# Protein Carbonyls Immunohistochemical Staining Kit (50 slides)

Product No. SML-R0IK04-EX

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| **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,<br>(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 containg 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 ($NaN_3$)* |
| **Reagents not included:** | Detection reagents for rabbit Ig.<br>(e.g., Elite ABC Kit (Rabbit IgG), Vector Labs) |

# Protein carbonyls immunohistochemical staining protocol

1. Deparaffinize (Methacarn fixed paraffin-embedded section)

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|---|---|
| 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

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|---|---|
| 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% $H_2O_2$ / 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)

7. Apply anti-rabbit Ig (e.g., Elite ABC Kit (VECTOR, Inc.))
   Biotinylated anti-rabbit IgG antibody 30 minutes
   (1:200 in PBS / 1.5% goat serum)
   (use 0.1 mL diluted secondary antibody solution per slide)
   PBS 5 minutes (3 times)

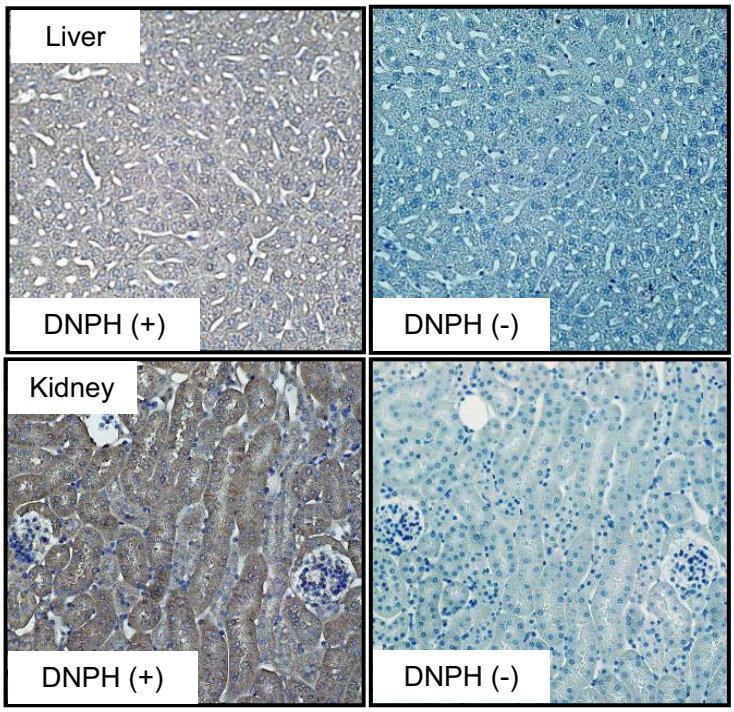
8. Avidin-Biotin-complex formation (for Elite ABC Kit (VECTOR, Inc.))
   2% A solution, B solution / PBS 30 minutes
   (Prepare 2% A solution, B solution / PBS about 30 minutes before use)
   (use 0.1 mL diluted Avidin-Biotin solution per slide)
   PBS 5 minutes (3 times)

9. Develop
   Dissolve DAB (3,3'-diaminobenzidine) tablet as recommended by manufacturer (e.g., SIGMA, Inc, DAKO, Inc.).
   (use 0.1 mL diluted DAB solution per slide)
   Rinse slides with DDW after Development

10. Counterstain
    Counterstain with Hematoxylin
    Rinse slides in tap water 10 - 15 minutes

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

12. Mount
    Mount coverslips on slides using mounting medium




**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 (DNPH(+)) or without (DNPH(-)) DNPH solution. Protein carbonyls are detected only in DNPH-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)