

# Product Datasheet

## Sirtuin 1/SIRT1 Antibody NBP1-49540

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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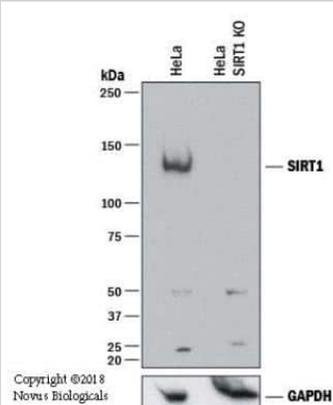
**NBP1-49540**

Sirtuin 1/SIRT1 Antibody

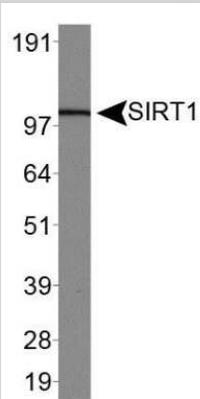
Product Information	
Unit Size	0.1 ml
Concentration	0.2 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS, 0.1% BSA, and 50% Glycerol
Product Description	
Host	Rabbit
Gene ID	23411
Gene Symbol	SIRT1
Species	Human
Immunogen	A genomic peptide made to an internal region of the human SIRT1 protein (within residues 400-550). [Swiss-Prot Q96EB6]
Notes	Manufactured by Genomic Antibody Technology™. GAT <a href="#">FAQs</a>
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:100, Immunohistochemistry 1:150, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunohistochemistry-Paraffin 1:150, Knockout Validated, Knockdown Validated
Application Notes	<p>This SIRT1 antibody is useful in Immunocytochemistry/Immunofluorescence, Immunohistochemistry paraffin embedded sections and Western blot. In Western blot a band is seen ~105 kDa.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.</p> <p>See <a href="#">Simple Western Antibody Database</a> for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100, apparent MW was 109 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

## Images

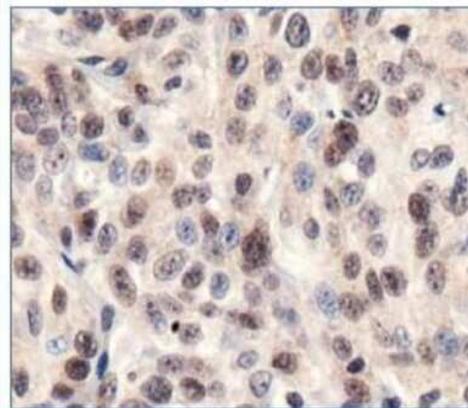
Western Blot: Sirtuin 1/SIRT1 Antibody [NBP1-49540] - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and SIRT1 knockout (KO) HeLa cell line. PVDF membrane was probed with 1:1000 of Rabbit Anti-Human SIRT1 Polyclonal Antibody (Catalog # NBP1-49540) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was detected for SIRT1 at approximately 130 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.



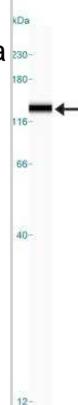
Western Blot: Sirtuin 1/SIRT1 Antibody [NBP1-49540] - Analysis of SIRT1 in HeLa whole cell lysates.



Immunohistochemistry: Sirtuin 1/SIRT1 Antibody [NBP1-49540] - Immunohistochemical analysis of SIRT1 in human renal cell carcinoma.



Simple Western: Sirtuin 1/SIRT1 Antibody [NBP1-49540] - Simple Western lane view shows a specific band for SIRT1 in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



## Publications

Yanes R, Zhang H, Shen Y et al. Metabolic reprogramming in memory CD4 T cell responses of old adults Clinical Immunology 2019-07-01 [PMID: 31279855] (Simple Western, Human)

## Procedures

### Western Blot protocol specific for SIRT1 Antibody (NBP1-49540)

Sirtuin 1/SIRT1 Antibody:

Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 40 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 dH<sub>2</sub>O 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% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH<sub>2</sub>O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-SIRT1 primary antibody (NBP1-49540) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH<sub>2</sub>O 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 1 hour 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 ECL).

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

### Immunohistochemistry-Paraffin protocol for Sirtuin 1/SIRT1 Antibody (NBP1-49540)

Sirtuin 1/SIRT1 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 dH<sub>2</sub>O 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 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 dH<sub>2</sub>O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH<sub>2</sub>O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.



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### **Products Related to NBP1-49540**

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

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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.

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