ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Anti-Nitrotyrosine Antibodies and positive control

Cat # NITT11-M	Mouse Monoclonal Anti-Nitrotyrosine IgG #1	**SIZE**: 100 ug
Cat # NITT12-A	Rabbit Anti-Nitrotyrosine IgG #2, aff pure	**SIZE**: 100 ug
Cat # NITT13-S	Goat Anti-Nitrotyrosine antiserum #3	**SIZE**: 100 ul
Cat # NITT12-C	Nitrated Mol. Wt protein Standard for Western	**SIZE**: 100 ul

Nitric oxide (NO) and reactive oxygen species (ROS) are important mediators to produce harmful or protective functions. NO can react with superoxide anions (O2.-), yielding the toxic oxidizing agent peroxynitrite (ONOO-). Peroxynitrite induces nitration of tyrosine residues leading to changes of protein structure and function, and alterations in signaling pathways. Antibodies to nitrotyrosine have been used to monitor the status of peroxynitrite-induced modification of proteins.

Source of Antigen and Antibodies

#NITT12-A

Antigen	Nitrated-KLH conjugate
Ab Host/type	Rabbit, Polyclonal antiserum # NITT12-S and IgG, purified over antigen-agarose (Cat # NITT12-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates
-ve control IgG	Cat # 20009-1, Rabbit (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

#NITT13-S

Antigen	Nitrated-KLH conjugate
Ab Host/type	Goat, Polyclonal antiserum (Cat # NITT13-S)
2-Ab	**Rabbit Anti-goat IgG-HRP conjugate** Cat # 30220 (AP, biotin, FITC conjugates
-ve control IgG	**# 20011-1, Goat (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control**

#NITT11-M

Antigen	Nitrated-KLH conjugate
Ab Host/type	Balb/c **mouse**. The clone producing an IgG1 isotype anti-nitrotyrosine has been expanded into mice as ascites and antibody purified by protein G affinity chromatography (Cat # **NITT1-M**). Polyclonal rabbit antibodies was also immunoaffinity
2-Ab	**Goat Anti-mouse IgG-HRP conjugate Cat # 40320 (AP, biotin, FITC conjugates also available)**)
-ve control IgG	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

#NITT12-C (10-20 blots) has a mixture of mol. Wt calibrated proteins (superoxide dismutase, ~16 kDa, occasionally a dimmer of ~32 kDa may also be seen; BSA ~66 kDa; and muscle myosin ~215 kDa) that were nitrated with peroxynitrite for use in Western blotting using the polyclonal anti-nitrotyrosine. Not recommended for use with monoclonal anti-nitrotyrosine. NITT12-C is supplied in SDS-PAGE sample buffer (reduced) for Western. Load 10 ul/lane of **NITT12**-C for good visibility with antibody Cat # **NITT12**-A or **# NITT13-S**. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **NITT12**-C solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. This preparation is intended for qualitative purpose and not to serve as standard of known concentration. Do not freeze, thaw, or hear repeatedly

Form & Storage

Monoclonal Antibody is supplied as protein A/G purified IgG in 10mM PBS, pH 7.4 and 0.1% sodium azide in solution or powder. Rabbit **polyclonal** IgG is provided in 10mM PBS, pH 7.4, 0.1% BSA, and 0.1% sodium azide. **Lyophilized** products should be reconstituted in 100 ul water and gently mixed for 15 min at room temp. All peptide/antibody received in solution or reconstituted from lyophilized vials should be stored frozen at -20°C or below in suitable aliquots. It is not recommended to store diluted solutions. Avoid repeated freeze and thaw.

Recommended Usage

Western Blotting: An initial testing of antibodies is recommended at 1-5 ug/ml for Western. Proteins extracted from human A431 cells or other cells pretreated with peroxynitrite for 5 min may serve as good positive control for Western. Users must optimize antibody dilution depending upon the nature of samples and other technical conditions.
ELISA (1:10-50K; using 50-100 ng control nitrated proteins/well).
Histochemistry & Immunofluorescence: An initial testing of 2-10 ug/ml is recommended. Users must optimize antibody dilution depending upon the nature of samples and other technical conditions.
Immunoprecipitation: We recommend antibody testing at ~5 ug/ml.

Specificity & Cross-reactivity

Antibodies should react with nitrated proteins from all species.

General References: **(1)** Viera L (1999) Methods Enzymol, 301:373; Beckman et al (1993) Nature 364, 584; Haddad et al (1994) J Clin. Invest. 94, 2407, Ayata (1997) J Neurosci. 17, 6908

(2) Citations of ADI's Antibodies (see web site for updated list)
Penkowa M, 2001, Neuroscience, 102, 805-818, **IHC.**
Carrasco,J, 2000, Eur. J. Neurosci. 12, 2311, **IHC.**
Penkowaa, M, 2001, J Neuroimmunol 119, 248-260 (**IHC**); Penkowa, 2003, Exp. Neurol. 181, 130 (IHC/IF)
Carrasco (2003) Neurobiol. Dis. 13, 22 (IHC);

*This product is for In vitro research use only.

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