Product Datasheet

HMGB1/HMG-1 Antibody
NB100-2322SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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Reviews: 1 Publications: 7

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NB100-2322SS

HMGB1/HMG-1 Antibody

Product Information

Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-citrate/phosphate, pH 7-8
Target Molecular Weight	29 kDa

Product Description

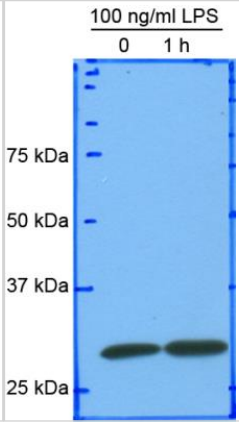
Host	Rabbit
Gene ID	3146
Gene Symbol	HMGB1
Species	Human, Mouse, Rat, Bovine, Canine, Sheep
Species Reactivity	Human, mouse, rat, dog, bovine, sheep. Predicted to react with porcine, equine and rabbit based on 100% sequence homology.
Immunogen	A synthetic peptide made to an internal portion of the human HMGB1 protein sequence (between residues 100-200). [UniProt #P09429]

Product Application Details

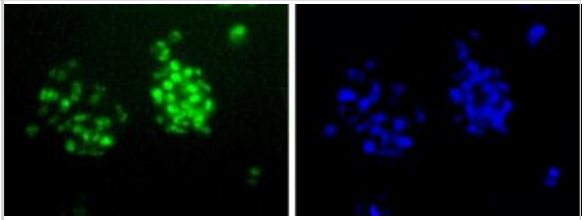
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry 1:100, Immunohistochemistry 1:100-1:250, Immunohistochemistry-Paraffin 1:100-1:250, Western Blot 0.5-1.0 ug/ml, Immunocytochemistry/Immunofluorescence 0.05 ug/ml, Simple Western 1:2000
Application Notes	This HMGB1 antibody is useful for Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry paraffin embedded sections and Western blot, where a band is seen ~29 kDa.In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

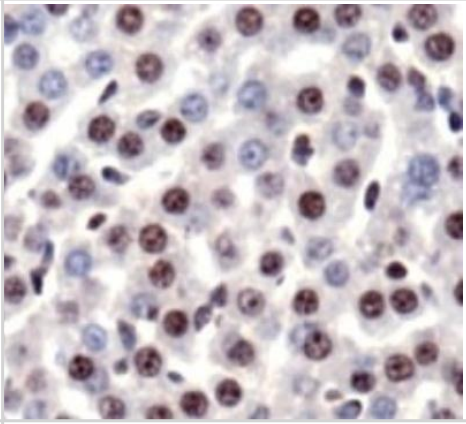
Western Blot: HMGB1 Antibody [NB100-2322] - Hepatocyte protein lysate at 1:1000 4C overnight. Image from verfified customer review.



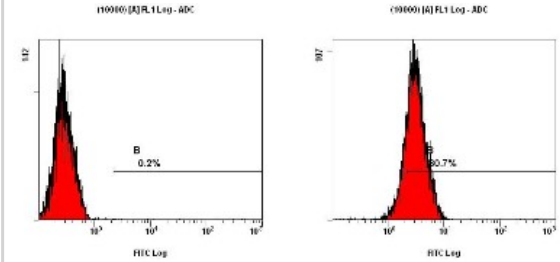
Immunocytochemistry/Immunofluorescence: HMGB1 Antibody [NB100-2322] - Detection of HMGB1 (Green) in Hela cells using NB100-2322. Nuclei (Blue) are counterstained with Hoecsht 33258.



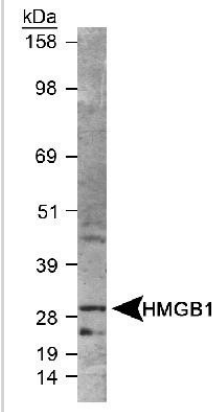
Immunohistochemistry-Paraffin: HMGB1 Antibody [NB100-2322] - Staining of HMGB1 in mouse liver using NB100-2322.

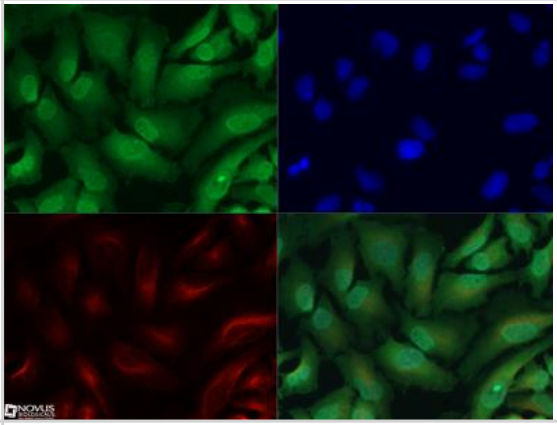


Flow Cytometry: HMGB1 Antibody [NB100-2322] - Staining of NTERA-2 cells using NB100-2322 at a 1:50 dilution detected using Dylight-488 conjugated goat anti-rabbit IgG secondary antibody.



Western Blot: HMGB1 Antibody [NB100-2322] - Detection of HMGB1 in whole cell HeLa lysate using NB100-2322. ECL exposure, 1 min.



Western Blot: HMGB1 Antibody [NB100-2322] - HMGB1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red). A concentration of 0.05 ug/ml was used. Image objective 40x.	
Simple Western: HMGB1/HMG-1 Antibody [NB100-2322] - Simple Western lane view shows a specific band for HMGB1 in 0.05 mg/ml of Jurkat lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	

Publications

Zahedi K, Barone S, Wang Y et al. Proximal tubule epithelial cell specific ablation of the spermidine/spermine n1-acetyltransferase gene reduces the severity of renal ischemia/reperfusion injury. PLoS OnE. 2014 Nov 13 [PMID: 25390069] (WB, Human)

Robinson AP, Caldis MW, Harp CT et al. High-mobility group box 1 protein (HMGB1) neutralization ameliorates experimental autoimmune encephalomyelitis J Autoimmun 2013 Mar 17 [PMID: 23514872] (ICC/IF, Mouse)

Kang R, Tang D, Schapiro NE et al. The HMGB1/RAGE inflammatory pathway promotes pancreatic tumor growth by regulating mitochondrial bioenergetics. Oncogene 2013 Jan 14 [PMID: 23318458] (WB, Mouse)

Fazio EN, Dimattia GE, Chadi S et al. Stanniocalcin 2 alters PERK signalling and reduces cellular injury during cerulein induced pancreatitis in mice BMC Cell Biol 2011 May 5 [PMID: 21545732] (IHC, ICC/IF, Mouse)

Tang D, Kang R, Livesey KM et al. High-mobility group box 1 is essential for mitochondrial quality control Cell Metab 2011 Jun 8 [PMID: 21641551] (ICC/IF, WB, Mouse)

Montie HL, Pestell RG, Merry DE. SIRT1 Modulates Aggregation and Toxicity through Deacetylation of the Androgen Receptor in Cell Models of SBMA. J Neurosci 3031(48):17425-17436. 2011 Nov. [PMID: 22131404]

Barlan AU, Griffin TM, McGuire KA, Wiethoff CM. Adenovirus membrane penetration activates the NLRP3 inflammasome. J Virol. 2010 Oct 27. [PMID: 20980503] (WB, Human)

Procedures

WB Protocol specific for HMGB1 Antibody (NB100-2322)
Western Blot Protocol
1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 35 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 dH2O 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% non-fat dry milk + 1% BSA in TBS for 2 hours at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-HMGB1 primary antibody (NB 100-2322) in blocking buffer and incubate 3 hours at room temperature.
8. Rinse the membrane in dH2O 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 2 hours 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's ECL).

Note: Tween-20 can be added to the blocking or antibody diultion buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

IHC-P Protocol specific for HMGB1 Antibody (NB100-2322)
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 dH2O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. 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 secondary antibody, diluted in 1X PBST, 5% goat serum. 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 Striptavidin-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 dH2O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH2O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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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 guarantee, please visit www.novusbio.com/guarantee.