Product Datasheet

Ubiquitin Antibody (Ubi-1) - BSA Free
NB300-130

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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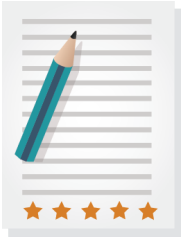
Updated 2/21/2025 v.20.1



NB300-130

Ubiquitin Antibody (Ubi-1) - BSA Free

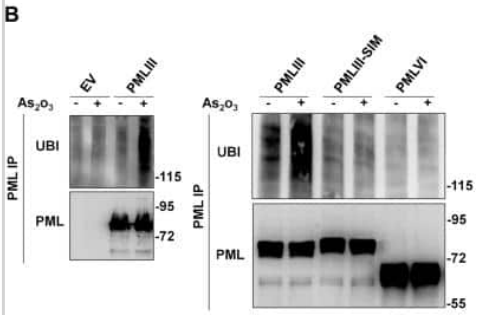
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	Ubi-1
Preservative	0.035% Sodium Azide
Isotype	IgG1
Purity	Affinity purified
Buffer	50% PBS, 50% glycerol
Target Molecular Weight	8.5 kDa

Product Description	
Host	Mouse
Gene ID	6233
Gene Symbol	RPS27A
Species	Human, Mouse, Rat, Porcine, Bovine, C. elegans, Chicken, Drosophila, Equine, Plant, Monkey, Zebrafish
Reactivity Notes	Plant reactivity reported in scientific literature (PMID: 19147500).
Specificity/Sensitivity	Recognizes polyubiquitin chains much more strongly than monoubiquitinated molecules or free ubiquitin. Specifically recognizes ubiquitinated cytoplasmic inclusion bodies.
Immunogen	Purified bovine erythrocyte ubiquitin cross-linked with glutaraldehyde to KLH

Product Application Details	
Applications	Western Blot, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000-1:2000, Immunohistochemistry 1:2000, Immunocytochemistry/ Immunofluorescence 1:2000, Immunohistochemistry-Paraffin 1:2000, Immunohistochemistry-Frozen 1:2000, Immunoblotting
Application Notes	This Ubiquitin Antibody (Ubi-1) is useful for Western blot, ELISA, Immunocytochemistry/Immunofluorescence, and Immunohistochemistry on both paraffin-embedded and frozen sections. In Western blot a band can be seen at 8.5 kDa. This antibody is excellent for the detection of ubiquitinated inclusions seen in human neurodegenerative diseases such as the neurofibrillary tangles of Alzheimer's disease. Use in immunoblotting reported in scientific literature (PMID: 31727601).

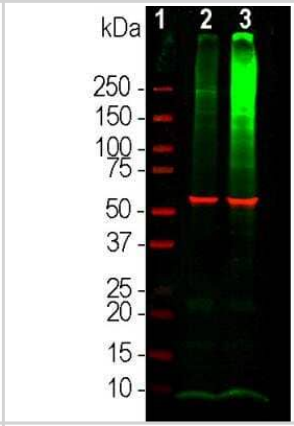
Images

Western Blot: Ubiquitin Antibody (Ubi-1) [NB300-130] - Ubiquitination of PML in response to As2O3. PML-/- MEFs were transiently transfected with the empty vector (EV) or PMLIII (left panel) or contructs encoding PMLIII, PMLIII-SIM or PMLVI (right panel). Two days later, they were treated with As2O3 for 1 h. The cell extracts were immunoprecipitated with rabbit anti-PML antibody and the immunoprecipitate was analyzed by Western blot using anti-ubiquitin and anti-PML antibodies. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0044949) licensed under a CC-BY license.

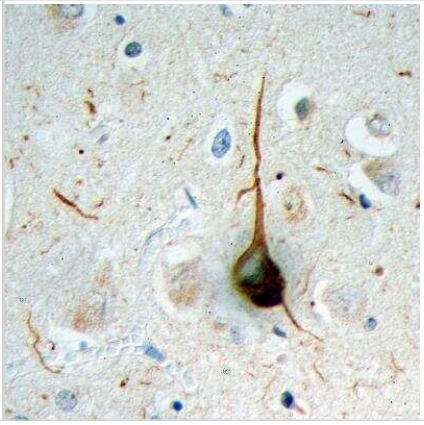


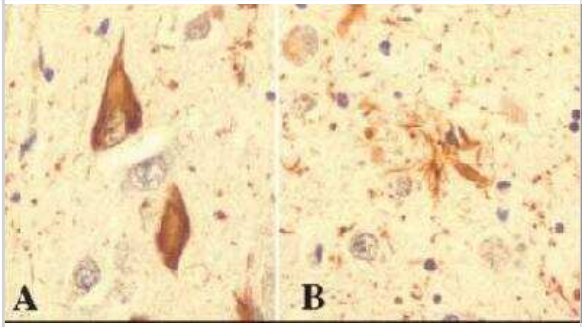
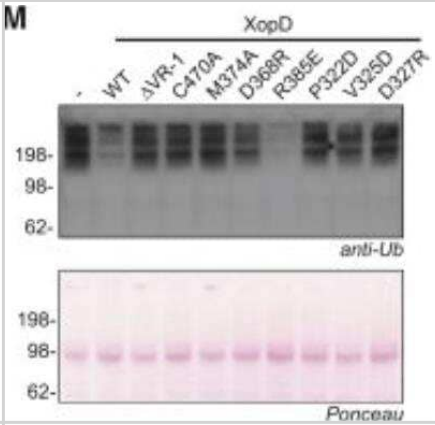
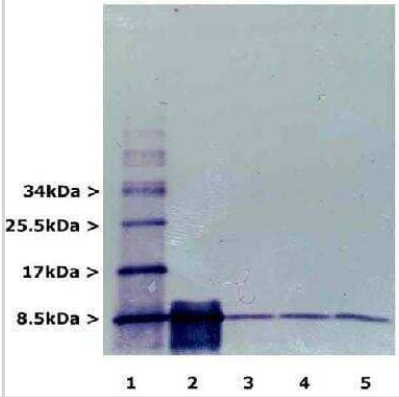
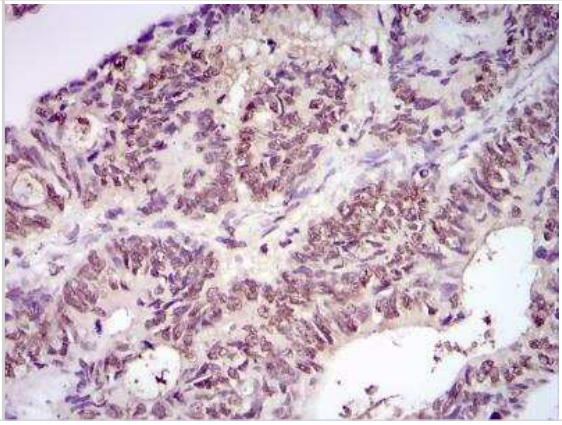
Immunocytochemistry/Immunofluorescence: Ubiquitin Antibody (Ubi-1) [NB300-130] - Study of mutant BRAF protein found within the inclusions. BRAFV600E/ubiquitin double-IF labeling. A nucleus with a large inclusion is shown with positive immunoreactivity for both mutant BRAF (red staining) and ubiquitin (green staining); the merged color yellow (arrow) points out the co-localisation of BRAFV600E and ubiquitin in the same inclusion. Image collected and cropped by CiteAb from the following publication (////doi.org/10.1371/journal.pone.0226199) licensed under a CC-BY license.

Western Blot: Ubiquitin Antibody (Ubi-1) [NB300-130] - Analysis of HEK293 cell lysates using mouse mAb Ubiquitin, dilution 1:1000 (Green). [1] protein standard (Red), [2] cells maintained in normal medium, [3] cells treated with 10uM of proteasome inhibitor lactacystin (Lc) for 16hrs. Lysed cells were electrophoresed on 4-20% SDS-PAGE and transferred to PVDF membranes. The smear detected above the 200kDa standard represent accumulation of ubiquitinated proteins in proteasome inhibitor-Lc treated cells. The prominent band at 8kDa corresponds to monoubiquitin. Rabbit pAb to HSP60, dilution 1:5000 (Red) was used as a loading control.

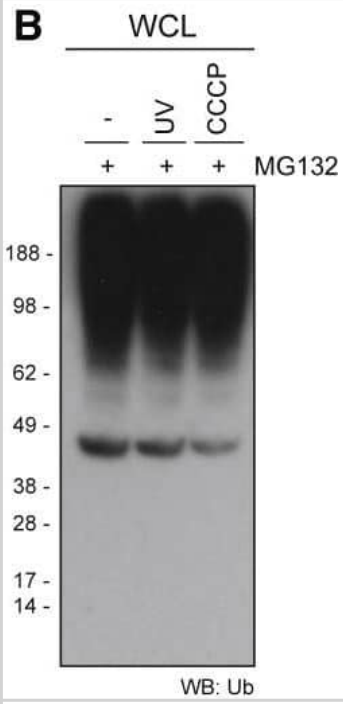


Immunohistochemistry-Paraffin: Ubiquitin Antibody (Ubi-1) [NB300-130] - FFPE section of cerebral cortex of an Alzheimer patient processed with Ubiquitin antibody using HRP/DAB (Brown) and also stained with haemotoxylin (Blue). A typical flame shaped tangle is seen in a pyramidal neuron in the center and is surrounded by some dystrophic neurites, also strongly ubiquitin positive. Both are commonly seen in cortical and hippocampal Alzheimer brain sections and are typical for this disease, but are rare or absent in healthy brain.

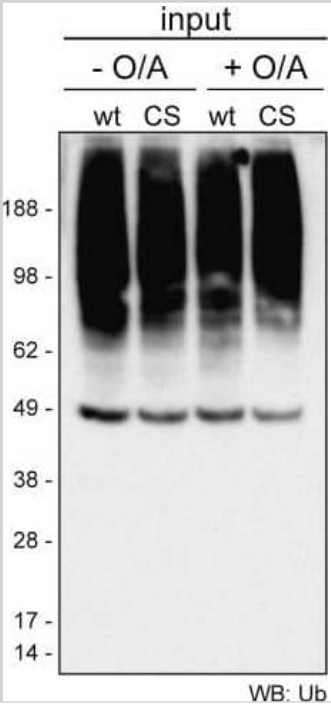


Immunohistochemistry-Paraffin: Ubiquitin Antibody (Ubi-1) [NB300-130] - Staining of Ubiquitin in hippocampal tissue from an Alzheimer patient.	
Western Blot: Ubiquitin Antibody (Ubi-1) [NB300-130] - Molecular Analysis of XopD Ub/Ubl Specificity. S. lycopersicum protein extract blotted for total Ub (Ubiquitin; Novus Biologicals) following a 1 hr room temperature treatment with the XopD variants used in (K) at 1 uM final concentration. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27425412/) licensed under a CC-BY license.	
Western Blot: Ubiquitin Antibody (Ubi-1) [NB300-130] - Western blot of Ubiquitin expression on (Lane 1) poly-ubiquitin (lys63 linked), (Lane 2) pure ubiquitin and crude homogenates of adult rat, (Lane 3) cortex, (Lane 4) cerebellum and (Lane 5) brain stem.	
Immunohistochemistry-Paraffin: Ubiquitin Antibody (Ubi-1) [NB300-130] - IHC staining of Ubiquitin in human rectal cancer using DAB with hematoxylin counterstain.	

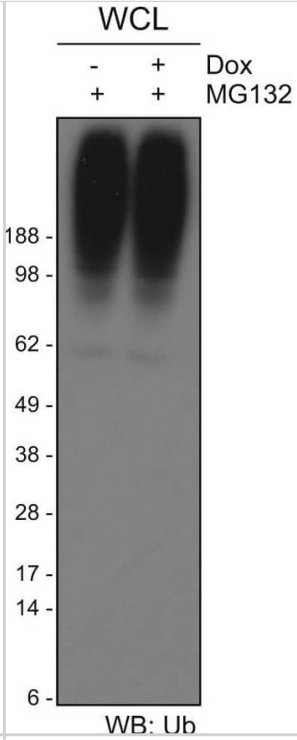
Western Blot: Ubiquitin Antibody (Ubi-1) [NB300-130] - In Vivo Applications of Affimers(A) HA-NleL 293 T-Rex cells were induced with 1 µg/mL doxycycline for 12 hr or left untreated. Whole-cell lysate (WCL) blots are shown for actin, HA(-NleL), Ub, & K6 chains. Western blots with the K6 affimer are also shown after Ub enrichment using TUBEs.(B) TUBE-PD of HEK293 cells after 1 hr of MG132 (10 µM) without further treatment (−) or with additional UV (40 J/m2) or CCCP treatment (10 µM for 1 hr) & subsequently blotted with the K6 affimer. Input controls are shown for total Ub & actin & γH2AX. The relative signal increase from two experiments is shown below the respective lanes.(C) Expression of WT or catalytically inactive (C431S) Parkin in HeLa Flp-In cells was induced with 0.2 µg/mL doxycycline for 16 hr. Mitochondria were depolarized with O/A for 2 hr. WCL inputs are shown for total Ub, expressed Parkin, TOM20, & actin. The TUBE-PD was also blotted using the K6 affimer & total Ub.(D) Confocal fluorescence microscopy images of cells as in (B) stained with K6 affimer (green), TOM20 (red), & DAPI (blue). Cells & a magnified area are outlined in white. Scale bars correspond to 20 µm.See also Figure S5. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28943312), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



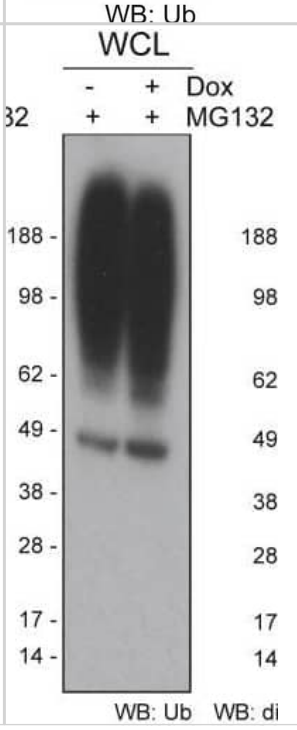
Western Blot: Ubiquitin Antibody (Ubi-1) [NB300-130] - In Vivo Applications of Affimers(A) HA-NleL 293 T-Rex cells were induced with 1 µg/mL doxycycline for 12 hr or left untreated. Whole-cell lysate (WCL) blots are shown for actin, HA(-NleL), Ub, & K6 chains. Western blots with the K6 affimer are also shown after Ub enrichment using TUBEs.(B) TUBE-PD of HEK293 cells after 1 hr of MG132 (10 µM) without further treatment (−) or with additional UV (40 J/m2) or CCCP treatment (10 µM for 1 hr) & subsequently blotted with the K6 affimer. Input controls are shown for total Ub & actin & γH2AX. The relative signal increase from two experiments is shown below the respective lanes.(C) Expression of WT or catalytically inactive (C431S) Parkin in HeLa Flp-In cells was induced with 0.2 µg/mL doxycycline for 16 hr. Mitochondria were depolarized with O/A for 2 hr. WCL inputs are shown for total Ub, expressed Parkin, TOM20, & actin. The TUBE-PD was also blotted using the K6 affimer & total Ub.(D) Confocal fluorescence microscopy images of cells as in (B) stained with K6 affimer (green), TOM20 (red), & DAPI (blue). Cells & a magnified area are outlined in white. Scale bars correspond to 20 µm.See also Figure S5. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28943312), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Ubiquitin Antibody (Ubi-1) [NB300-130] - HUWE1 Assembles K6 Chains In Vitro & In Vivo(A) Table summarizing proteins identified with the corresponding number peptide-spectrum matches (PSMs) in three replicates of DUB-treated K6 affimer pull-downs.(B) In vitro assembly reaction of the HECT E3 HUWE1 with Ub WT, Ub K6R, Ub K11R, & Ub K48R on Coomassie. Arrows indicate K6 diUb.(C) Linkage composition of HUWE1-generated diUb after 1 hr as determined by AQUA MS.(D) AQUA-derived total cellular chain composition of HUWE1−/− HeLa & parental cells after TUBE-based enrichment. Error bars indicate mean ± SD from n = 3. □p < 0.05, according to a two-tailed Student's t test. N.S., not significant.(E) TUBE-PD from a doxycycline-inducible HUWE1 shRNA Ls174T cell line blotted with the K6-specific affimer, with input controls for actin, total Ub, & HUWE1.(F) K6 affimer pull-down in doxycycline-inducible HUWE1 shRNA Ls174T cells blotted against Mfn2. Cells were left untreated or treated with 10 µg/mL MG132 for 4 hr and/or 1 µg/mL doxycycline for 72 hr. Pull-downs were incubated with 250 nM USP21 as indicated, with a K48 blot to show completeness of the deubiquitination reaction. Input controls are shown for Mfn2, actin, & HUWE1.See also Figure S7. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28943312), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Ubiquitin Antibody (Ubi-1) [NB300-130] - In Vivo Applications of Affimers(A) HA-NleL 293 T-Rex cells were induced with 1 µg/mL doxycycline for 12 hr or left untreated. Whole-cell lysate (WCL) blots are shown for actin, HA(-NleL), Ub, & K6 chains. Western blots with the K6 affimer are also shown after Ub enrichment using TUBEs.(B) TUBE-PD of HEK293 cells after 1 hr of MG132 (10 µM) without further treatment (−) or with additional UV (40 J/m2) or CCCP treatment (10 µM for 1 hr) & subsequently blotted with the K6 affimer. Input controls are shown for total Ub & actin & γH2AX. The relative signal increase from two experiments is shown below the respective lanes.(C) Expression of WT or catalytically inactive (C431S) Parkin in HeLa Flp-In cells was induced with 0.2 µg/mL doxycycline for 16 hr. Mitochondria were depolarized with O/A for 2 hr. WCL inputs are shown for total Ub, expressed Parkin, TOM20, & actin. The TUBE-PD was also blotted using the K6 affimer & total Ub.(D) Confocal fluorescence microscopy images of cells as in (B) stained with K6 affimer (green), TOM20 (red), & DAPI (blue). Cells & a magnified area are outlined in white. Scale bars correspond to 20 µm.See also Figure S5. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28943312), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Hamamoto K, Liang X, Ito A et Al. Unveiling the physiological impact of ESCRT-dependent autophagosome closure by targeting the VPS37A ubiquitin E2 variant-like domain Cell Rep 2024-11-27 [PMID: 39607828]

Michel MA, Scutts S, Komander D., et Al. Secondary interactions in ubiquitin-binding domains achieve linkage or substrate specificity Cell Rep 2024-07-25 [PMID: 39052481]

Volik PI, Zamaraev AV, Egorshina AY et Al. Ally or traitor: the dual role of p62 in caspase-2 regulation Cell Death Dis 2024-11-14 [PMID: 39543123]

Muhammad T, Edwards SL, Morphis AC et al. Non-cell-autonomous regulation of germline proteostasis by insulin/IGF-1 signaling-induced dietary peptide uptake via PEPT-1 The EMBO Journal 2024-09-16 [PMID: 39284915]

RB Damgaard, PR Elliott, KN Swatek, ER Maher, P Stepensky, O Elpeleg, D Komander, Y Berkun OTULIN deficiency in ORAS causes cell type-specific LUBAC degradation, dysregulated TNF signalling and cell death EMBO Mol Med, 2019-03-01;11(3):. 2019-03-01 [PMID: 30804083]

Haiquan Lu, Yajing Lyu, Linh Tran, Jie Lan, Yangyiran Xie, Yongkang Yang, Naveena L Murugan, Yueyang J Wang, Gregg L Semenza HIF-1 recruits NANOG as a coactivator for TERT gene transcription in hypoxic breast cancer stem cells. Cell reports 2022-02-10 [PMID: 34592152]

Qingpeng Xie, Bin Hu, Haosong Li Acetylation- and ubiquitination-regulated SFMBT2 acts as a tumor suppressor in clear cell renal cell carcinoma Biology Direct 2024-05-11 [PMID: 38734627]

Robichaud S, Fairman G, Vijithakumar V et al. Identification of novel lipid droplet factors that regulate lipophagy and cholesterol efflux in macrophage foam cells Autophagy 2021-02-26 [PMID: 33590792]

Li L, Guturi KKN, Gautreau B et al. Ubiquitin ligase RNF8 suppresses Notch signaling to regulate mammary development and tumorigenesis. J. Clin. Invest. 2018-10-01 [PMID: 30222135]

Mohan AK Regulation of Inflammatory Signalling by Caspases and M1-linked Ubiquitin Chains in Drosophila melanogaster Thesis 2023-01-01 [PMID: 35263507]

Chomiak AA, Guo Y, Kopsidas CA et al. Nde1 is required for heterochromatin compaction and stability in neocortical neurons iScience 2022-06-17 [PMID: 35601919]

Hackerova L, Klusackova B, Zigo M et al. Modulatory effect of MG-132 proteasomal inhibition on boar sperm motility during in vitro capacitation Frontiers in veterinary science 2023-03-23 [PMID: 37035827] (FLOW, Porcine)

Details:
Duroc boars sperm; dilution: 1:10

More publications at http://www.novusbio.com/NB300-130

Procedures

Immunohistochemistry-Paraffin Protocol Specific for NB300-130: Ubiquitin Antibody (Ubi-1)

Materials

1) 1 Phosphate buffered saline (pH 7.6): NaCl 137mmol/L, KCl 2.7mmol/L, Na2HPO4 4.3mmol/L, KH2PO4 1.4 mmol/L
2) Citrate buffer, 0.01 M, pH6.0, Sodium Citrate 3g, Citric acid 0.4g
3) 3% Hydrogen peroxide
4) Primary antibody
5) Blocking serum (normal serum)
6) Biotinylated secondary antibody
7) DAB staining kit

Methods

1. Dewax and hydration of slides using xylene and EtOH:
Dry slides for 20 min in a 60 C oven
Add Xylene, 2 x 10 min
100%, 95%, 80%, and 70% EtOH, 5 min each EtOH concentration
Rinse in PBS, 5'

2 Antigen retrieval method (only for paraffin slides)
1a. High-pressure antigen retrieval procedure (recommended method)
Place slides in a glass slide holder (ensure that the slide holder is completely filled with slides, slides without sections if necessary, to ensure even heating. The entire slide holder is immersed in 1000 ml of Citrate buffer (0.01M, pH6.0) within a pressure cooker
Once steam is produced, and ONLY when steam is visible, from the pressure cooker (usually 15-20 min), the required high-pressure will have been reached, and slides will be incubated for 2 min.
Turn off heat, and allow buffer and slides to cool to room temperature
Slides are then rinsed in PBS for 5 minutes
2. Add 3% hydrogen peroxide solution, 10'at RT, then PBS, 3X5'
3. Normal blocking serum, 20'at RT
4. Incubate with Primary Ab, 4C overnight or 1.5 hours at 37C
5. Rinse with PBS, 3 X 5' each rinse
6. Add Biotin-conjugated second antibody, 10'at RT
7. Rinse with PBS, 3 X 5' each rinse
8. Add Streptavidin-Peroxidase, 10'at RT
9. Rinse with PBS, 3 X 5' each rinse
10. Staining with DAB solution, 2-5'under microscope
11. Stop the reaction by washing in tap water
12. Counterstain in Haematoxylin for 3-5 minutes
13. 75%, 80%, 95% and 100% ethanol, 5x2', xylene 2 x 10'

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Products Related to NB300-130

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NBP2-58398PEP	Ubiquitin Recombinant Protein Antigen

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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