COSMO BIO CO., LTD.
Inspiration for Life Science

Protein carbonyls western blot detection kit

15 Blots (7.5 cm X 8.5 cm)

Catalog number: SML-ROIK03-EX

Kit component

Antibody : Rabbit anti-DNP antibody 0.075 mL

10 mM Tris (pH 7.6), 0.14 M NaCl

★This kit does not contain NaN_3

The property of the antibody see below [1)]

DNPH solution（shade the light）: 10X 2,4-Dinitrophenylhydrazine (DNPH) solution 15 mL

Oxidized protein : oxidized BSA , soluble in SDS-PAGE sample buffer [2)] 0.15 mL

Storage and Stability : antibody, DNPH solution, oxidized protein 4°C, 1 year

1)【property of the antibody】

Rabbit Polyclonal Antibody

2,4-dinitrophenyl (DNP) IgG

Purified IgG Fraction

Rabbit anti-DNP IgG

Volume : 0.075 mL

Antigen : DNP-KLH

Host : Rabbit

Supplied As: IgG fraction purified from rabbit serum.

Prepared in 10 mM Tris (pH 7.6), 0.14 M NaCl.

Storage and Stability: 4 °C, 1 year

2)【SDS-PAGE sample buffer】

62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.05% bromophenol blue

Protein carbonyls western blot protocol

Electrophoresis and transfer

1. Prepare the electrophoresis sample
2. Electrophoresis the sample. Oxidized protein use 10 μl per lane.
3. Transfer a PVDF membrane.

   ※We recommend PVDF membrane because nitrocellulose membrane is high background.

DNPH derivatization（all steps are at room temperature, with shaking）

1. Immerse the transferred PVDF membrane in 100% Methanol for 1 minute (this step doesn't need nitrocellulose membrane).
2. Wash the membrane in 20% methanol - 80% TBS (10 mM Tris-HCl, pH 7.4, 0.14 M NaCl) for 5 minutes.
3. Wash the membrane in 2 N HCl for 5 minutes.
4. Prepare a 10 ml 1X DNPH solution (mixed a 10X DNPH solution and diluting in 2 N HCl).

   Incubate the membrane in 1X DNPH solution **for exactly 5 minutes.**

   ※if you check the specificity of reaction. Incubate the membrane in 2 N HCl.
5. Wash the membrane three times in 2 N HCl for 5 minutes each time.
6. Wash the membrane seven times in 100% methanol (PVDF membrane) or 50% methanol (Nitrocellulose membrane), 5 minutes each time.
7. Wash the membrane in TBS for 5 minutes.

Immunoreactions and detection

1. Blocking

   Block the membrane in 5% Skim milk/TBST (10 mM Tris-HCl, pH 7.4, 0.14 M NaCl, 0.1% Tween-20) for 1 hour at room temperature with constant agitation.
2. Reaction of primary antibody

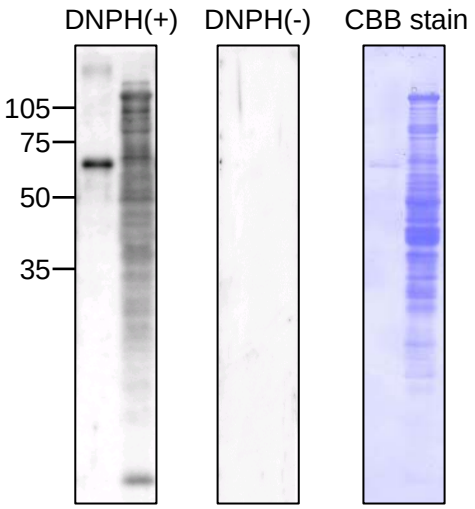
   Incubate the membrane with rabbit anti-DNP antibody diluted 1:2,000 (5 μl) in 10 ml 5% Skim milk/TBST for 1 hour at room temperature.
3. Wash the membrane three times in TBST for 5 minutes each time.
4. Reaction of secondary antibody (you can use a commercial antibody)

   Method of using a Goat Anti-Rabbit IgG, HRP-conjugate for secondary antibody

   Incubate the membrane with secondary antibody diluted in 5% Skim milk/TBST for 1 hour at room temperature.

5. Wash the membrane three times in TBST for 5 minutes each time.
6. Detection

   Use the detection method of your choice. We recommend enhanced chemiluminescence reagent.



【Western Blot Analysis】

DNPH(+) : DNPH in 2 N HCl

Left lane: oxidized BSA 0.1 μg

Right lane: mouse liver extract 5 μg

DNPH(-) : 2 N HCl

Left lane: oxidized BSA 0.1 μg

Right lane: mouse liver extract 5 μg

CBB stain : proteins stained by Coomassie brilliant blue

★ DNP antibody at 1:2,000 dilution used.

References:

1. Nakamura A. et al., Analysis of protein carbonyls with 2,4-dinitrophenyl hydrazine and its antibodies by immunoblot in two-dimensional gel electrophoresis. *J Biochem (Tokyo)*. 119 768-774 (1996)
2. Goto S. et al., Age-associated, oxidatively modified proteins: A critical evaluation. *Age* 20 81-89 (1997)
3. Goto S. et al., Carbonylated Proteins in Aging and Exercise: Immunoblot Approaches. *Mech Ageing Dev* 107 245-253 (1999)
4. Nakamura A. et al., Vitellogenin-6 is a major carbonylated protein in aged nematode, *Caenorhabditis elegans*. *Biochem Biophys Res Commun.* 264 580-583 (1999)
5. Robinson CE. et al., Determination of protein carbonyl groups by immunoblotting. *Anal Biochem.* 266 48-57 (1999)
6. Sato T. et al., Senescence marker protein-30 protects mice lungs from oxidative stress, aging, and smoking. *Am J Respir Crit Care Med*. 174 530-537 (2006)

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME, KOTO-KU, TOKYO 135-0016 JAPAN
TEL: (81)3-5632-9617 / FAX: (81)3-5632-9618 / e-mail: export@cosmobio.co.jp / URL:www.cosmobio.com