

# Product Datasheet

## S100B Antibody (15F9NB) - BSA Free NBP2-45267

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NBP2-45267**

S100B Antibody (15F9NB) - BSA Free

**Product Information**

<b>Unit Size</b>	0.1 mg
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	15F9NB
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG2b Kappa
<b>Purity</b>	Protein G purified
<b>Buffer</b>	PBS

**Product Description**

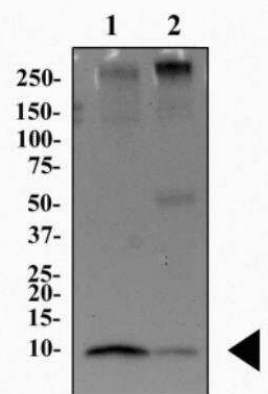
<b>Host</b>	Mouse
<b>Gene ID</b>	6285
<b>Gene Symbol</b>	S100B
<b>Species</b>	Human
<b>Immunogen</b>	Partial recombinant human S100B protein (amino acids 2-92) [Uniprot: P04271].

**Product Application Details**

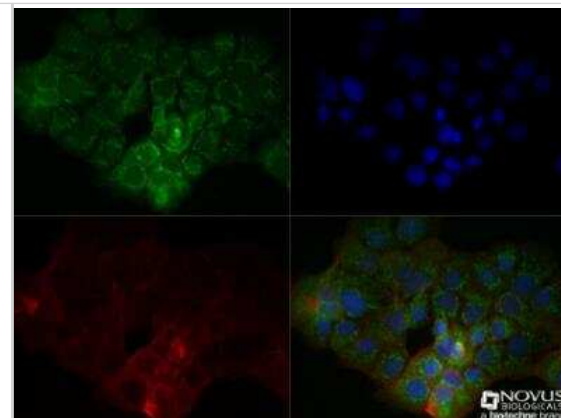
<b>Applications</b>	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
<b>Recommended Dilutions</b>	Western Blot 2 ug/ml, Flow Cytometry 1:500, Immunohistochemistry 1:100 - 1:500, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 1:100 - 1:500

**Images**

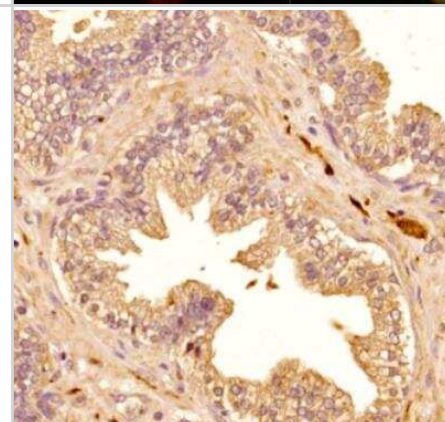
Western Blot: S100B Antibody (15F9NB) [NBP2-45267] - Total protein from human brain (1) and mouse brain (2) was separated on a 4-15% gel by SDS-PAGE, transferred to 0.2 um PVDF membrane for 30 minutes and blocked in 5% non-fat milk in TBST. The membrane was probed with 2 ug/ml anti-S100B in 1% milk and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



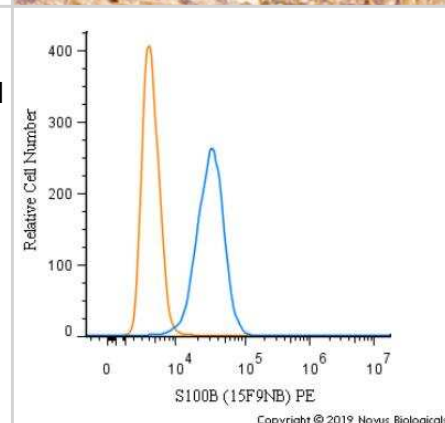
**Immunocytochemistry/Immunofluorescence: S100B Antibody (15F9NB) [NBP2-45267]** - Analysis in A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with S100B (15F4NB) at a 1:100 dilution overnight at 4 degrees Celsius and detected with DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were detected with DAPI (Blue) at 2.0 ug/ml in 1X PBS. Cells were imaged using a 40X objective.



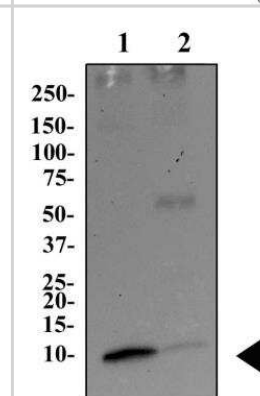
**Immunohistochemistry-Paraffin: S100B Antibody (15F9NB) [NBP2-45267]** - Analysis in human prostate tissue.



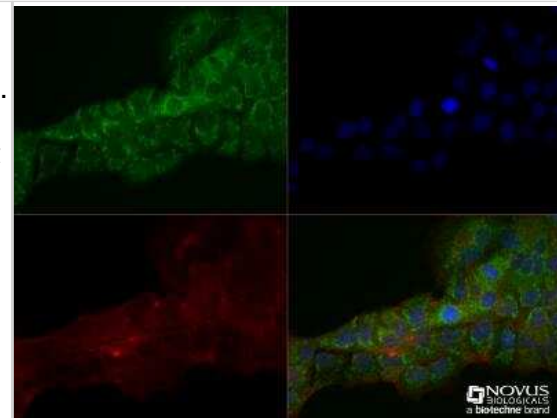
**Flow Cytometry: S100B Antibody (15F9NB) [NBP2-45267]** - An intracellular stain was performed on SK-MEL-28 cells with S100B [15F9NB] Antibody NBP2-45267PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.



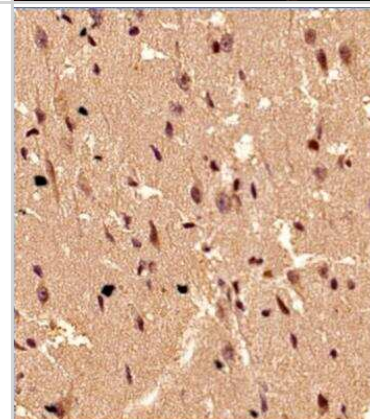
**Western Blot: S100B Antibody (15F9NB) [NBP2-45267]** - Total protein from human brain (1) and mouse brain (2) was separated on a 4-15% gel by SDS-PAGE, transferred to 0.2 um PVDF membrane for 30 minutes and blocked in 5% non-fat milk in TBST. The membrane was probed with 2 ug/ml anti-S100B in 1% milk and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



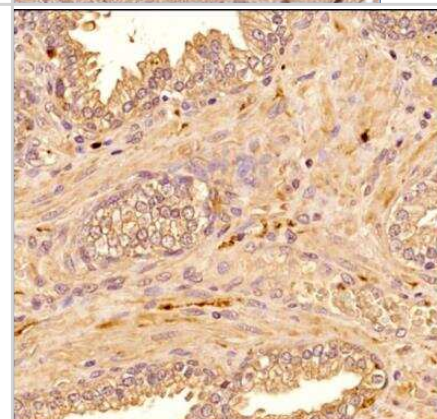
**Immunocytochemistry/Immunofluorescence: S100B Antibody (15F9NB) [NBP2-45267]** - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with S100B (15F9NB) at a 1:100 dilution overnight at 4 degrees Celsius and detected with DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were detected with DAPI (Blue) at 2.0 ug/ml in 1X PBS. Cells were imaged using a 40X objective.



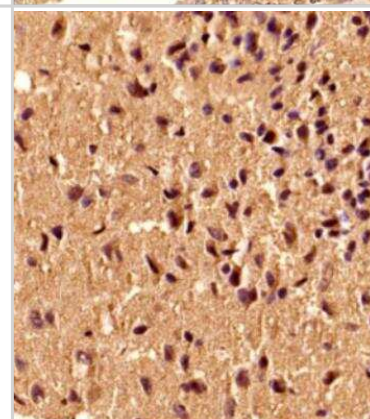
**Immunohistochemistry-Paraffin: S100B Antibody (15F9NB) [NBP2-45267]** - Analysis of S100B (15F9NB) in human brain.



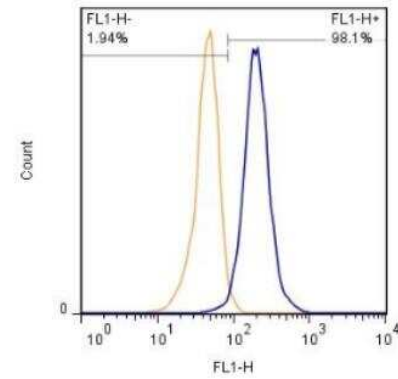
**Immunohistochemistry-Paraffin: S100B Antibody (15F9NB) [NBP2-45267]** - Analysis of S100B in human prostate.



**Immunohistochemistry-Paraffin: S100B Antibody (15F9NB) [NBP2-45267]** - Analysis in human brain tissue.



Flow Cytometry: S100B Antibody (15F9NB) [NBP2-45267] - Staining of A431 cells ( $1 \times 10^6$  cells/ml) with S100B antibody, clone 15F4.1D2 (Orange) stained at a dilution of 1:500. Detected with a GtxRb DyLight 488 secondary. Shown with the secondary control (Blue).



## Publications

Gao S, Tang Y, Sun W et al. 3D-bioprinted GelMA nerve guidance conduits promoted peripheral nerve regeneration by inducing trans-differentiation of MSCs into SCLCs via PIEZO1/YAP axis Materials Today Advances 2023-03-01 (ICC/IF, WB, Mouse, Rat)

## Procedures

### Western Blot protocol for S100B Antibody (NBP2-45267)

1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
  2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
  3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
  4. Rinse the blot.
  5. Block the membrane using standard blocking buffer for at least 1 hour.
  6. Wash the membrane in wash buffer three times for 10 minutes each.
  7. Dilute anti-S100B primary antibody in blocking buffer and incubate 1 hour at room temperature.
  8. Wash the membrane in wash buffer three times for 10 minutes each.
  9. Apply the diluted 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 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.
- Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

### Immunocytochemistry/Immunofluorescence protocol for S100B Antibody (NBP2-45267)

#### S100B Antibody (15F9NB):

#### 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 1:25,000 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



**Immunohistochemistry-Paraffin protocol for S100B Antibody (NBP2-45267)**

1. Deparaffinize the tissue sections by immersing the slides in Xylene with two changes for 10 min each. Sections should not get dried at any stage from this point.
2. Rehydrate the tissue sections by immersing the slides in decreasing grades of ethanol as follows:
  - a. Immerse in 100% ethanol with 2 changes for 5 minutes each
  - b. Immerse in 95% ethanol with 2 changes for 5 minutes each
  - c. Immerse in 90% ethanol for 5 minutes
  - d. Immerse in 70% ethanol for 5 minutes
  - e. Immerse in 50% ethanol for 5 minutes
  - f. Immerse in distilled water for 5 minutes
3. Antigen Retrieval (Microwave Method):
  - a. Immerse the slides in a microwave compatible tray containing 10 mM Sodium Citrate buffer (pH 6.0) with 0.05% Tween 20.
  - b. Boil the slides and maintain the sub-boiling temperature for 5 minutes in the microwave. Thereafter, take out the tray very carefully and cool it at room temperature (RT) for about 30 minutes.
  - c. Wash the slides 3 times, 3 minutes each by immersing them in TBST (Tris Buffered Saline having 0.05% Tween 20).
4. Quenching of Endogenous Peroxidase:
  - a. Incubate the slides in 3% hydrogen peroxide prepared in methanol for 15 minutes (at RT, in dark conditions).
  - b. Wash the slides in TBST 3 times, 3 minutes each.
5. Protein Blocking:
  - a. Incubate the sections with background sniper solution at RT for 15 minutes (Biocare Medicals, USA).
  - b. Wash the sections 3 times, 3 min each by immersing the slides in TBST.
6. Primary Antibody:
  - a. Dilute the primary antibody at 5ug/ml concentration using PBS as a diluent.
  - b. Incubate the sections with diluted primary antibody for 90 minutes at RT in a humidified chamber.
  - c. Thereafter, wash the slides 4 times, 5 minutes each with TBST.
7. Probe (Secondary Reagent):
  - a. Incubate with MACH 1 Mouse probe for 15 minutes at RT.
  - b. Incubate for 30 min at room temperature with HRP-Polymer (Biocare Medical, USA).
  - c. Wash the slides with TBST 4 times, 5 minutes each
8. Chromogen:
  - a. Mix 32ul of DAB Chromogen with 1 ml of DAB substrate buffer (Biocare Medical, USA).
  - b. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds - 5 minutes).
  - c. As soon as an appropriate color develops, rinse the slides with deionized water (2-3 brief rinses).
9. Counter stain:
  - a. Counter stain with Hematoxylin for 30 seconds (Vector Labs, USA).
  - b. Wash in deionized water for 1-2 minutes to clear the extra stain.
  - c. Incubate the slides in bluing solution or Scott's water twice for 2 minutes each time.
10. Dehydrate the sections in increasing grades of alcohols:
  - a. 50% alcohol for 1 minute
  - b. 70% for 1 minute
  - c. 90% for 1 minute
  - d. 95% for 1 minute
  - e. 100% for 1 minute
  - f. Xylene with 2 changes for 2 minutes each
11. Mount with DPX mount and cover-slip glass (Fisher Scientific, USA), carefully not allowing any air bubbles to enter.

**NOTE:-** This protocol is provided as a reference tool only. Depending upon the type of tissues /tissue processing and reagents employed, the end user will need to optimize the final conditions for achieving an expected staining.



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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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