Product Datasheet

APE Antibody - BSA Free
NB100-101

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Reviews: 3 Publications: 77

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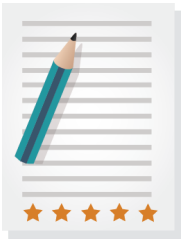
Updated 4/13/2025 v.20.1



NB100-101

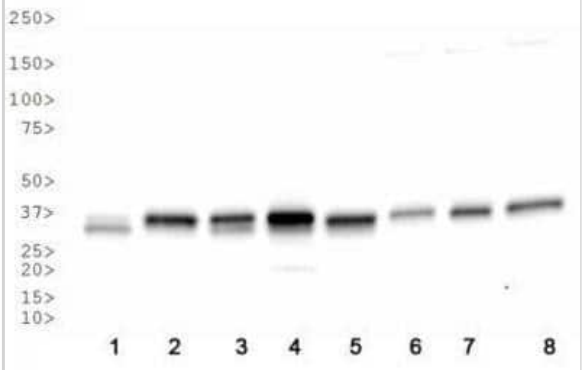
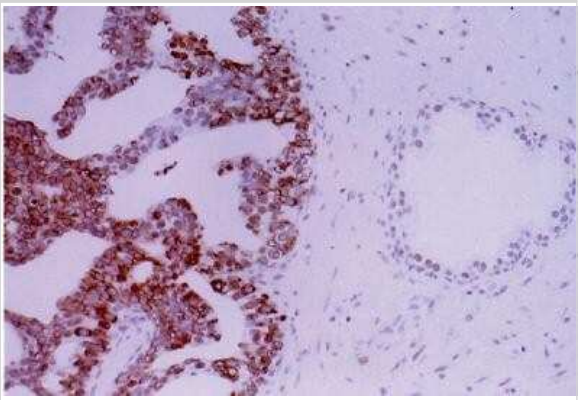
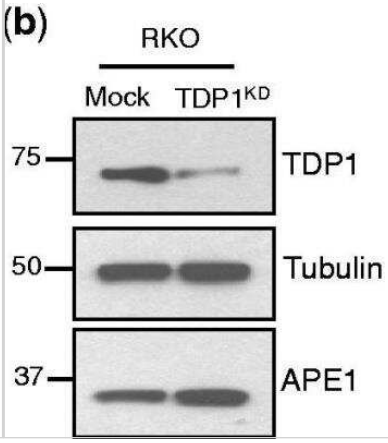
APE Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	37 kDa

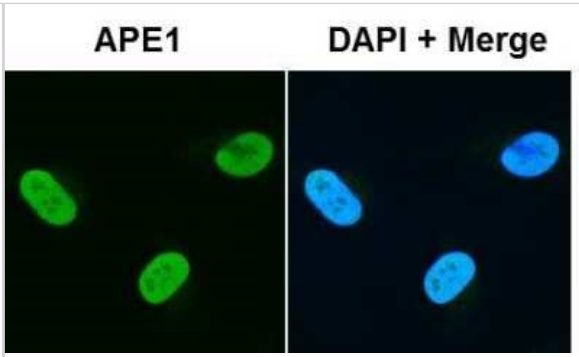
Product Description	
Host	Rabbit
Gene ID	328
Gene Symbol	APEX1
Species	Human, Mouse, Rat, Primate, Rabbit
Reactivity Notes	Rabbit reactivity reported in the scientific literature (PMID: 15276530).
Immunogen	Affinity purified human APE1 [UniProt# P27695]

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Block/Neutralize, Chromatin Immunoprecipitation (ChIP), Immunohistochemistry Free-Floating
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:12.5, Flow Cytometry 1.0 ug/ml, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunoprecipitation 7 ug/ml, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen 1:100, Flow (Intracellular) 1.0 ug/ml, Immunohistochemistry Free-Floating reported in scientific literature (PMID 17332344), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Block/Neutralize
Application Notes	In WB this antibody detects a single band at 37 kDa. SH-SY5Y Lysate (nuclear extract) image in western blot provided via verified customer review. In IHC it can be competitively inhibited from recognizing the APE1 antigen in tissues using APE1 protein. This antibody can be used on frozen sections, fixed-paraffin sections and cytospin preps. NB100-101 can also be used following the apoptosis (TUNEL) procedure with the Boehringer-Mannheim TUNEL assay kit. Antibody staining should be performed AFTER the TUNEL assay. NB100-101 can inhibit the repair activity of APE1 protein. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.1 mg/mL, separated by Size, antibody dilution of 1:12.5, apparent MW was 46 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

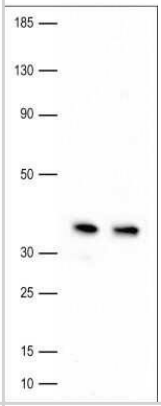
Images

Simple Western: APE Antibody [NB100-101] - Lane view shows a specific band for APE1 in 0.1 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: APE Antibody [NB100-101] - Analysis of APE1 in cell lysates: 1. HeLa, 2. Ntera2, 3. A431, 4. HepG2, 5. MCF7, 6. NIH 3T3, 7. PC12, and 8. Cos 7.	
Immunohistochemistry: APE Antibody [NB100-101] - Immunohistochemical staining of APE-ref-1 in prostate cancer.	
Western Blot: APE Antibody [NB100-101] - RKO cells were subjected to scrambled siRNA (Mock) or siRNA against TDP1 (TDP1KD) and cell lysates analyzed by immunoblotting (left). Control RKO (RKOSc siRNA) and RKO cells in which TDP1 levels were depleted (RKOTDP1 siRNA) were examined for their survival following exposure to the indicated doses of MMS, as described earlier in the text (right). Increasing the load of unrepaired methylated purines by exploiting the limited availability of TDP1 alone or in combination with canonical BER factors such as APE1 provides a new synthetic lethal setting to improve the clinical outcome of temozolomide-based cancer therapy. Image collected and cropped by CiteAb from the following publication (academic.oup.com/nar/article/42/5/3089/1051107), licensed under a CC-BY license.	

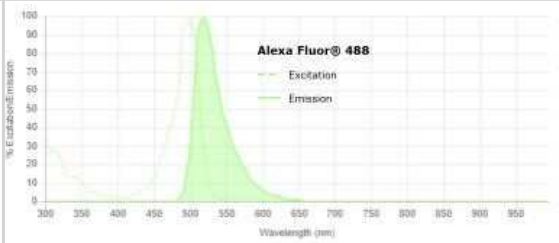
Immunocytochemistry/Immunofluorescence: APE Antibody [NB100-101] - IF on HeLa cells. Image from verified customer review.



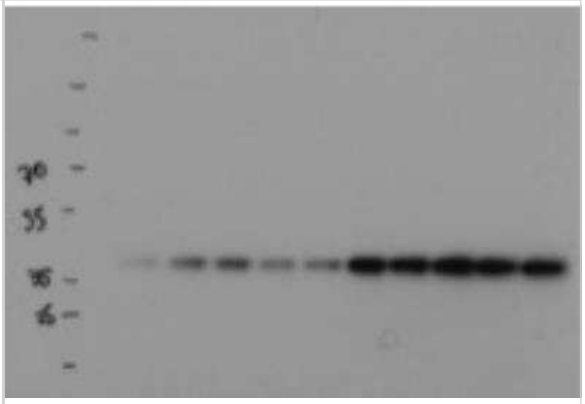
Western Blot: APE Antibody [NB100-101] - SH-SY5Y (nuclear extract) tested at 1:1000 dilution. Image provided by verified customer review.



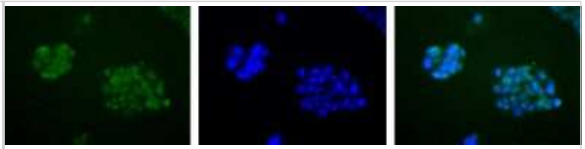
Flow Cytometry: APE Antibody [NB100-101] - Spectral properties of Alexa Fluor(R) 488: an excitation maximum of 490 nm and an emission maximum of 525 nm. Use the Novus Spectra Viewer to identify the optimal laser(s) and filters for Alexa Fluor(R) 488 and to determine its compatibility with other fluorophores when designing a multicolor experiment. https://www.novusbio.com/spectraviewer



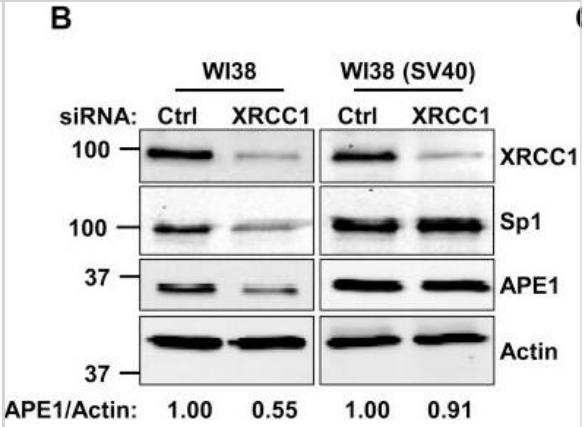
Western Blot: APE Antibody [NB100-101] - Analysis of APE in human melanoma cell lysate using anti-APE antibody. Image from verified customer review.



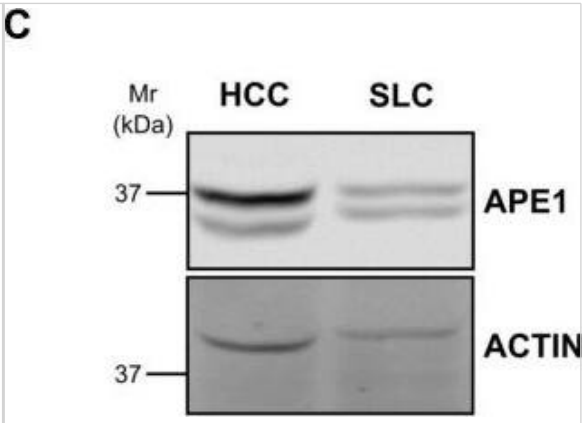
Immunocytochemistry/Immunofluorescence: APE Antibody [NB100-101] - Detection of APE1 (Green) in HepG2 cells using NB100-101. Nuclei (Blue) are counterstained with Hoechst 33258.



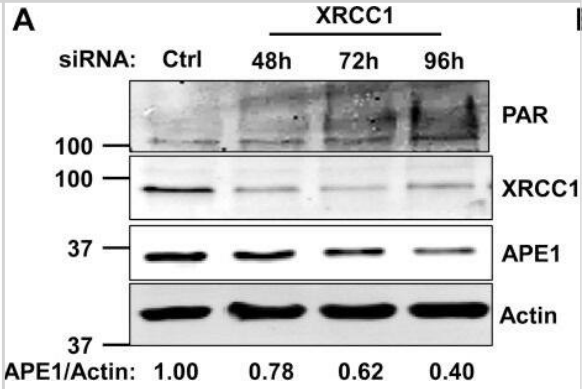
Western Blot: APE Antibody - BSA Free [NB100-101] - Defective p53 activity leads to failure of the BER coordination system. (A) High-throughput microscopy analysis of TIG-1 cells transfected with plasmids expressing wild-type or mutant p53. Boxplots showing the distribution of APE1 staining intensity (in arbitrary units) in p53 low versus p53 overexpressing cells. Each p53 mutant is reported on top of the relevant plot. The dashed line highlights the median APE1 intensity in p53 low cells (N > 5000). NS: not statistically significant at P < 0.05. (B) Representative western blotting analysis comparing WI38 & WI38 (SV40) cells upon transfection with the indicated siRNAs. Failure to downregulate Sp1 correlates with the inability to modulate APE1. Actin was used as loading control. (C) qPCR analysis on APE1 transcript in WI38 & WI38 (SV40) cells upon transfection with the indicated siRNAs. APE1 transcription is reduced in WI38 cells only. Note the higher transcript content in WI38 (SV40) cells. (D) Representative Western blotting analysis comparing WI38 & WI38 (SV40) cells upon transfection with the indicated siRNAs. Failure to modulate BER correlates with γH2AX staining in WI38 (SV40) cells; γH2AX increases further upon XRCC1 depletion. (E) Neutral Comet assay on WI38 & WI38 (SV40) fibroblasts shows accumulation of DSBs upon XRCC1-depletion in transformed cells only. Results are expressed as mean ± SD of three independent experiments. Image collected & cropped by CiteAb from the following publication (https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkw015), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

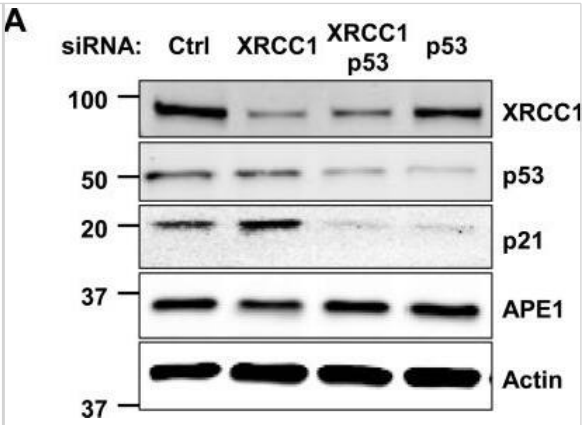
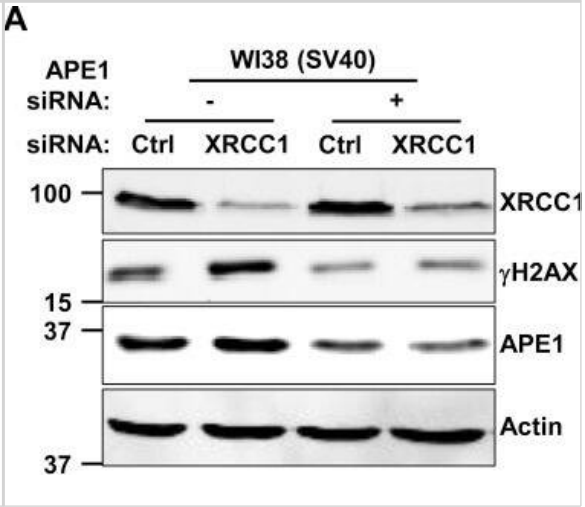


Western Blot: APE Antibody - BSA Free [NB100-101] - APE1 expression in HCC tissues(A) qPCR of tumor (HCC), peri-HCC, & surrounding liver cirrhosis (SLC), & normal liver (CTRL) (left panel). Ratio between HCC & SLC & HCC & peri-HCC within the same patients (right panel). APE1 mRNA quantification was normalized to two reference genes 18srRNA & Actin. Bar graphs indicate mean & SEM. (B) APE1 protein quantification in HCC & SLC tissue lysates from HCC cancer patients. Graphs indicate the different distributions of the fold of protein expression for each sample as the ratio between APE1 & actin. (see Supplementary Figure 1). *P < 0.05. (C) Western blot analysis of HCC & SLC tissue lysates patients performed on pooled samples from HCC cancer. Actin was used as loading control & for the relative normalization. A representative image of western blot analysis is shown. Data represent the means of ± SD of three independent experiments. *P < 0.05. (D) Immunohistochemistry of HCC, SLC, & normal (CTRL) tissue. Red & yellow arrows indicate nuclear & cytoplasmic positivity, respectively. (E) Scan of HCC nodules & its corresponding sAPE1 from 3 patients representing for each low, median, & high sAPE1. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30719231), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: APE Antibody - BSA Free [NB100-101] - Persistent SSBs decrease APE1 levels by affecting its transcription. (A) Western blotting analysis on a representative XRCC1 knockdown time-course. TIG-1 cells were incubated with either a control siRNA (72 h), or a XRCC1-targeting siRNA, & harvested at the indicated time points. DNA damage accumulation is highlighted by PAR formation; APE1 is downregulated in a time-dependent manner. Actin was used as loading control. (B) Alkaline Comet assay on TIG-1 cells harvested 72 h after XRCC1 depletion shows accumulation of SSBs (N = 9). (C) Histogram showing the downregulation in APE1 protein amount 72 h after XRCC1 knockdown, as measured by western blotting (N = 10). (D) Histogram showing the downregulation in APE1 transcript level 72 h after XRCC1 depletion, as measured by qPCR (N = 7). Results depicted in histograms are presented as mean ± SD of the indicated number (N) of independent experiments. Image collected & cropped by CiteAb from the following publication (https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkw015), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: APE Antibody - BSA Free [NB100-101] - Downregulation of APE1 in response to BER unbalance is dependent of p53. (A) Representative western blotting analysis on TIG-1 cells depleted of XRCC1 & p53. APE1 is downregulated upon XRCC1 knockdown in a p53-dependent manner. Actin was used as loading control. (B) Left: histogram showing the quantification of APE1 protein amount in the experiment showed in panel A (N = 5). Right: histogram illustrating the amount of APE1 transcript upon combined XRCC1/p53 depletion, as measured through qPCR (N = 3). Results are expressed as mean ± SD of the indicated number (N) of independent experiments. (C) Boxplot showing the distribution of APE1 staining intensity (in arbitrary units) in p53 low versus p53 overexpressing cells. The dashed line highlights the median APE1 intensity in p53 low cells (N > 8000). (D) Representative high-throughput immuno-fluorescence pictures showing TIG-1 cells stained for APE1 (right panel) & p53 (middle panel) after transfection with a p53 expressing plasmid. Cells that downregulating APE1 in response to p53 overexpression are marked by a contour line. Image collected & cropped by CiteAb from the following publication (https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkw015), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: APE Antibody - BSA Free [NB100-101] - Excessive AP-endonuclease activity in cells with p53 impairment leads to accumulation of genomic instability. (A) Representative western blotting analysis on WI38 (SV40) cells shows rescue of γH2AX staining upon co-depletion of XRCC1 & APE1. (B) Neutral Comet assay on WI38 (SV40) fibroblasts shows accumulation of DSBs upon XRCC1-depletion & rescue after co-depletion of XRCC1 & APE1. Results are expressed as mean ± SD of three independent experiments. (C) Representative western blotting analysis on WI38 (SV40) cells shows rescue of γH2AX staining after depletion of XRCC1 in presence of APE1 inhibitor III (APE1i III, 5 μM for 24 h), or AR03 (2.5 μM for 24 h). Actin was used as loading control in all western blotting experiments. Image collected & cropped by CiteAb from the following publication (https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkw015), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Gabriella DH, Anbukkarasi M, Kamakshi S et al. Ref-1 redox activity regulates retinal neovascularization by modulating transcriptional activation of HIF-1α FASEB J. 2025-02-15 [PMID: 39902892] (Immunohistochemistry, Mouse)

Abbotts R, Jewell R, Nsengimana J et Al. Targeting human apurinic/apyrimidinic endonuclease 1 (APE1) in phosphatase and tensin homolog (PTEN) deficient melanoma cells for personalized therapy Oncotarget 2014-05-01 [PMID: 24830350] (Western Blot)

Gotoh N, Oda T, Kitamura Y et Al. APEX1 Polymorphisms Affect Acute Myeloid Leukemia Risk, and Its Expression Is Involved in Cell Proliferation and Differentiation Int J Lab Hematol 2024-11-13 [PMID: 39536468]

Adel Alblihy, Ahmed Shoqafi, Michael S. Toss, Mashael Algethami, Anna E. Harris, Jennie N. Jeyapalan, Tarek Abdel-Fatah, Juliette Servante, Stephen Y. T. Chan, Andrew Green, Nigel P. Mongan, Emad A. Rakha, Srinivasan Madhusudan Untangling the clinicopathological significance of MRE11-RAD50-NBS1 complex in sporadic breast cancers NPJ Breast Cancer 2021-11-15 [PMID: 34782604]

Tarek M.A. Abdel-Fatah, Arvind Arora, Paul M. Moseley, Christina Perry, Emad A. Rakha, Andrew R. Green, Stephen Y.T. Chan, Ian O. Ellis, Srinivasan Madhusudan DNA repair prognostic index modelling reveals an essential role for base excision repair in influencing clinical outcomes in ER negative and triple negative breast cancers Oncotarget 2015-09-08 [PMID: 26267318]

Mijit, M;Kpenu, E;Chowdhury, NN;Gampala, S;Wireman, R;Liu, S;Babb, O;Georgiadis, MM;Wan, J;Fishel, ML;Kelley, MR; In vitro and In vivo evidence demonstrating chronic absence of Ref-1 Cysteine 65 impacts Ref-1 folding configuration, redox signaling, proliferation and metastasis in pancreatic cancer Redox biology 2023-12-01 [PMID: 38056311]

Mashael Algethami, Michael S. Toss, Corinne L. Woodcock, Chandar Jaipal, Juliette Brownlie, Ahmed Shoqafi, Adel Alblihy, Katia A. Mesquita, Andrew R. Green, Nigel P. Mongan, Jennie N. Jeyapalan, Emad A. Rakha, Srinivasan Madhusudan Unravelling the clinicopathological and functional significance of replication protein A (RPA) heterotrimeric complex in breast cancers NPJ Breast Cancer 2023-03-30 [PMID: 36997566]

Xue Z, Demple B. Knockout and Inhibition of Ape1: Roles of Ape1 in Base Excision DNA Repair and Modulation of Gene Expression Antioxidants (Basel) 2022-09-15 [PMID: 36139891] (Western Blot, Block/Neutralize)

Kim DV, Kulishova LM, Torgasheva NA et al. Mild phenotype of knockouts of the major apurinic/apyrimidinic endonuclease APEX1 in a non-cancer human cell line PLOS ONE 2021-09-16 [PMID: 34529719]

Caston RA, Fortini P, Chen K et al. Maintenance of Flap Endonucleases for Long-Patch Base Excision DNA Repair in Mouse Muscle and Neuronal Cells Differentiated In Vitro International Journal of Molecular Sciences 2023-08-12 [PMID: 37628896] (Western Blot)

Ito M, Ducasa GM, Molina JD et al. ABCA1 deficiency contributes to podocyte pyroptosis priming via the APE1/IRF1 axis in diabetic kidney disease Scientific reports 2023-06-14 [PMID: 37316538] (IHC, Mouse)

Details:
1:500 dilution, fresh sections used

Miner KM, Jamenis AS, Bhatia TN et al. alpha-synucleinopathy exerts sex-dimorphic effects on the multipurpose DNA repair/redox protein APE1 in mice and humans Progress in neurobiology 2022-06-13 [PMID: 35710046]

More publications at http://www.novusbio.com/NB100-101

Procedures

Western Blot Protocol for APE1 Antibody (NB100-101)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three 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 manufacturer's instructions.

Immunocytochemistry/ Immunofluorescence Protocol for APE Antibody (NB100-101)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Flow (Intracellular) Protocol for APE Antibody (NB100-101)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Immunohistochemistry-Paraffin Protocol for APE Antibody (NB100-101)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

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Products Related to NB100-101

NB800-PC1	HeLa Whole Cell Lysate
NBP1-49581	APE1 Redox Inhibitor
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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