# 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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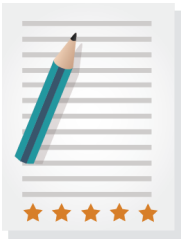
Updated 1/9/2024 v.20.1





# NB100-101

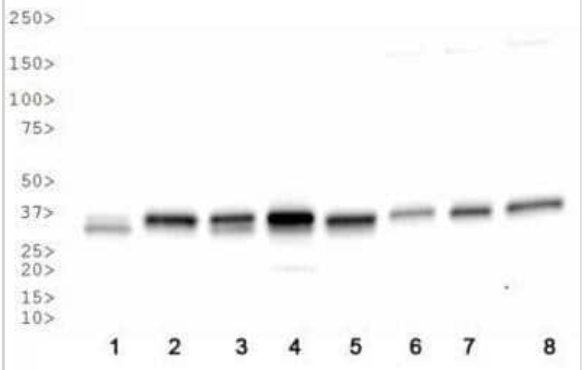
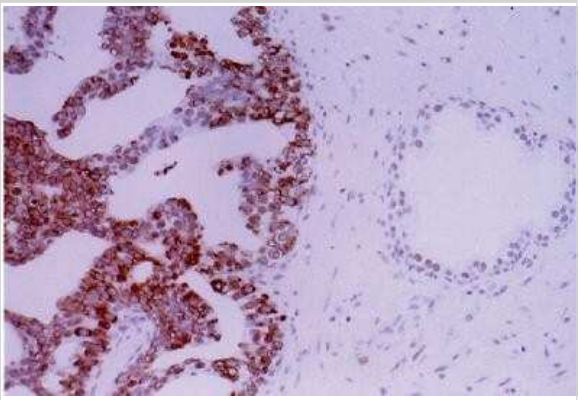
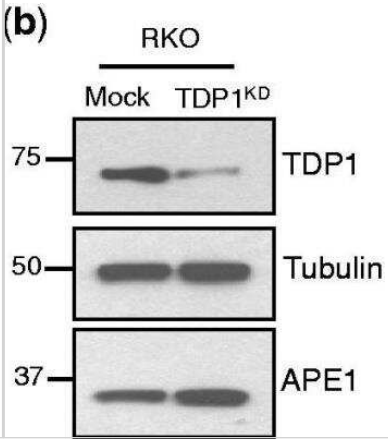
APE Antibody - BSA Free

| 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 |

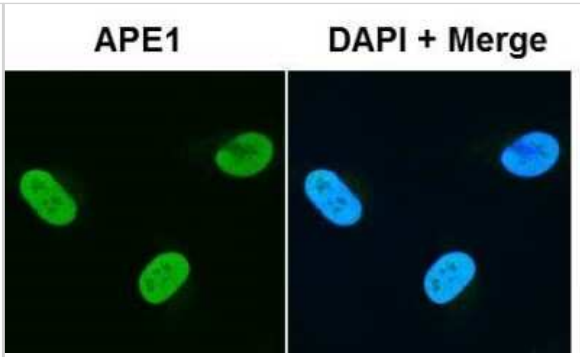
| Product Description | |
|---|---|
| **Host** | Rabbit |
| **Gene ID** | 328 |
| **Gene Symbol** | APEX1 |
| **Species** | Human, Mouse, Rat, Primate, Rabbit |
| **Reactivity Notes** | Rabbit reactivity reported in the scientific literature (PMID: 15276530). |
| **Immunogen** | Affinity purified human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

| Product Application Details | |
|---|---|
| **Applications** | Western Blot, Simple Western, Flow Cytometry, 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, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunoprecipitation 7 ug/ml, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen 1:100, 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. Separated by Size-Wes, Sally Sue/Peggy Sue. |

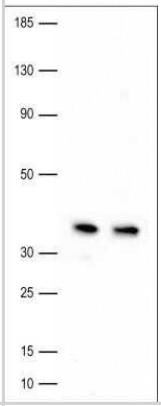
## Images

| | |
|---|---|
| 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. |   |

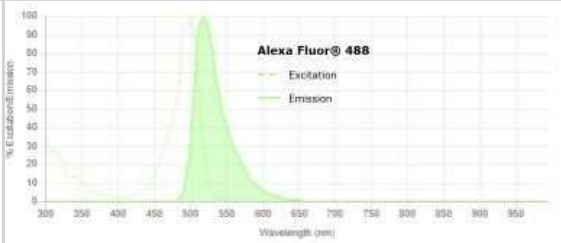
Immunocytochemistry/Immunofluorescence: APE Antibody [NB100-101] - IF on HeLa cells. Image from verified customer review.



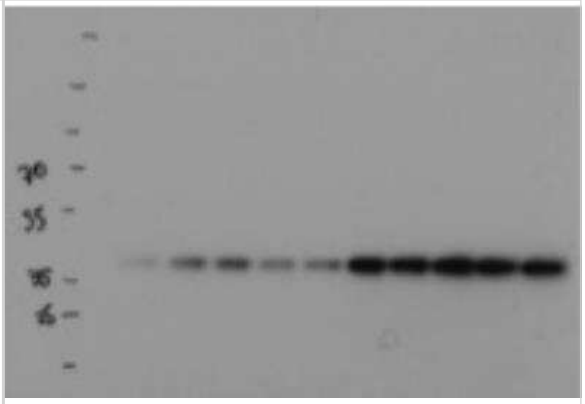

Western Blot: APE Antibody [NB100-101] - SH-SY5Y (nuclear extract) tested at 1:1000 dilution. Image provided by verified customer review.



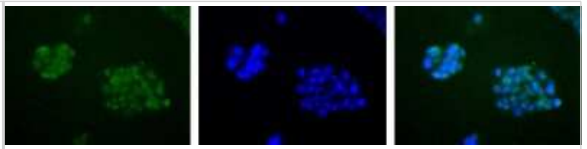

Flow Cytometry: APE Antibody [NB100-101] - Spectral properties of Alexa Fluor(R) 488: an excitation maximum of 490 nm and an emission maximum of 525 nm. Use the Novus Spectra Viewer to identify the optimal laser(s) and filters for Alexa Fluor(R) 488 and to determine its compatibility with other fluorophores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



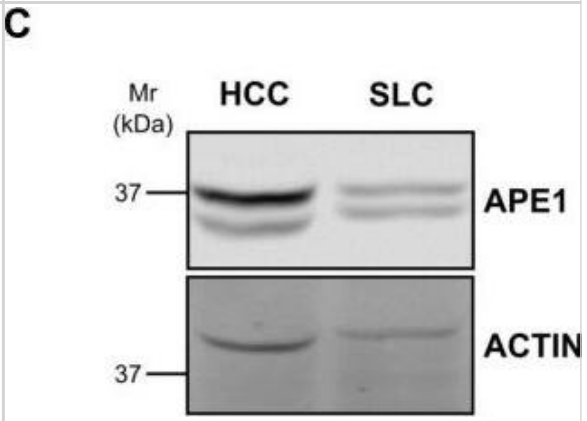

Western Blot: APE Antibody [NB100-101] - Analysis of APE in human melanoma cell lysate using anti-APE antibody. Image from verified customer review.



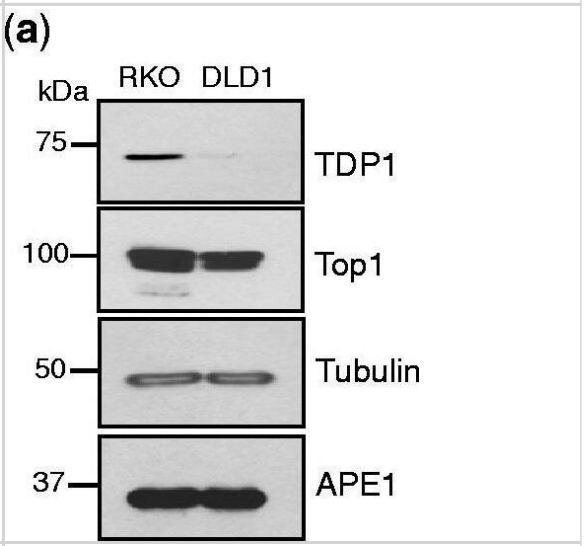
Immunocytochemistry/Immunofluorescence: APE Antibody [NB100-101] - Detection of APE1 (Green) in HepG2 cells using NB100-101. Nuclei (Blue) are counterstained with Hoechst 33258.



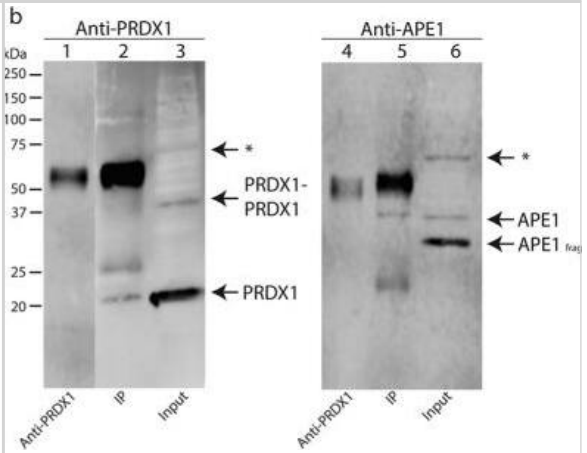
APE1 expression in HCC tissues. (C) Western blot analysis of HCC and SLC tissue lysates patients performed on pooled samples from HCC cancer. Actin was used as loading control and for the relative normalization. A representative image of western blot analysis is shown. Data represent the means of +/- SD of three independent experiments. *P < 0.05. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30719231), licensed under a CC-BY licence.



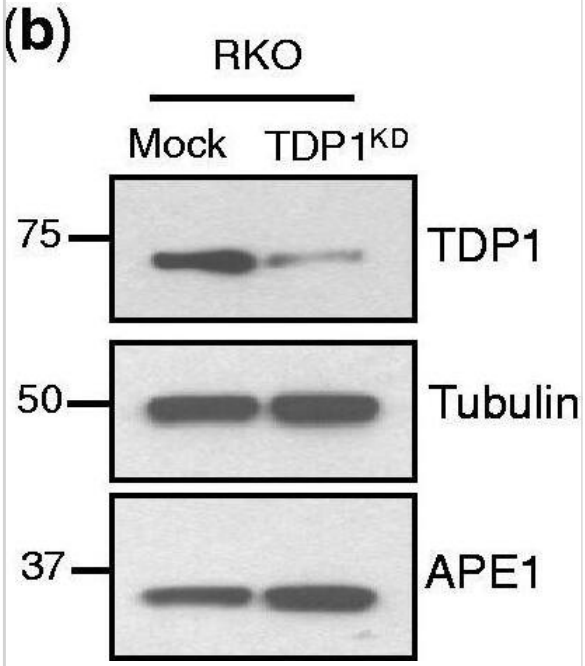

Aphidicolin-induced ATM signaling in immortalized MEFs. (A) Cells (WT, MJI-53; KO, MJI-1) were treated for 24 h using 0 or 3 μM aphidicolin. (B) Cells (lane 1–3, WT, MJI-91; lanes 4–6, KO, MJI-186; lanes 7–9, WT, MJI-105; lanes 1–12, KO, MJI-84) were treated for 24 h using 0, 1 or 3 μM aphidicolin. Actin and 53BP1 serve as loading controls. DYNLL1 is used as a surrogate marker for loss of ASCIZ. (C) Quantification of western blot band intensities. An arbitrary unit of 100 represents the average band intensity for the respective phospho-protein in the 3 μM aphidicolin-treated wildtype samples on each membrane. Graphs indicate the mean ± standard error, n = 3. Additional loading controls for total KAP1, p53 and H2AX are shown in Supplementary Fig. S1A and B. Image collected and cropped by CiteAb from the following open publication (https://linkinghub.elsevier.com/retrieve/pii/S1568786417301349), licensed under a CC-BY license. Not internally tested by Novus Biologicals.




Autophagy responds rapidly to changing glucose conditions. Immortalized MSCs were cultured in physiologic (also called low in culture parlance; 1 g/L; 5.5 mM) or high (4.5 g/L; 25 mM) glucose media for 2 days and then changed to the corresponding opposite glucose concentration for up to 96 h. Myosin light chain 3 (LC3) levels were probed via immunoblot to assess autophagic response (a). The role of oxygen in the glucose response was also assessed by culturing the MSCs in a Biospherix hypoxic chamber at 4% and 1% oxygen in high and low glucose media for 4 days, followed by comparable LC3 blots (b). Shown are representative blots of three repeated studies. α-Actinin was used as a housekeeping control for all blots Image collected and cropped by CiteAb from the following open publication (https://stemcellres.biomedcentral.com/articles/10.1186/s13287-016-0436-7), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Chromatin opening activates DDR via a mechanism that requires catalytically functional LOXL2 but can be independent of DNA damage. a γ-H2AX and 53BP1 staining and foci quantification are shown by immunofluorescence with a specific antibody for γ-H2AX (left image) or for 53BP1 (right image). Dot graphs indicate the number of foci for γ-H2AX (upper graph) and 53BP1 (lower graph) per cell in control and LOXL2 KD conditions. b γ-H2AX staining and foci quantification are shown by immunofluorescence with the indicated antibody after LOXL2 reinfection. MDA-MB-231 cells were first infected with control or LOXL2 KD lentivirus and then, after puromycin selection, again with GFP (MOCKGFP+), wild-type LOXL2-IRES-GFP (LOXL2GFP+), or LOXL2mut-IRES-GFP (LOXL2mutGFP+). Cells were fixed after 24 h. Dot graphs indicate the number of γ-H2AX foci per GFP-positive cells containing MOCKGFP+ (upper graph), LOXL2GFP+ (middle graph), or LOXL2mutGFP+ (lower graph). c Dot graphs indicate the number of γ-H2AX (upper graph) and 53BP1 (lower graph) foci per cell in control and LOXL2 KD cells after treatment with 200 μM cordycepin for the indicated timepoints. d γ-H2AX and 53BP1 staining and foci quantification are shown after immunofluorescence with the indicated antibodies in non-replicative conditions. Dot graphs indicate the number of the γ-H2AX (left graph) and 53BP1 (right graph) foci in control and LOXL2 KD cells. e Representative image showing DNA damage in control or LOXL2 KD MDA-MB-231 cells, visualized by the alkaline comet assay. Cells were treated with 0.3 μM doxorubicin for 24 h. The graph shows the percentage of MDA-MB-231 positive cells. f Chromosome alterations in control and LOXL2 KD MDA-MB-231 cells. Error bars indicate the SD from at least three independent experiments. *P < 0.05, **P < 0.01, and ***P < 0.001, ****P < 0.0001 Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31462706), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

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] (B/N, WB)

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] (WB)

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]

Quinones JL, Thapar U, Wilson SH et al. Oxidative DNA-protein crosslinks formed in mammalian cells by abasic site lyases involved in DNA repair DNA Repair (Amst.) 2020-01-09 [PMID: 31945542] (WB, Human)

Zhang, Q;Chao, TC;Patil, VS;Qin, Y;Tiwari, SK;Chiou, J;Dobin, A;Tsai, CM;Li, Z;Dang, J;Gupta, S;Urdahl, K;Nizet, V;Gingeras, TR;Gaulton, KJ;Rana, TM; The long noncoding RNA ROCKI regulates inflammatory gene expression EMBO J. 2019-04-15 [PMID: 30918008] (WB, Human)

Shah F, Logsdon D, Messmann RA et al. Exploiting the Ref-1-APE1 node in cancer signaling and other diseases: from bench to clinic NPJ Precis Oncol 2017-08-21 [PMID: 28825044] (WB, Human)

Pascut D, Sukowati CHC, Antoniali G et al. Serum AP-endonuclease 1 (sAPE1) as novel biomarker for hepatocellular carcinoma Oncotarget 2019-01-08 [PMID: 30719231] (IHC-P, Human)

Kumar S, Talluri S, Pal J et al. Role of apurinic/apyrimidinic nucleases in the regulation of homologous recombination in myeloma: mechanisms and translational significance. Blood Cancer J. 2018-09-25 [PMID: 30301882] (WB, Human)

Logsdon DP, Shah F, Carta F et al. Blocking HIF signaling via novel inhibitors of CA9 and APE1/Ref-1 dramatically affects pancreatic cancer cell survival. Sci Rep 2018-09-13 [PMID: 30214007] (WB, Human)

Fehrenbacher JC, Guo C, Kelley MR, Vasko MR. DNA damage mediates changes in neuronal sensitivity induced by the inflammatory mediators, MCP-1 and LPS, and can be reversed by enhancing the DNA repair function of APE1. Neuroscience. 2017-09-28 [PMID: 28965839] (WB)

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.

**Novus Biologicals USA**
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

**Bio-Techne Canada**
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

**Bio-Techne Ltd**
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

**General Contact Information**
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

## 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.

**For more information on our 100% guarantee, please visit www.novusbio.com/guarantee**

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