

# Product Datasheet

## Palladin Antibody (1E6) - BSA Free NBP1-25959

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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**NBP1-25959**

Palladin Antibody (1E6) - BSA Free

<b>Product Information</b>	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	1E6
<b>Preservative</b>	0.05% Sodium Azide
<b>Isotype</b>	IgG1
<b>Purity</b>	Protein G purified
<b>Buffer</b>	Tris-Glycine and 0.15M NaCl
<b>Target Molecular Weight</b>	85 kDa
<b>Product Description</b>	
<b>Description</b>	Novus Biologicals Mouse Palladin Antibody (1E6) - BSA Free (NBP1-25959) is a monoclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-Palladin Antibody: Cited in 20 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Mouse
<b>Gene ID</b>	23022
<b>Gene Symbol</b>	PALLD
<b>Species</b>	Human, Mouse, Rat, Canine, Chicken
<b>Reactivity Notes</b>	Canine reactivity reported in scientific literature (PMID: 23451256).
<b>Immunogen</b>	Endogenous Palladin immunoprecipitated from chicken embryo fibroblasts.
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Knockdown Validated
<b>Recommended Dilutions</b>	Western Blot 1 ug/ml, Flow Cytometry 5 ug/ml, Immunohistochemistry 1:50-1:200, Immunocytochemistry/ Immunofluorescence 1:20-1:100, Immunoprecipitation 0.05 ml / 2 ml lysate, Immunohistochemistry-Paraffin 1:50-1:100, Knockdown Validated
<b>Application Notes</b>	In WB a band is seen at ~85 kDa. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

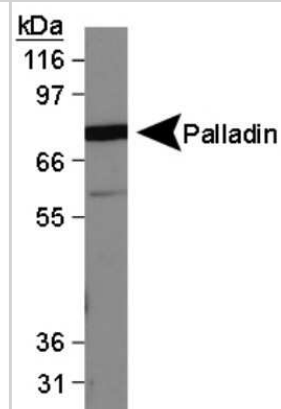


## Images

Immunocytochemistry/ Immunofluorescence: Palladin Antibody (1E6) [NBP1-25959] - C2C12 cells were transfected with shRNA targeting palladin or nontargeting shRNA as a control. Stable transfectants were submitted to a differentiation medium and followed for 5 days in culture (D0 to D5). Myogenic differentiation was assessed at the indicated time points using RNA/protein analysis and immunofluorescence staining. Immunofluorescence images of stable transfectants labeled with palladin antibody (green) and F-actin (red). Blue is DAPI-stained nuclei. Scale bar is 10  $\mu$ m. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0124762>) licensed under a CC-BY license.



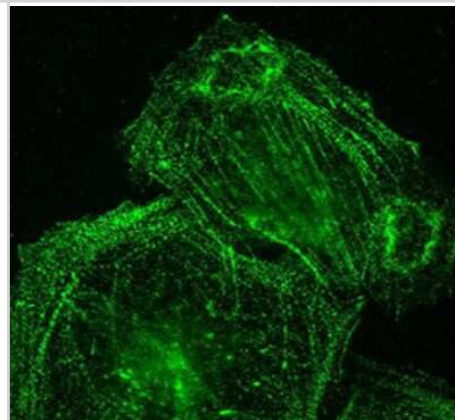
Western Blot: Palladin Antibody (1E6) [NBP1-25959] - MDA-MB-231 lysate.



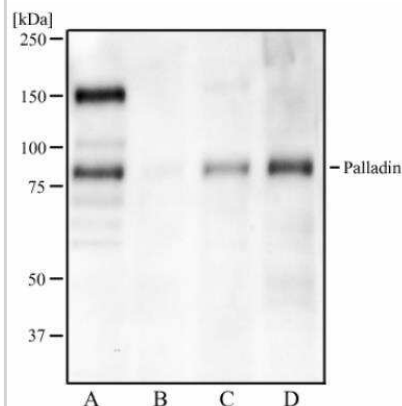
Western Blot: Palladin Antibody (1E6) [NBP1-25959] - Western blots analysis of C2C12 cells labeled with a monoclonal antibody against 90-kDa palladin or a polyclonal antibody against 140- and 200-kDa palladin, MyHC, myogenin, and MLC. The blots clearly show the decrease in palladin expression concomitant with the elevation of differentiation markers. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0124762>) licensed under a CC-BY license.



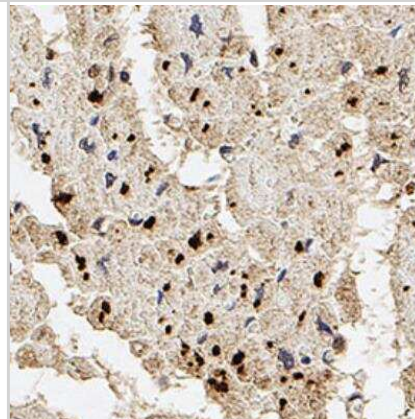
Immunocytochemistry/Immunofluorescence: Palladin Antibody (1E6) [NBP1-25959] - Immunofluorescence on a7r5 cells.



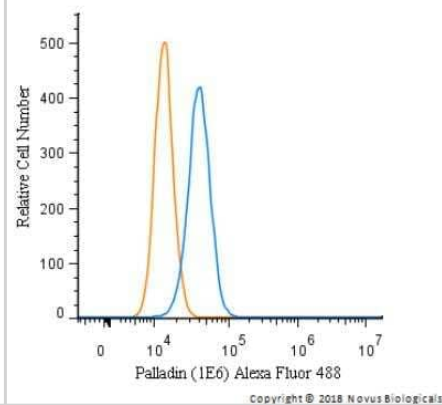
**Western Blot: Palladin Antibody (1E6) [NBP1-25959]** - Western blot analysis of HeLa (A), A431 (B), NIH3T3 (C), and PC-12 (D) cell line extracts using Palladin antibody at 1ug/ml.



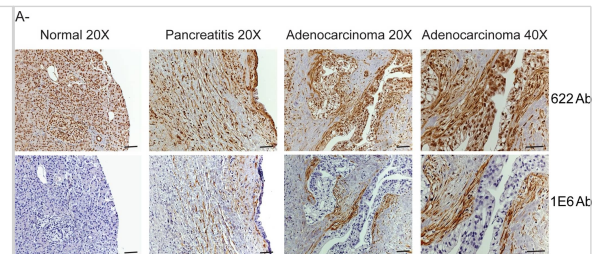
**Immunohistochemistry-Paraffin: Palladin Antibody (1E6) [NBP1-25959]** - Palladin was detected in immersion fixed paraffin-embedded sections of human heart using Mouse Anti-Human Palladin (1E6) Monoclonal Antibody (Catalog # NBP1-25959) at 1:100 for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cytoplasm in cardiomyocytes.



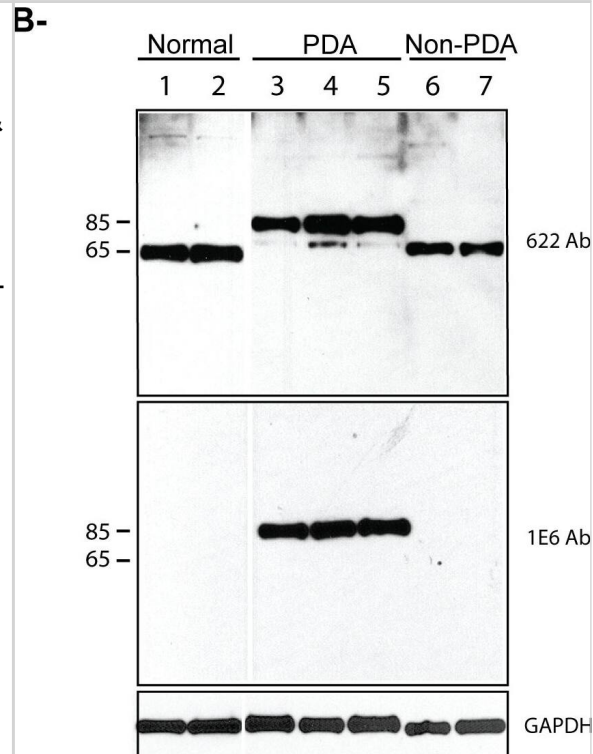
**Flow Cytometry: Palladin Antibody (1E6) [NBP1-25959]** - An intracellular stain was performed on HeLa cells with Palladin Antibody [1E6] NBP1-25959AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



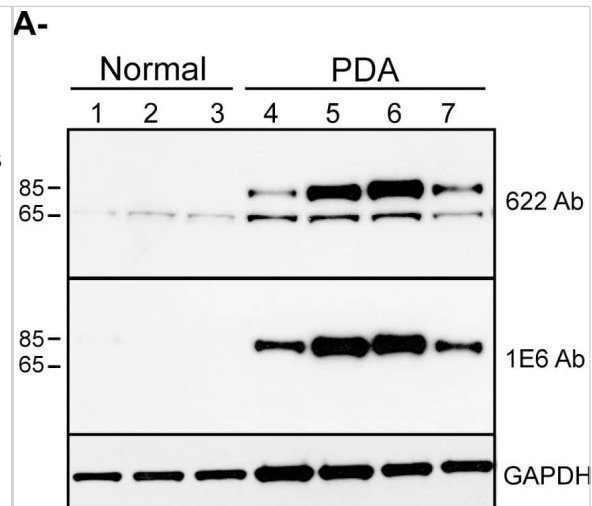
**Immunohistochemistry: Palladin Antibody (1E6) [NBP1-25959] - Palladin staining of paraffin-embedded patient tissues.** A. IHC staining was performed using standard antigen-retrieval protocols, & counter-stained with hematoxylin. Tissue sections were stained for palladin using two palladin antibodies: polyclonal 622 & monoclonal 1E6. Palladin stain is detected with brown reaction product. In tumor sections, palladin is detected at dramatically elevated levels in the stromal fibroblasts. Note also the expanded stroma around the neoplastic cells, which is characteristic of the desmoplastic reaction. Scale bars, 200  $\mu$ M. B. Quantification of immunohistochemistry results. Ten sections each of normal pancreatitis & adenocarcinoma specimens were stained with four different antibodies (622, COM, 1E6 & 4D10) & scored by two pathologists, as described in the text. Results for both ductal epithelium (left) & stroma (right) stained with various palladin antibodies are shown for normal pancreas (n=9, blue), pancreatitis (n=7, red) & pancreatic adenocarcinoma (n=10, yellow). The results confirmed that palladin levels are increased in the stroma, & not the epithelial tumor cells, of the adenocarcinomas. Although palladin levels are also increased in cases of chronic pancreatitis, they do not reach the same levels as in the tumors. Compared to the polyclonal 622 & COM, the monoclonal antibodies 1E6 & 4D10 are effective at distinguishing between pancreatitis & cancer. C. Double-label immunostaining for palladin (1E6 Ab) &  $\alpha$ -SMA in sections of pancreatic tumors confirms that palladin is strongly detected in a population of activated TAFs that surround the neoplastic cells. Scale bars, 200  $\mu$ M. Image collected & cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0010347>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



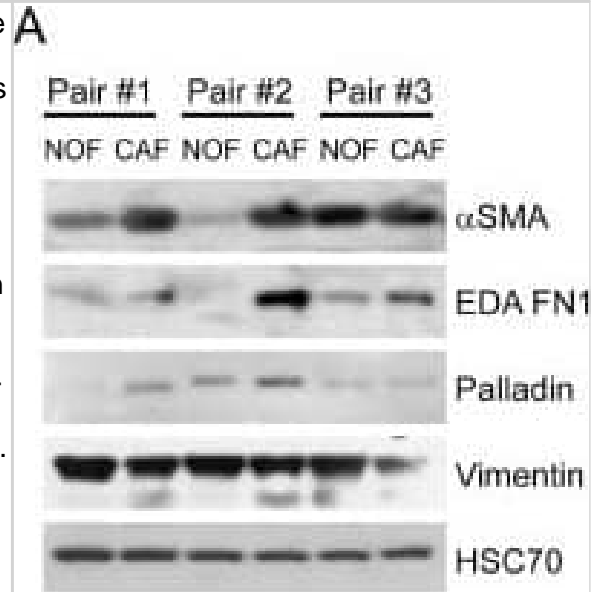
**Western Blot: Palladin Antibody (1E6) [NBP1-25959] - Analysis of human palladin isoforms in pancreatic tissues.** A. Human palladin isoforms. Proline-rich domains are represented by red boxes, & Ig-like domains are shown as blue boxes. The epitope recognized by the 1E6 & 4D10 antibodies is highlighted in green. The region amplified by RT-qPCR is highlighted in light blue. Isoform #1, 3 & 4 are the primary products of the palladin gene & have been detected by immunoblotting. The sequences of these isoforms are published. The sequences of isoforms #2, 5, 6, & 7 were obtained from genomic databases. "ND": not-determined. B. Western blot analysis of pancreas samples. Small pieces of fresh tissue were snap-frozen in liquid nitrogen, ground in a chilled mortar & pestle, extracted in a detergent-containing lysis buffer, & centrifuged at 15,000 $\times$ g to remove any unsolubilized particulates. The supernatant was boiled in Laemmli sample buffer & resolved by SDS-PAGE, with 15  $\mu$ g protein loaded per lane. The samples were immunoblotted & probed with two anti-palladin antibodies & an antibody to GAPDH (a housekeeping gene) as a control for equal loading. Lanes 1–2: normal pancreas. Lanes 3–5: primary adenocarcinoma tumors (PDA). Lane 6–7: Non-primary adenocarcinoma tumors (Non-PDA) (Lane 6: solid pseudopapillary tumor, Lane 7: neuroendocrine tumor). C. RT-qPCR. Total RNA was isolated from normal tissue (patients 1–4) & PDA tumors (patients 1–3), reverse transcribed, & subjected to RT-qPCR using gene-specific primers. Each bar represents the mean + SEM (0.06–0.35%) from three or more independent determinations. Image collected & cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0010347>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



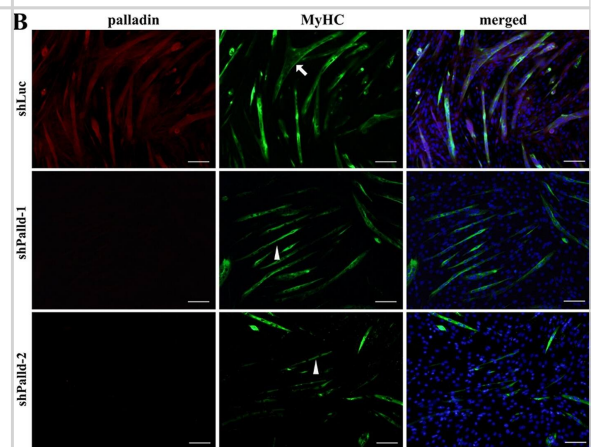
Western Blot: Palladin Antibody (1E6) [NBP1-25959] - Detection of palladin in post-surgical samples collected with 18-gauge needles. A. Samples of normal (lane 1 to 3) & pancreatic adenocarcinoma (lanes 4 to 7) were obtained from donated post-surgical organs using 18-gauge needles. Tissue samples were snap-frozen, ground, lysed & analyzed as in Figure 1. The blot was stained with both monoclonal (1E6) & polyclonal (622) palladin antibodies, & the major band (85–90 kDa) was detected by both antibodies in all tumor samples. B. Same samples (normal, lane 1–3 & PDA, lane 4–7) were analyzed for epithelial vs myofibroblast markers. The blot was stained with both, anti-E-cadherin antibody (as an epithelial cell marker) & anti- $\alpha$ SMA antibody (as a myofibroblast marker). Blots were stained for tubulin as a control for equal loading. Image collected & cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0010347>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Palladin Antibody (1E6) [NBP1-25959] - CAFs & NOFs are biochemically & morphologically different & CAF exosomes can also be transferred to CRC cells(A) Western blot of paired primary NOFs & CAFs for myofibroblastic markers alpha-smooth muscle actin ( $\alpha$ -SMA), fibronectin ED-A (ED-A FN1), palladin & vimentin. HSC-70 was used as an equal loading control. (B) Light microscopy of representative primary NOF & CAF cells (10x). (C) Fluorescence microscopy demonstrating phalloidin staining of F-actin filaments (green), counterstained with DAPI (blue; 40x). (D) Mean surface area & (E) intensity of phalloidin staining in a representative NOF-CAF pair. (F) Flow cytometry of DLD1 cells (control) & DLD1 cells co-cultured with CAF exosomes (exosome). The proportion of cells under the M1 region is given as a percentage. (G) Co-culture of CAF exosomes with DLD1 & SW480 cells with resultant increase in miR-199b & miR-21-5p. Data is presented as mean  $\pm$  SEM. Student's t-test (D, E) or paired t-test (F, G): \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/29283887>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Palladin Antibody (1E6) [NBP1-25959] - Effect of palladin depletion on terminal differentiation of C2C12 cells.(A) Phase contrast images of stable transfectants at late stages of differentiation. Scale bar is 50  $\mu$ m. (B) Representative immunofluorescence images of stable transfectants at day 5 of differentiation. Cells were labeled with palladin (red), MyHC (green), & DAPI (blue). Scale bar is 100  $\mu$ m. (C) Fusion index analysis of stable transfectants at day 5 (left) & day 7 (right) of differentiation. A minimum of 4,000 nuclei were counted from random fields of each cell line. Note that palladin depletion resulted in a decrease of the fusion index at the late stage of differentiation. (D) Quantification of multinucleated myotubes throughout the course of differentiation. (E) Quantification of the number of MyHC-positive cells in stable transfectants. All error bars indicate the means  $\pm$  SD of at least four independent experiments. \* indicates statistically significant difference from control cells, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  by Student's t-test. ns = not significant. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/25875253>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Mastrototaro G, Carullo P, Zhang J et al. Ablation of palladin in adult heart causes dilated cardiomyopathy associated with intercalated disc abnormalities eLife 2023-03-16 [PMID: 36927816] (Immunoprecipitation, Western Blot, Immunocytochemistry/ Immunofluorescence, Avian - Chicken, Mouse)

Wang Y, Wang Y, Zhu Y, Yu P et Al. Angiomin cleavage promotes leader formation and collective cell migration Dev Cell 2024-10-10 [PMID: 39389053]

Massimiliano Mellone, Christopher J. Hanley, Steve Thirdborough, Toby Mellows, Edwin Garcia, Jeongmin Woo et al. Induction of fibroblast senescence generates a non-fibroblastic myofibroblast phenotype that differentially impacts on cancer prognosis Aging (Albany NY) 2016-12-15 [PMID: 27992856]

Mayer O, Bugis J, Kozlova D et al. Cytoskeletal Protein Palladin in Adult Gliomas Predicts Disease Incidence, Progression, and Prognosis Cancers 2022-10-19 [PMID: 36291914] (IF/IHC, Mouse)

Davidson B, Bock Aj, Holth A, Nymoen Da Expression of palladin is associated with disease progression in metastatic high-grade serous carcinoma Cytopathology 2020-08-02 [PMID: 32741023] (IF/IHC, Human)

Dhanda A Morphological and functional characterization of host proteins during infections by actin-hijacking bacterial pathogens Thesis 2020-01-01 (ICC/IF, Human, Mammal)

Details:

Potorous tridactylus kidney

Dhanda AS, Vogl AW, Albraiki SE et al. Palladin Compensates for the Arp2/3 Complex and Supports Actin Structures during Listeria Infections MBio 2018-04-10 [PMID: 29636431] (ICC/IF, WB, Human)

Stasiak M, Gawryś K, Popielarski M et al. Differential Quantitative Proteomics of Human Microvascular Endothelial Cells 1 by iTRAQ Reveals Palladin to be a New Biomarker During TGF- $\beta$ 1 Induced Endothelial Mesenchymal Transition Journal of Proteomics & Bioinformatics 2017-10-11 (WB, Human)

Bhome R, Goh RW, Bullock MD et al. Exosomal microRNAs derived from colorectal cancer-associated fibroblasts: role in driving cancer progression Aging (Albany NY) 2017-12-28 [PMID: 29283887] (WB, Human)

Li S, Liu C, Gu L et al. Autophagy protects cardiomyocytes from the myocardial ischaemia-reperfusion injury through the clearance of CLP36. Open Biol. 2016-08-01 [PMID: 27512143] (Mouse)

Morishige N, Murata S, Nakamura Y et al. Coordinated Regulation of Palladin and alpha-Smooth Muscle Actin by Transforming Growth Factor- $\beta$  in Human Corneal Fibroblasts. Invest. Ophthalmol. Vis. Sci. 1905-07-08 [PMID: 27367503] (ICC/IF, Human)

van Steenbeek FG, Van den Bossche L, Grinwis GC et al. Aberrant Gene Expression in Dogs with Portosystemic Shunts. PLoS One 2013-01-01 [PMID: 23451256] (IHC-P, Canine)

More publications at <http://www.novusbio.com/NBP1-25959>

## Procedures

### Protocol specific for PALLD Antibody (NBP1-25959)

Procedure Guide for NBP1-25959 - Palladin Antibody

Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH<sub>2</sub>O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH<sub>2</sub>O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the mouse anti-Palladin primary antibody (NBP1-25959) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH<sub>2</sub>O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce ECL).

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided

it does not interfere with antibody-antigen binding.

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### **Products Related to NBP1-25959**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

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### **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.

For more information on our 100% guarantee, please visit [www.novusbio.com/guarantee](http://www.novusbio.com/guarantee)

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