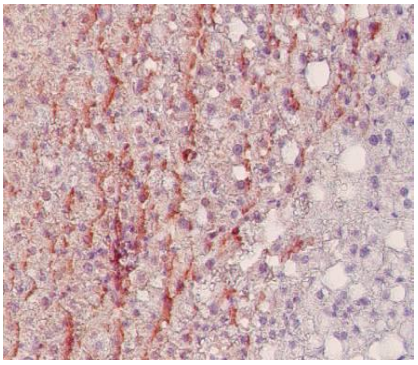
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	Nitrotyrosine, clone HM.11		
Catalog number	HM5001-20UG		
Lot number	-	**Expiry date**	-
Volume	200 µl	**Amount**	20 µg
Formulation	0.2 µm filtered in PBS+0.1%BSA	**Concentration**	100 µg/ml
Host Species	Mouse IgG2b	**Conjugate**	None
Endotoxin	N.A.	**Purification**	Protein G
Storage	4°C		

Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	1,3	4,5				1		2-8
Yes	●	●				●		●
No								
N.D.			●	●	●		●	

N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot



IHC: Nitrotyrosine in human liver of severely obesed patients. Staining of paraffin tissue section with clone HM.11 (Cat. # HM5001). Anti-nitrotyrosine at 2µg/ml (1h, RT).

Dilutions to be used depend on detection system applied. It is recommended that users test the reagent and determine their own optimal dilutions. The typical starting working dilution is 1:50.

- IHC-F: also cytospins: acetone fixation 10 min -20 °C; block endogenous peroxidase by 0.3 % H2O2 in PBS (or methanol for intracellular staining); blocking with 10% NGS or 5 % BSA for 30 min. use at assay dependent concentration.
- IHC-P:10% formalin fixation; 3% H2O2 to block endogenous peroxidases; Citrate buffer pH 6.0 for 1 min at 100 °C as antigen retrieval treatment; use at assay dependent concentration (1:200/400) (Ref 1).
- W: reduced and no-reduced samples; block with 5% BSA or skimmed milk, use at assay dependent concentration.
- Positive control: W: mouse kidney lysate, mouse optic nerve, retina, spinal cord and brain lysates, rat aorta lysate. IHC-P: human lung tissue.

General Information

Description

The monoclonal antibody HM.11 recognizes modified amino acid nitrotyrosine in all different species. Nitrotyrosine is formed in tissues in presence of the active metabolite NO and is a stable end product of nitrosylation of tyrosine. Inflammation is characterized by increased nitric oxide (NO) production. NO reacts rapidly with superoxide to form peroxynitrite. At physiological pH and in the presence of transition metals, peroxynitrite undergoes heterolytic cleavage to form hydroxyl anion and nitronium ion, the latter of which nitrates protein tyrosine residues. The presence of nitrotyrosine has been detected in various inflammatory processes including atherosclerotic plaques, Amyotrophic Lateral Sclerosis (ALS) and Multiple Sclerosis (MS). Thus, the presence of nitrotyrosine on proteins can be used as a marker for peroxynitrite formation in vivo and consequently as a marker of NO-mediated tissue damage. The

monoclonal antibody HM.11 recognizes nitrotyrosine, both with the free amino acid as well as with proteins containing nitrotyrosine.

Immunogen Nitrated KLH

Cross reactivity Phosphotyrosine: No; Chlorotyrosine: No

References

1. Ter Steege, J et al; Nitrotyrosine in plasma of celiac disease patients as detected by a new sandwich ELISA. Free Radic Biol Med 1998, *25*: 953
2. Klausz, G et al; Local and peripheral cytokine response and CagA status of Helicobacter pylori-positive patients with duodenal ulcer. Eur Cytokine Netw 2003, *14*: 143
3. Casoni, F et al; Protein nitration in a mouse model of familial amyotrophic lateral sclerosis. J Biol Chem 2005, *280*: 16295
4. Han, F et al; Protein nitration and poly-ADP-ribosylation in brain after rapid exsanguinations cardiac arrest in a rat modelof emergency preservation and resuscitation. Resuscitation 2008, *79*: 301
5. Tsuhako, H et al; Tempol ameliorates murine viral encephalomyelitis by preserving the blood-brain barrier, reducing viral load, and lessening inflammation. Free radic Biol Med 2010, *48*: 704
6. Brunelli, L et al. Exploratory investigation on nitro- and phospho-proteome cerebellum changes in hyperammonemia and hepatic encephalopathy rat models. Metabolic brain disease 2012, *27*:37
7. Nardo, G et al. Amyotrophic Lateral Sclerosis Multiprotein Biomarkers in Peripheral Blood Mononuclear Cells. Plos one 2011, *6*:e25545
8. Yip, P.K. et al. The Omega-3 Fatty Acid Eicosapentaenoic Acid Accelerates Disease Progression in a Model of Amyotrophic Lateral Sclerosis. Plos One 2013, *8*:61626

Storage&stability Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.

Precautions For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

We hereby certify that the above-stated information is correct and that this product has been successfully tested by the Quality Control Department. This product was released for sale according to the existing specifications. This document has been produced electronically and is valid without a signature.

Approved by Manager of QC	Date
Brenda Teunissen	13/01/2021

Do you have any questions or comments regarding this product? Please contact us via support@hycultbiotech.com.