stress for most types of ROS.

Many of the current assays involve pre-derivatization of the carbonyl group with dinitrophenylhydrazine (DNPH) prior to electrophoresis, followed by immunoblotting with an anti-DNP antibody. Unfortunately, this pre-derivatization alters the electrophoretic (and electrofocusing) properties of proteins. Consequently, it is not possible to directly compare the patterns from "oxidized" fingerprints with those from "non-oxidized" protein fingerprints. Cell Biolabs' Protein Carbonyl Immunoblot Kit has the ability to conduct all derivatization and staining **after** electrophoresis and transblotting. This allows one to directly compare oxidized vs. non-oxidized protein fingerprints.

The OxiSelect™ Protein Carbonyl Immunoblot Kit offers a simple and complete system for the detection of protein oxidation. This kit also includes a Protein Oxidation Immunoblot Control as positive control. Each kit provides sufficient quantities to perform at least 10 blots (7.5 cm X 8.5 cm).

Assay Principle



Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-309: Oxidized Protein Immunoblot Control (Carbonyl-BSA)
3. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
4. STA-318: OxiSelect™ AOPP Assay Kit
5. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA

Kit Components

1. Rabbit Anti-DNP Antibody (Part No. 230801): One 100 µL tube
2. Secondary Antibody, HRP-conjugate (Part No. 230805): One 100 µL tube
3. Protein Oxidation Immunoblot Control (Part No. 230803): One 100 µL tube (provided ready-to-use oxidized BSA in 1X reducing SDS-PAGE Sample Buffer, pre-boiled)
4. 10X DNPH Solution (Part No. 230804): One 20 mL amber bottle

Materials Not Supplied

1. Protein MW standards
2. Reducing SDS-PAGE Sample Buffer
3. Polyacrylamide gels such as precast gels available from Invitrogen or BioRad
4. Electrophoresis Buffers
5. Electrophoresis and Western Blot Transfer Systems
6. Immunoblotting Buffers such as TBST (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween-20)
7. PVDF or Nitrocellulose Membrane (PVDF is recommended)
8. Methanol
9. 2N HCl
10. Non-fat Dry Milk
11. ECL Reagents

Storage

Upon receipt, store the 10X DNPH Solution at 4ºC. Aliquot and store all other components at -20ºC 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 Protein Oxidation 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. Derivatization (all steps are at room temperature, with shaking)

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. Equilibrate the membrane in TBS containing 20% Methanol for 5 minutes.
3. Wash the membrane in 2N HCl for 5 minutes.
4. Prepare sufficient amount of 1X DNPH solution by diluting the 10X DNPH Solution in 2N HCl. Incubate the membrane with 1X DNPH solution for exactly 5 minutes.

   Note: 1X DNPH Solution is stable for one week when stored in the dark at 4ºC. Do not freeze.

5. Wash the membrane three times in 2N HCl, 5 minutes each time.
6. Next, wash the membrane five times in 100% methanol (PVDF) or 50% methanol (Nitrocellulose), 5 minutes each time.

III. Immunoblotting

1. Block the DNPH-treated membrane with 5% non-fat dry milk in TBST for 1 hr at room temperature with constant agitation.
2. Wash the blocked membrane three times with TBST, 5 minutes each time.
3. Incubate the membrane with Rabbit Anti-DNP Antibody, freshly diluted 1:1000 in 5% non-fat dry milk/TBST, for 1-2 hr at room temperature with constant agitation.
4. Wash the blotted membrane three times with TBST, 5 minutes each time.
5. 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.
6. Wash the blotted membrane five times with TBST, 5 minutes each time.
7. 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 oxidized BSA after DNPH derivation. One should use the data below for reference only. This data should not be used to interpret actual results.

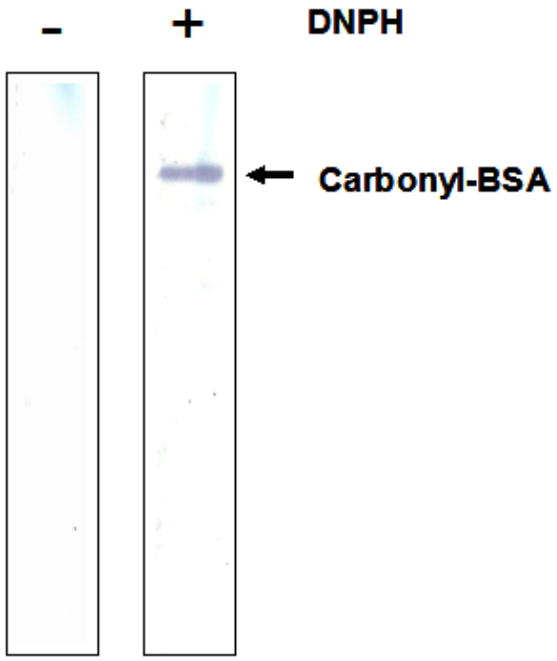


Figure 1: Immunoblotting of Oxidized BSA. Carbonyl-BSA, Oxidation Immunoblot Control, was first electroblotted onto nitrocellulose membrane. Following the electroblotting procedure, the membrane was incubated with (right strip) or without (left strip) DNPH solution. The derivatized Carbonyl-BSA is detected by immunoblotting with anti-DNP antibody as described in the Assay Protocol.

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Recent Product Citations

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