



Protein Carbonyls Immunohistochemical Staining Kit (50 slides)

Product No. SML-R0IK04-EX

Tested Application: Immunohistochemistry with methacarn-fixed paraffin-embedded tissue (see note 1 below)

Kit Components: 50 slides

Antibody: Rabbit anti-DNP, 0.06 mL, purified Ig from rabbit serum (10 mM Tris (pH 7.6), 0.14 M NaCl). Immunizing antigen: DNP-KLH

Control Specimen: Mouse Kidney, 2 slides,
(Methacarn-fixed paraffin-embedded sections)

DNPH solution: 2,4-Dinitrophenylhydrazine (DNPH) solution, 6 mL **shade from light**

Storage and Stability: Antibody, Specimen, DNPH solution: 1 year at 4°C

Note 1: *This kit cannot be used for tissues fixed with aldehyde containing fixatives such as formaldehyde, paraformaldehyde or glutaraldehyde. The use of Methacarn fixative is strongly advised. Methacarn fixing solution: (Methanol/Chloroform/Acetic Acid (6:3:1)).*

Note 2: *This kit does not contain Sodium Azide (NaN₃)*

Reagents not included: Detection reagents for rabbit Ig.
(e.g., Elite ABC Kit (Rabbit IgG), Vector Labs)



Protein carbonyls immunohistochemical staining protocol

1. Deparaffinize (Methacarn fixed paraffin-embedded section)

Xylene	5 minutes (3 times)
100% Ethanol	1 minute (3 times)
95% Ethanol	1 minute
90% Ethanol	1 minute
80% Ethanol	1 minute
70% Ethanol	1 minute
PBS	5 minutes (3 times)

2. DNPH derivatization

DNPH solution (included with kit)	exactly 30 minutes (use 0.1 mL DNPH solution per slide. Control slide use 2% HCl)
-----------------------------------	--

3. Wash

2% HCl	5 minutes
80% Ethanol	5 minutes
100% Ethanol	5 minutes
50% Ethanol / 50% Ethyl acetate	5 minutes
80% Ethanol	1 minute
DDW (distilled deionized water)	1 minute (2 times)

4. Quench

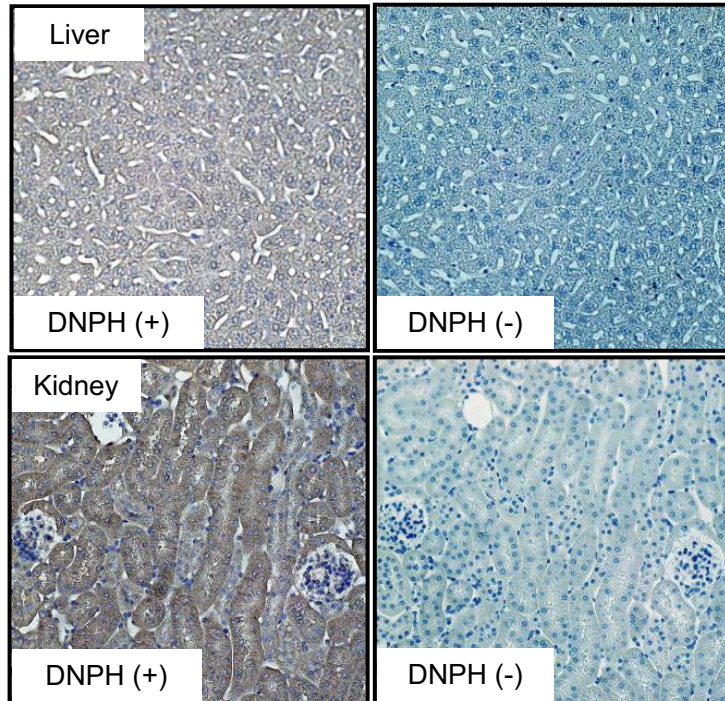
1% H ₂ O ₂ / Methanol	15 minutes
PBS	5 minutes (3 times)

5. Block

2%BSA / PBS-2% goat serum	30 minutes
(In the case of Avidin-Biotin system, Avidin solution (VECTOR, Inc.) should be add to blocking solution.)	
(use 0.1 mL blocking solution per slide)	
PBS	5 minutes (3 times)

6. Stain with anti-DNP

Rabbit anti-DNP antibody	1 hour
(1:100 in blocking solution)	
(In the case of Avidin-Biotin system, Biotin solution (e.g.VECTOR, Inc.) should be add to blocking solution.)	
(use 0.1 mL diluted primary antibody solution per slide)	
PBS	5 minutes (3 times)



Immunohistochemical staining

Mouse liver and kidney sections stained with rabbit anti-DNP antibody at 1:100 dilution and developed by 3,3'-diaminobenzidine (DAB). Specimens were incubated with (DNP(+)) or without (DNP(-)) DNP solution. Protein carbonyls are detected only in DNP-treated specimen and not detected at all in untreated specimen.

Reference:

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)

