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Nitrotyrosine mAb (Clone 2E11) antibody

Quality Control Certificate of Analysis
Catalogue No.: A010-2E11
Unit Size: 20 µl
Lot No: 1211-01

Background: A large number of physiological and pathological events lead to so called stress conditions to which the organism can adapt within certain limits by regulating the activity of a series of enzymatic cascades. Most stress responses are characterized by an increased generation of “free radicals” which encompass both reactive oxygen (ROS) and nitrogen species (RNS). Most of these species react with macromolecules of the organism, i.e. amino acids, proteins, lipids and DNA, leading to functional alterations which can either participate to adaptation or lead to cell death. Oxidative stress however, has different functional consequences depending not only upon its intensity and duration, but also upon the nature of the free radicals, ROS or RNS, generated. Interestingly, the nature of these reactive species depends on the ratio between the initially produced ROS, superoxide anion (O2.-), and nitric oxide (NO). Indeed, O2.- very rapidly reacts with NO and therefore, as long as the NO/ O2.- ratio is = 1, O2.- will therefore preferentially react with NO rather than with macromolecules, thus generating reactive nitrogen species (RNS): nitrosonium (NO+), N2O3 and peroxinitrite (ONOO-). These RNS induce posttranslational modifications: for NO+ and N2O3, S-nitrosation (Cys-SNO) and for ONOO- tyrosine nitration (Tyr-NO2), methionine sulfoxidation (Met-SOH) and thiol oxidation (RS-S-R). When the O2./ NO ratio becomes > 1, the O2.- and NO.2 ions and thereafter the OH. radicals cause irreversible oxidations and peroxidations of macromolecules which generally lead to cell death(1-4). The monoclonal antibodies have been selected for their high affinity and specificity towards Tyr-NO2 residues. They do not cross-react with Tyr or Tyr derivatives such as aminotyrosine, chlorotyrosine or phosphotyrosine, neither with nitroTrp which can also be generated in response to peroxynitrite. They also recognize Tyr-NO2 residues in various sequences as shown by their ability to recognize nitrated proteins including albumin, ovalbumin, insulin, hemoglobin, KLH and various cytoplasmic and mitochondrial proteins.

Description: Lyophilised **Mouse** monoclonal affinity purified antibody (A010-2E11AP) containing IgG2b antibody specific for Nitrotyrosine

Immunogen: Protein-bound nitrotyrosine

Antibody Isotype: IgG2b.

Antibody Purity: Protein A affinity purified.

Vial Constituents: Lyophilised A010-2E11 (20 µl) in Phosphate Buffered Saline 10 mM, NaCl 0.15 M (pH 7.4) Thimerosal 0,01% may be used as preservative

Storage Instructions: Lyophilised antibody is stable at 4 °C when stored with desiccant. Reconstitute lyophilised powder in 20 µl of 18 MΩ H_2O, aliquot and store frozen at -80 °C for 1 year. Avoid freeze - thaw cycles.

Tested Applications: **WB 1:2000**

Antibody specificity / Cross Reactivity: Nitrotyrosine either free or incorporated in proteins such as human serum nitro albumin, nitro haemoglobin and nitro insulin. All species

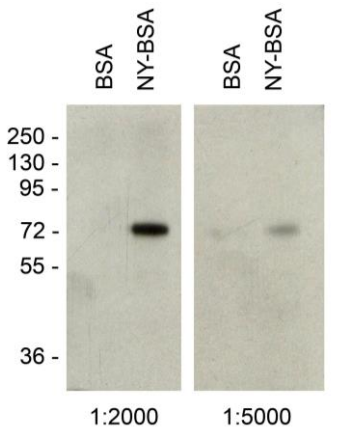

Figure 1

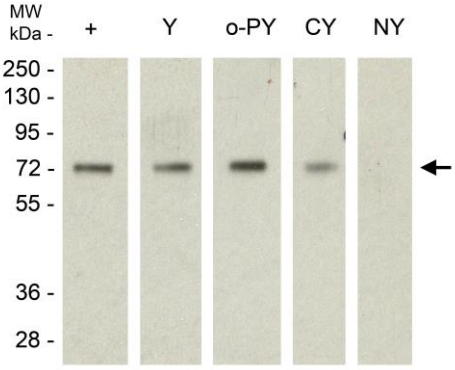

Figure 2

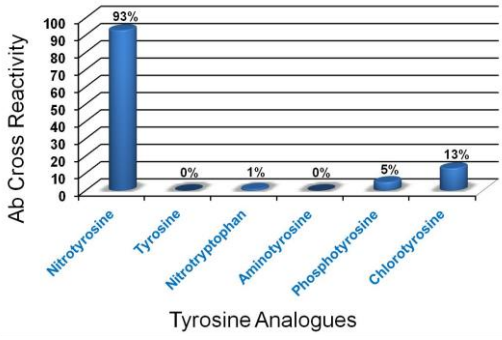

Figure 3

Figure 1: Western Blot using 1:2000 and 1:5000 Nitrotyrosine Clone 2E11-3D3 mouse monoclonal Ab (A010-2E11AP) against 10µg of BSA and NY-BSA. 10% gel, PVDF membrane

Figure 2: Western Blot using 1:2000 Clone 2E11-3D3 (A010-2E11AP) Ab against 10µg of NY-BSA +/- 100 µg/ml Tyrosine (Y), O-Phospho-L-Tyrosine (o-PY), 3-Chloro-L-Tyrosin (CY) and Nitrotyrosine (NY). 10% gel, PVDF membrane, 10 second film exposure. NY completely blocked Ab staining, CY caused some inhibition whereas Y and o-PY had no effect.

Figure 3:The cross reactivity of Tyrosine analogues with 2E11 Ab determined by competitive ELISA

Related Products: Anti-Nitro Tyrosine Clone 6B2 (A010-6B2AP)

Background references:

- García-Heredia, J. M., Díaz-Moreno, I., Nieto, P. M., Orzáez, M., Kocanis, S., Teixeira, M., Pérez-Payá, E., Díaz-Quintana, A., and De la Rosa, M. A. (2010) Biochim Biophys Acta 1797, 981-993

- Csibi, A., Communi, D., Müller, N., and Bottari, S. P. (2010) PLoS One 5, e10070