Product Datasheet

HMGB1/HMG-1 Antibody - BSA Free
NB100-2322

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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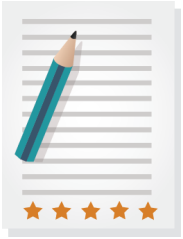
Updated 2/21/2025 v.20.1



NB100-2322

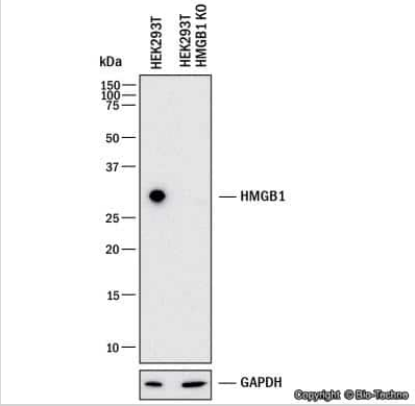
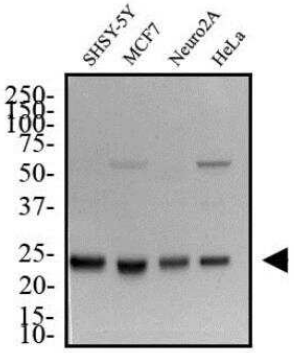
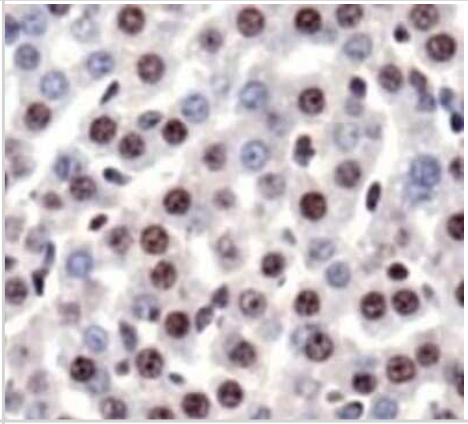
HMGB1/HMG-1 Antibody - BSA Free

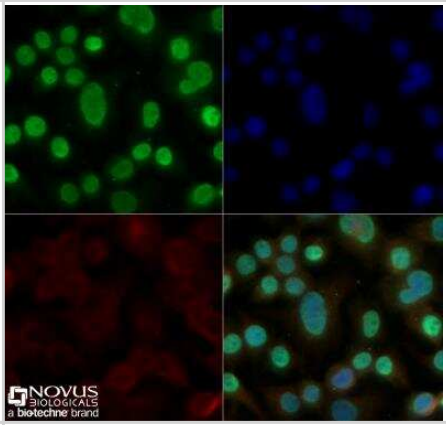
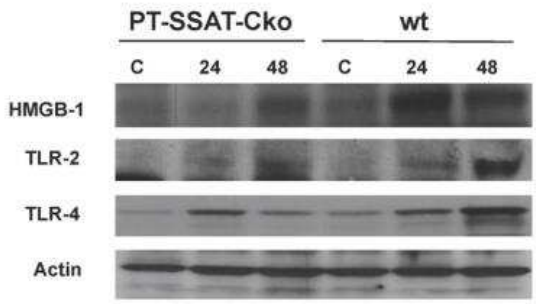
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	29 kDa

Product Description	
Host	Rabbit
Gene ID	3146
Gene Symbol	HMGB1
Species	Human, Mouse, Rat, Bovine, Canine, Rabbit, Sheep
Reactivity Notes	Rabbit reactivity reported in scientific literature (PMID: 27401639).
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, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 0.5-1.0 ug/ml, Simple Western 1:2000, Flow Cytometry 1:100, ELISA, Immunohistochemistry 1:100-1:250, Immunocytochemistry/ Immunofluorescence 0.05 ug/ml, Immunohistochemistry-Paraffin 1:100-1:250, Flow (Intracellular), Knockdown Validated
Application Notes	In Western blot a band is seen approx. 29 kDa. It has been reported that the HRP conjugated format (Catalog# NB100-2322H) of this antibody works well for use in ELISA. 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 Jurkat lysate 0.05 mg/mL, separated by Size, antibody dilution of 1:2000. Separated by Size-Wes, Sally Sue/Peggy Sue. 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.

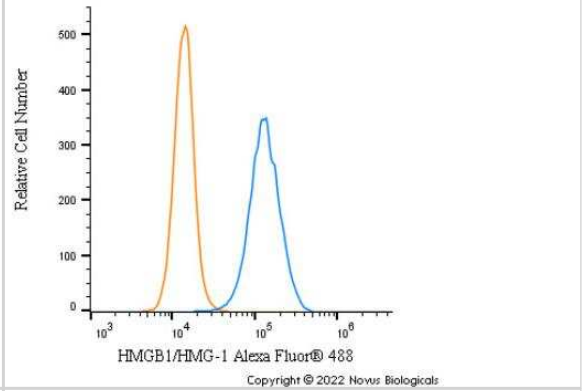
Images

Western Blot: HMGB1/HMG-1 Antibody [NB100-2322] - Western blot shows lysates of HEK293T human embryonic kidney parental cell line and HMGB1 knockout (KO) HEK293T cell line. PVDF membrane was probed with 1.0 ug/ml of Rabbit Anti-Human HMGB1 Polyclonal Antibody (Catalog # NB100-2322) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was detected for HMGB1 at approximately 30 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in the knockout HEK293T cell line. This experiment was conducted under reducing conditions.	
Western Blot: HMGB1/HMG-1 Antibody [NB100-2322] - Total protein from SHSY-5Y, MCF7, Neuro2A and HeLa 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 2.0 ug/mL anti-HMGB1 in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.	
Immunohistochemistry-Paraffin: HMGB1/HMG-1 Antibody [NB100-2322] - Staining of HMGB1 in mouse liver using NB100-2322.	
Simple Western: HMGB1/HMG-1 Antibody [NB100-2322] - Image 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.	

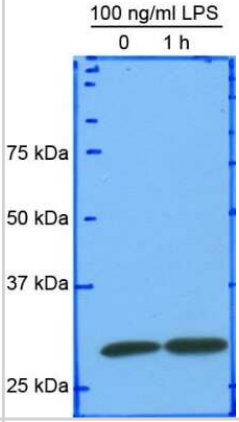
Immunocytochemistry/Immunofluorescence: HMGB1/HMG-1 Antibody [NB100-2322] - MCF7 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-HMGB1/HMG-1 Antibody NB100-2322 at 1 ug/ml for overnight at 4C and detected 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 100X objective and digitally deconvolved.	
Immunocytochemistry/Immunofluorescence: HMGB1/HMG-1 Antibody [NB100-2322] - Neuro2a cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-HMGB1 at 5 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Immunohistochemistry-Paraffin: HMGB1/HMG-1 Antibody [NB100-2322] - Rabbit blood vessel. Image from verified customer review.	
Western Blot: HMGB1/HMG-1 Antibody [NB100-2322] - The onset of innate immune response after I/R injury was compared in wt and PT-SSAT-Cko animals. Time course of the expression of HMGB1, TLR2 and 4 were compared in the kidneys of sham-operated and injured wt and PT-SSAT-Cko mice. The data are representative of three independent experiments. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0110161) licensed under a CC-BY license.	

Immunocytochemistry/Immunofluorescence: HMGB1/HMG-1 Antibody [NB100-2322] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-HMGB1/HMG-1 Antibody NB100-2322 at 1 ug/ml for 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 100X objective and digitally deconvolved.

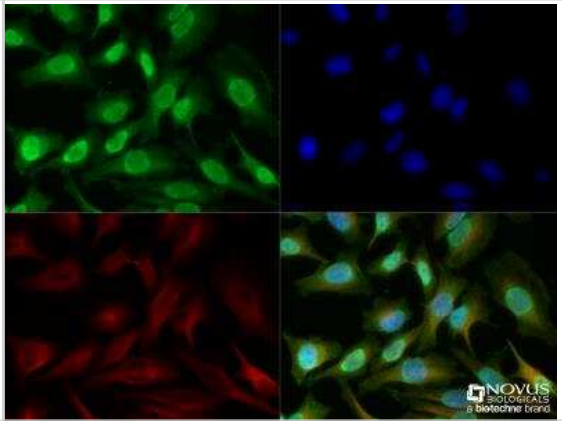
Flow Cytometry: HMGB1/HMG-1 Antibody [NB100-2322] - An intracellular stain was performed on HeLa cells with HMGB1/HMG-1 Antibody NB100-2322AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.

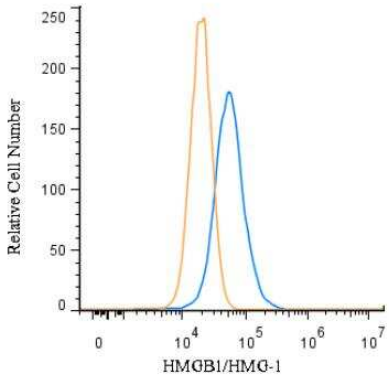
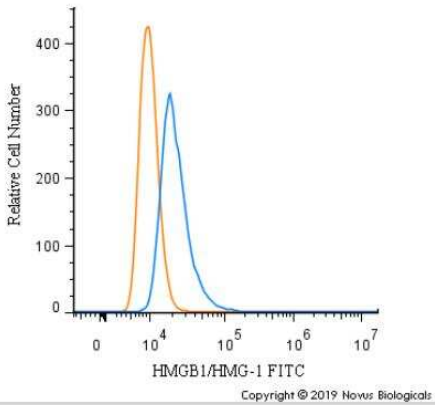
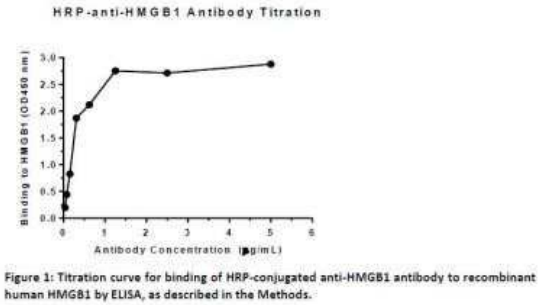
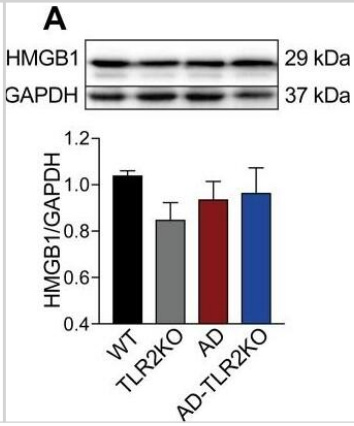


Western Blot: HMGB1/HMG-1 Antibody [NB100-2322] - Hepatocyte protein lysate at 1:1000 4C overnight. Image from verfified customer review.

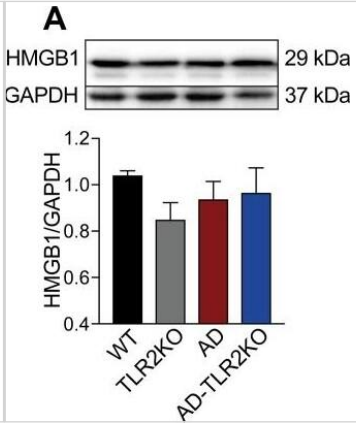


Immunocytochemistry/Immunofluorescence: HMGB1/HMG-1 Antibody [NB100-2322] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-HMGB1 [NB100-2322] at a 1:200 dilution overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) [NB100-690] was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Flow (Intracellular): HMGB1/HMG-1 Antibody [NB100-2322] - An intracellular stain was performed on HeLa with NB100-2322 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody.	
Flow Cytometry: HMGB1/HMG-1 Antibody [NB100-2322] - An intracellular stain was performed on RH-30 cells with NB100-2322F (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 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.	
ELISA: HMGB1/HMG-1 Antibody [NB100-2322] - A dose-dependent titration of the HRP conjugated anti-HMGB1 antibody on recombinant human HMGB1 protein. Image from verified customer review. Image using the HRP format of this antibody.	 Figure 1: Titration curve for binding of HRP-conjugated anti-HMGB1 antibody to recombinant human HMGB1 by ELISA, as described in the Methods.
Expression of endogenous ligands for TLR2. (A) Expression of biglycan in AD-TLR2KO mice increased significantly compared with that in WT, AD, and TLR2KO mice ($p<0.05$). (B) HMGB1 in the four groups did not show a significant difference ($p>0.05$).	

Western Blot: HMGB1/HMG-1 Antibody [NB100-2322] - Expression of endogenous ligands for TLR2. (A) Expression of biglycan in AD-TLR2KO mice increased significantly compared with that in WT, AD, & TLR2KO mice ($p<0.05$). (B) HMGB1 in the four groups did not show a significant difference ($p>0.05$). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31509519), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Dalvi S, Roll M, Chatterjee A, Kumar LK et Al. Human iPSC-based disease modeling studies identify a common mechanistic defect and potential therapies for AMD and related macular dystrophies Dev Cell 2024-10-03 [PMID: 39362220]

J Wu, F Bai, W Mao, B Liu, X Yang, J Zhang, T Li, G Borjigin, J Cao Anti-inflammatory effects of the prostaglandin D2/prostaglandin DP1 receptor and lipocalin-type prostaglandin D2 synthase/prostaglandin D2 pathways in bacteria-induced bovine endometrial tissue Veterinary research, 2022-11-26;53(1):98. 2022-11-26 [PMID: 36435808]

Vilca SJ, Margetts AV, Höglund L et Al. Microglia contribute to methamphetamine reinforcement and reflect persistent transcriptional and morphological adaptations to the drug Brain Behav Immun 2024-07-25 [PMID: 38838836]

Montes de Oca R, Alavi AS, Vitali N et al. Belantamab Mafodotin (GSK2857916) Drives Immunogenic Cell Death and Immune-mediated Antitumor Responses In Vivo Molecular Cancer Therapeutics 2021-10-01 [PMID: 34253590] (Flow Cytometry)

Shih-Hong Khoo, Pei-Ru Wu, Kun-Tu Yeh, Shih-Lan Hsu, Chi-Hao Wu Biological and clinical significance of the AGE-RAGE axis in the aggressiveness and prognosis of prostate cancer Journal of Food and Drug Analysis 2023-01-01 [PMID: 38526823]

Iram Zafar, Shajer Manzoor, Nithya Mariappan, Shama Ahmad, Mohammad Athar, Veena Antony, Aftab Ahmad A Murine Model of Vesicant-Induced Acute Lung Injury. The Journal of pharmacology and experimental therapeutics 2024-01-19 [PMID: 38050084]

Silvia Ezquerro, Fátima Mocha, Gema Frühbeck, Rocío Guzmán-Ruiz, Víctor Valentí, Carmen Mugueta, Sara Becerril, Victoria Catalán, Javier Gómez-Ambrosi, Camilo Silva, Javier Salvador, Inmaculada Colina, María M Malagón, Amaia Rodríguez Ghrelin Reduces TNF-α-Induced Human Hepatocyte Apoptosis, Autophagy, and Pyroptosis: Role in Obesity-Associated NAFLD. The Journal of clinical endocrinology and metabolism 2019-12-03 [PMID: 30137403]

Xiao J, Yao J, Jia L et al. Protective Effect of Met12, a Small Peptide Inhibitor of Fas, on the Retinal Pigment Epithelium and Photoreceptor After Sodium Iodate Injury. Invest. Ophthalmol. Vis. Sci. 2017-03-01 [PMID: 28346613]

Liu J, Liu Y, Wang Y et al. HMGB1 is a mediator of cuproptosis-related sterile inflammation Frontiers in Cell and Developmental Biology 2022-09-21 [PMID: 36211458]

Kuwar R, Rolfe A, Di L et al. A Novel Inhibitor Targeting NLRP3 Inflammasome Reduces Neuropathology and Improves Cognitive Function in Alzheimer's Disease Transgenic Mice Journal of Alzheimer's Disease 2021-08-17 [PMID: 34219728] (Western Blot)

Liu X, Cao H, Li J et al. Autophagy induced by DAMPs facilitates the inflammation response in lungs undergoing ischemia-reperfusion injury through promoting TRAF6 ubiquitination. Cell Death Differ. 2017-04-01 [PMID: 28157209] (B/N)

Yang W, Hong SA, Kim JM et al. The immunologic phenotype of thrombi is associated with future vascular events after cerebral infarction Journal of neurointerventional surgery 2023-05-17 [PMID: 37197936]

More publications at http://www.novusbio.com/NB100-2322

Procedures

WB Protocol specific for HMGB1 Antibody (NB100-2322)

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 manufacturers instructions.

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.

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 NB100-2322

NB800-PC1	HeLa Whole Cell Lysate
NB100-2322PEP	HMGB1/HMG-1 Antibody Blocking Peptide
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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