Product Datasheet

XBP1 Antibody - BSA Free
NBP1-77681

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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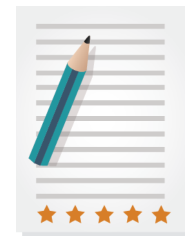


NBP1-77681

XBP1 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

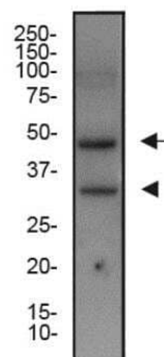
Description	Novus Biologicals Rabbit XBP1 Antibody - BSA Free (NBP1-77681) is a polyclonal antibody validated for use in IHC, WB, Flow, ICC/IF and ChIP. Anti-XBP1 Antibody: Cited in 18 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	7494
Gene Symbol	XBP1
Species	Human, Mouse
Specificity/Sensitivity	This antibody is specific for both XBP1s and XBP1u.
Immunogen	A genomic peptide made to an internal region of the human XBP1 protein (within residues 100-250). [Swiss-Prot P17861]
Notes	Manufactured by Genomic Antibody Technology™. GAT FAQs

Product Application Details

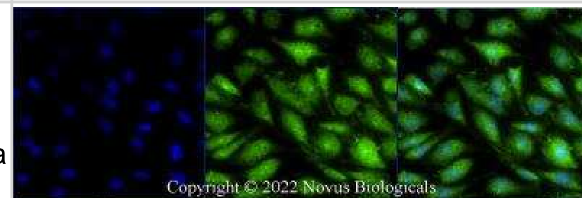
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Knockdown Validated
Recommended Dilutions	Western Blot 1 ug/mL, Flow Cytometry reported in scientific literature (PMID 31031094), Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:100, Knockdown Validated reported in scientific literature (PMID 33513694)
Application Notes	Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

Images

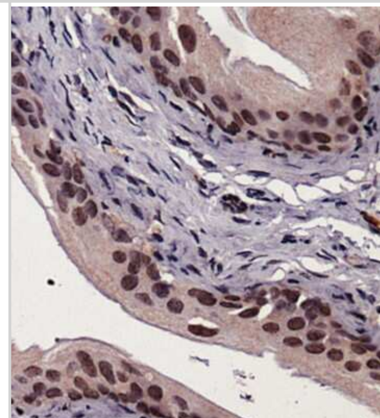
Western Blot: XBP1 Antibody [NBP1-77681] - Total protein from HeLa cells was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/ml anti-XBP1 in block buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Arrow delineates XBP1s and arrowhead XBP1u.



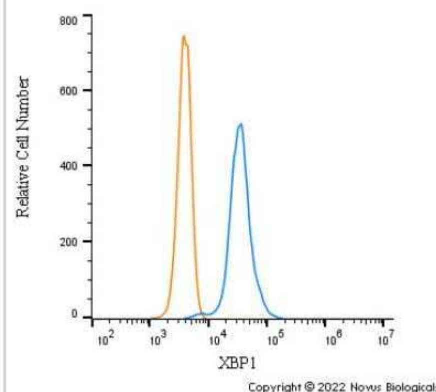
Immunocytochemistry/Immunofluorescence: XBP1 Antibody [NBP1-77681] - Mouse MS1 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with XBP1 Antibody (NBP1-77681) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

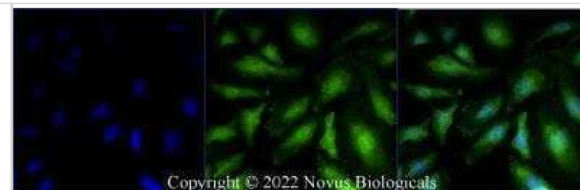
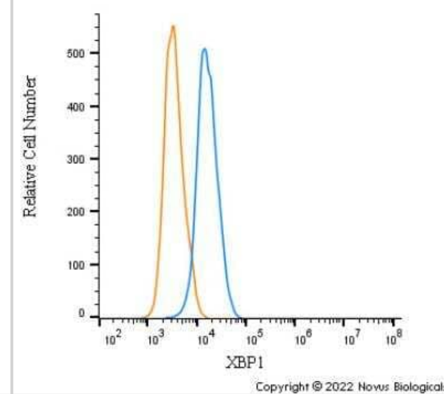
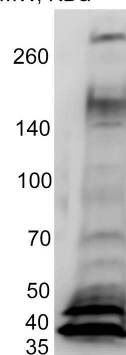
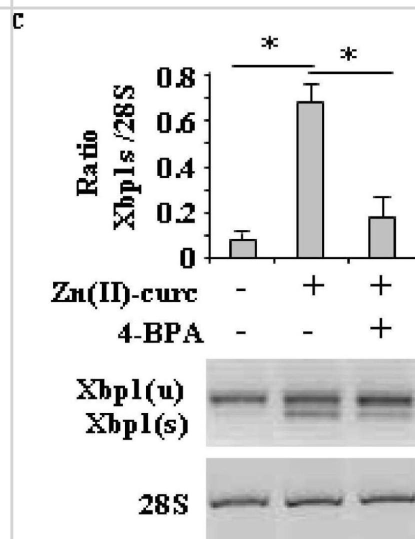


Immunohistochemistry: XBP1 Antibody [NBP1-77681] - Staining of XBP1 in mouse bladder using DAB with hematoxylin counterstain.

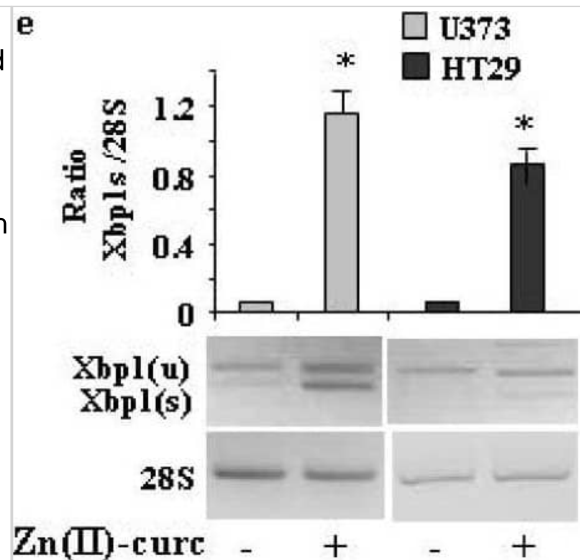


Flow Cytometry: XBP1 Antibody [NBP1-77681] - An intracellular stain was performed on NIH3T3 cells with XBP1 Antibody NBP1-77681 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).

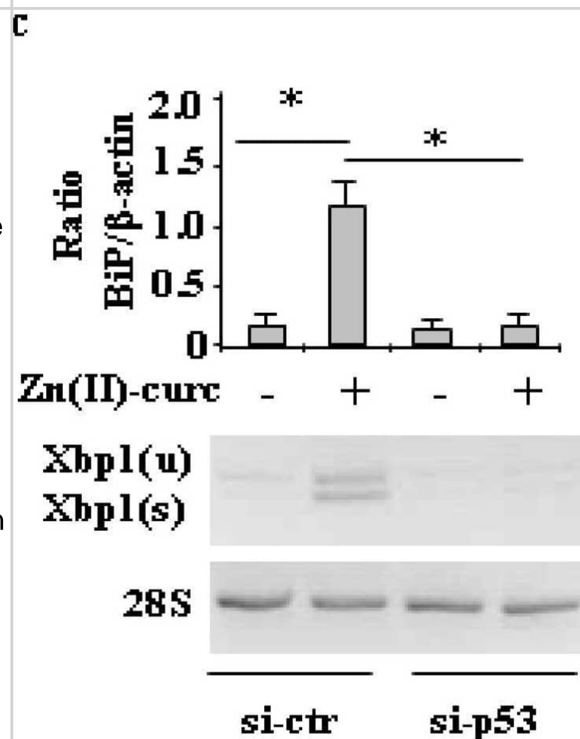


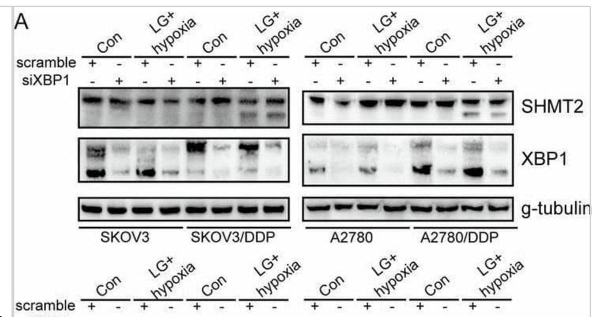
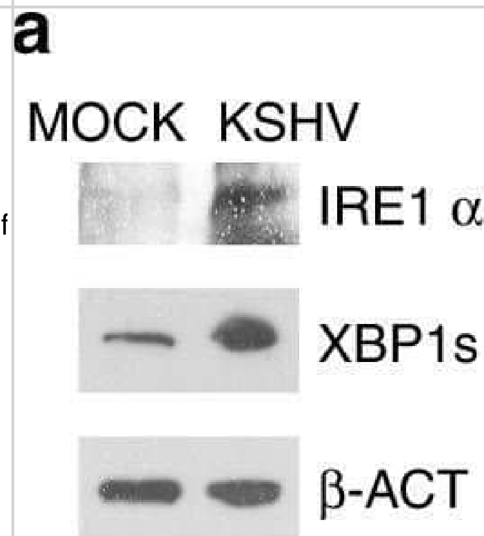
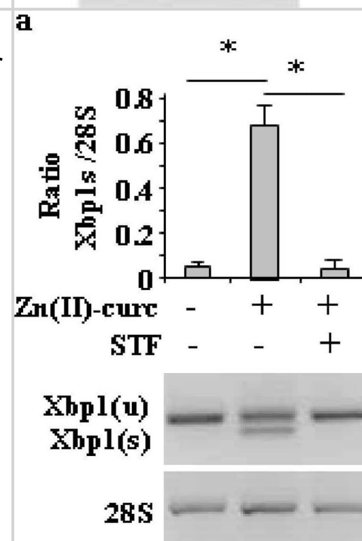
Immunocytochemistry/Immunofluorescence: XBP1 Antibody [NBP1-77681] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with XBP1 Antibody (NBP1-77681) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Flow Cytometry: XBP1 Antibody [NBP1-77681] - An intracellular stain was performed on HepG2 cells with XBP1 Antibody NBP1-77681 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Western Blot: XBP1 Antibody [NBP1-77681] - Western Blot of PC-3 cell lysate. Image from verified customer review.	
ER stress inhibition impairs Zn (II)-curc-induced autophagy and mutp53 degradation. (a) Western blot analysis of BiP, LC3I/II, and p53 protein levels in U373 cells untreated or treated with Zn(II)-curc (100 ug/mL) for 24 h, with or without 1 h pre-treatment with 4-BPA (2.5 mM). (b) Densitometric analysis was performed using Image J software to calculate the ratio of the protein levels, as detected in (a), vs. β-actin. Histograms represent the mean +/- SD of three independent experiments. * p ≤ 0.05. (c) Total mRNA was extracted from U373 cells untreated or treated as in (a). Spliced (s) and unspliced (u) Xbp1 gene expression were assayed by the PCR of reverse-transcribed cDNA. Densitometric analysis was performed using Image J software to calculate the Xbp1s/28S ratio. Histograms represent the mean +/- SD of three independent experiments. * p ≤ 0.05. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32138264), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Zn (II)-curc induces endoplasmic reticulum (ER) stress in mutant p53H273-carrying cells. (a) Representative photomicrographs of ER-Red Fluorescence staining in U373 cells untreated (Mock) or treated with Zn (II)-curc (100 ug/mL) for 16 h (Original magnification: 40×). (b) Quantization of ER content in U373 cells from ER-Red Fluorescence-stained cells. Mean fluorescence intensity (MFI) of each individual cell was normalized to cell size and expressed as fold-change compared with untreated cells at the same time point. Histograms represent the mean +/- SD of three independent experiments. * $p \leq 0.05$. (c) Western blot analysis of p53, BiP, total (tot) IRE1α, phosphorylated (p) IRE1α, and XBP1 spliced (s) protein levels evaluated in U373 and HT29 cells untreated or treated with Zn (II)-curc (100 ug/mL) for 24 h. (d) Densitometric analysis was performed using Image J software to calculate the ratio of the protein levels, as detected in (c), vs. β-actin. Histograms represent the mean +/- SD of three independent experiments. * $p \leq 0.05$. (e) Total mRNA was extracted from U373 and HT29 cells untreated or treated with Zn (II)-curc (100 ug/mL) for 24 h. Spliced (s) Xbp1 gene expression was assayed by the polymerase chain reaction (PCR) of reverse-transcribed cDNA. Densitometric analysis was performed using Image J software to calculate the Xbp1s/28S ratio. Histograms represent the mean +/- SD of three independent experiments. * $p \leq 0.05$. (f) p53 gene expression was assayed by PCR as in (e). The p53/28S ratio is indicated. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32138264), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Mutp53 knockdown abrogates the Zn (II)-curc-induced ER stress. (a) U373 cells were transfected with control pSuper (si-ctr) and pSuper-p53 (si-p53) vectors for p53 knockdown and 36 h after transfection, cells were treated with Zn (II)-curc (100 ug/mL) for 16 h, before undergoing ER-Red Fluorescence staining. Quantization of ER content in U373 cells from ER-Red Fluorescence-stained cells as evaluated by the mean fluorescence intensity (MFI) of each individual cell normalized to cell size and expressed as fold-change compared with untreated cells at the same time point. Histograms represent the mean +/- SD of three independent experiments. * $p \leq 0.05$. (b) Western blot analysis of BiP and p53 protein levels in U373 and HT29 cells transfected for 36 h with si-ctr and si-p53, and then treated with Zn (II)-curc (100 ug/mL) for 24 h. Densitometric analysis was performed using Image J software to calculate the ratio of BiP and p53 protein levels vs. β-actin, as indicated ns: not specific signal. (c) Total mRNA was extracted from U373 cells treated as in (b), and spliced (s) and unspliced (u) Xbp1 gene expression were assayed by the PCR of reverse-transcribed cDNA. (upper panel) Densitometric analysis was performed using Image J software to calculate the Xbp1s/28S ratio. Histograms represent the mean +/- SD of three independent experiments. * $p \leq 0.05$. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32138264), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Selective utilization of SHMT2 promoter 2 by HIF1α and TFE3 complex.A, B Western blot analysis of SHMT2, SHMT2α, XBP1, TFE3, HIF1α protein expression upon XBP1, TFE3, HIF1α knockdown respectively in cisplatin-sensitive (parental) and cisplatin-resistant (DDP) SKOV3 or A2780 cells under different cell culture. C Luciferase assay different promoter activity upon XBP1, TFE3, HIF1α knockdown respectively cisplatin-resistant (DDP) SKOV3 or A2780 cells under low-glucose and hypoxic environment. D Co-IP experiment testing the interaction between TFE3, HIF1α in cisplatin-resistant (DDP) SKOV3 or A2780 cells under low-glucose and hypoxic environment. E Binding pose of TFE3 with HIF1α in 3D. F, G PLA analyze showing the distribution of TFE3 with HIF1α in cisplatin-resistant (DDP) SKOV3 or A2780 cells under different cell culture. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/40097394), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
KSHV activates UPR and up-regulates PD-L1 on KSHV- infected macrophages.a Ire1α and XBP1s expression in mock- and KSHV-infected macrophages was evaluated by western blot analysis; b ATF4, CHOP and BIP expression in mock- and KSHV-infected macrophages was evaluated by western blot analysis. β-actin (β-ACT) was used as loading control. A representative experiment out of three is shown. Histograms represent the mean plus S.D. of the densitometric analysis of the ratio of each protein/β-ACT. *p-value < 0.05. c PD-L1 expression on mock- and KSHV-infected macrophages was evaluated by FACS analysis. A representative experiment is shown, and the mean of fluorescence intensity is indicated. Grey peaks represent the isotype controls. d Histograms representing the mean plus SD of PD-L1 MFI (Mean fluorescence Intensity) are also reported. *p-value < 0.05. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32418990), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
IRE1α inhibition impairs Zn (II)-curc-induced autophagy and mutp53 degradation. (a) Total mRNA was extracted from U373 cells untreated or treated with Zn(II)-curc (100 ug/mL) for 24 h, with or without inhibitor of XBP1 cleavage STF-083010 (STF) (60 μM). Spliced (s) and unspliced (u) Xbp1 gene expression was assayed by the PCR of reverse-transcribed cDNA. (lower panel) Densitometric analysis was performed using Image J software to calculate the Xbp1s/28S ratio. Histograms represent the mean +/- SD of three independent experiments. * p ≤ 0.05. (b) Western blot analysis of p53, BiP, and LC3I/II protein levels in U373 cells untreated or treated, as in (a). (c) Densitometric analysis was performed using Image J software to calculate the ratio of the protein levels, as detected in (b), vs. β-actin. Histograms represent the mean +/- SD of three independent experiments. * p ≤ 0.05. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32138264), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Hilan G, Daniel G, Collak F et al. Cancer-Targeting Peptides Functionalized With Polyarginine Enables GRP78-Dependent Cell Uptake and siRNA Delivery Within the DU145 Prostate Cancer Cells. Journal of peptide science : an official publication of the European Peptide Society 2025-02-19 [PMID: 39967318]

Gebert M, Sobolewska A, Bartoszewska S et al. Genome-wide mRNA profiling identifies X-box-binding protein 1 (XBP1) as an IRE1 and PUMA repressor Cellular and Molecular Life Sciences 2021-11-01 [PMID: 34636989]

Stein D, Slobodnik Z, Tam B et al. 4-phenylbutyric acid-Identity crisis; can it act as a translation inhibitor? Aging Cell 2022-12-01 [PMID: 36373957]

Weaver FE, White E, Peek AM et Al. 4-Phenylbutyric acid mitigates ER stress-induced neurodegeneration in the spinal cords of a GM2 gangliosidosis mouse model Hum Mol Genet 2024-11-12 [PMID: 39530163]

Luis O Correa-Medero, Shayna E Jankowski, Hanna S Hong, Nicholas D Armas, Aditi I Vijendra, Mack B Reynolds, Garrett M Fogo, Dominik Awad, Alexander T Dils, Kantaro A Inoki, Reid G Williams, Annabelle M Ye, Nadezhda Svezhova, Francisco Gomez-Rivera, Kathleen L Collins, Mary X O'Riordan, Thomas H Sanderson, Costas A Lyssiotis, Shannon A Carty ER-associated degradation adapter Sel1L is required for CD8 + T cell function and memory formation following acute viral infection. Cell reports 2024-04-29 [PMID: 38687642]

Nancy Ahuja, Shalini Gupta, Rashmi Arora, Ella Bhagyaraj, Drishti Tiwari, Sumit Kumar, Pawan Gupta Nr1h4 and Thrb ameliorate ER stress and provide protection in the MPTP mouse model of Parkinson’s Life Science Alliance 2024-04-12 [PMID: 38609183]

Pauline de Zeeuw, Lucas Treps, Melissa García-Caballero, Ulrike Harjes, Joanna Kalucka, Carla De Legher, Katleen Brepoels, Kristel Peeters, Stefan Vinckier, Joris Souffreau, Ann Bouché, Federico Taverna, Jonas Dehairs, Ali Talebi, Bart Ghesquière, Johan Swinnen, Luc Schoonjans, Guy Eelen, Mieke Dewerchin, Peter Carmeliet The gluconeogenesis enzyme PCK2 has a non-enzymatic role in proteostasis in endothelial cells Communications Biology 2024-05-23 [PMID: 38783087]

Denolly S, Guo H, Martens M et al. Dengue virus NS1 secretion is regulated via importin-subunit ?1 controlling expression of the chaperone GRp78 and targeted by the clinical drug ivermectin mBio 2023-09-13 [PMID: 37702492]

Navas-Madro□al M, Almendra-Pegueros R, Puertas-Umbert L et al. Targeting mitochondrial stress with SS31 prevents experimental abdominal aortic aneurysm: crosstalk with ER stress British journal of pharmacology 2023-03-25 [PMID: 36964990] (WB, Mouse)

Espina M, Di Franco N, Brañas-Navarro M et al. The GRP78-PERK axis contributes to memory and synaptic impairments in Huntington's disease R6/1 mice Neurobiology of disease 2023-07-11 [PMID: 37442396] (WB, Mouse)

Details:
Dilution: 1:1000

Lombardi S, Goldman AR, Tang HY et al. Targeting Fatty Acid Reprogramming Suppresses CARM1-expressing Ovarian Cancer Cancer research communications 2023-06-01 [PMID: 37377614] (ChIP, Human)

Pandit M, Kil YS, Ahn JH et al. Methionine consumption by cancer cells drives a progressive upregulation of PD-1 expression in CD4 T cells Nature communications 2023-05-05 [PMID: 37147330] (Western Blot, Mouse)

Details:
WB 1:1000

More publications at http://www.novusbio.com/NBP1-77681

Procedures

Immunohistochemistry-Paraffin protocol for XBP1 Antibody (NBP1-77681)
XBP1 Antibody:
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.

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

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunocytochemistry/Immunofluorescence protocol for XBP1 Antibody (NBP1-77681)
XBP1 Antibody:
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.
1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NBP1-77681

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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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