

# Product Datasheet

## Synaptotagmin 1 Antibody

### NB100-1938

Unit Size: 0.25 ml

Store at 4C in the dark.

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**NB100-1938**

## Synaptotagmin 1 Antibody

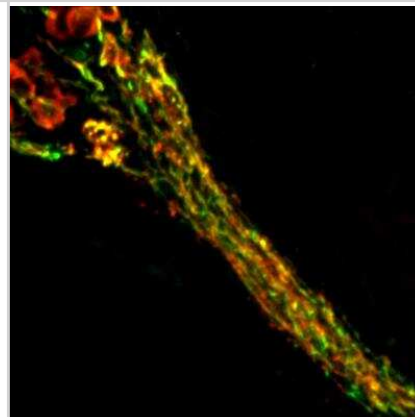
Product Information	
Unit Size	0.25 ml
Concentration	0.4 mg/ml
Storage	Store at 4C in the dark.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgY
Purity	Immunogen affinity purified
Buffer	10mM Sodium PBS (0.9% isotonic, w/v, pH 7.2)
Target Molecular Weight	48 kDa

Product Description	
Description	<p><b>Purification Notes</b> After repeated injections into the hens, immune eggs were collected, and the IgY fractions were purified from the yolks. These IgY fractions were then affinity-purified using a peptide column, and the concentrations of the eluates adjusted to 400 ug/ml. Finally, equal volumes of each of the three affinity-purified anti-peptide antibodies were mixed, and the preparation was filter-sterilized.</p> <p><b>Storage Notes</b> Store at 4C in the dark. Under these conditions, the antibodies should have a shelf life of at least 12 months (provided they remain sterile). Do not freeze these antibodies unless you want to store them for longer periods of time. Note, however, that each time an antibody preparation is frozen, about half of its binding activity is lost.</p>
Host	Chicken
Gene ID	6857
Gene Symbol	SYT1
Species	Human, Mouse, Rat
Marker	Neuron Cell Marker
Immunogen	Chickens were immunized with four synthetic peptide/keyhole limpet hemocyanin (KLH) conjugates. These synthetic peptides corresponded to different regions of Synaptotagmin 1, but are shared between the human (NP_005630, NCBI) and mouse (NP_033332, NCBI) sequences.

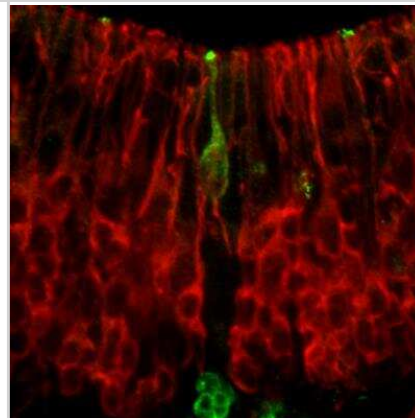
Product Application Details	
Applications	Western Blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000-1:2000, Immunohistochemistry 1:1000-1:2000, Immunocytochemistry/Immunofluorescence 1:10 - 1:500, Immunohistochemistry-Paraffin 1:1000-1:2000
Application Notes	Each of the three antibodies were analyzed by immunohistochemistry (1:2000) using fluorescein-labeled goat anti-chicken IgY (1:500) as the secondary reagent.

## Images

Immunocytochemistry/Immunofluorescence: Synaptotagmin 1 Antibody [NB100-1938] - Immunohistochemical staining of Synaptotagmin 1 (GREEN) and NFH (RED) in a cryostat section through the Cochlear nerve in an adult mouse.

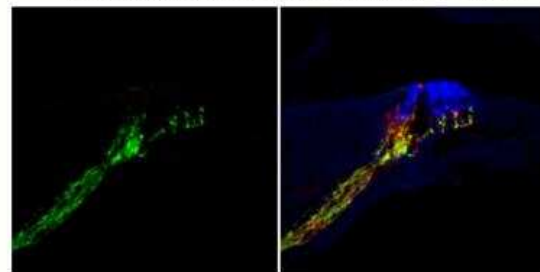


Immunocytochemistry/Immunofluorescence: Synaptotagmin 1 Antibody [NB100-1938] - Immunohistochemical staining of Synaptotagmin 1 (GREEN) and Vimentin (RED) in a cryostat section through the basal plate of an e16 mouse brain.



Immunocytochemistry/Immunofluorescence: Synaptotagmin 1 Antibody [NB100-1938] - Immunohistochemical staining of Synaptotagmin 1 (GREEN) and NFH (RED) and Vimentin (BLUE) in a cryostat section through the Cochlear nerve and Organ of Corti in an adult mouse.

Aves Chicken anti-Synaptotagmin (green), 1:1,000



## Procedures

### Immunohistochemistry Chicken IgY Protocol (NB100-1938)

Immunohistochemistry Chicken IgY Protocol (NB100-1938): [https://www.novusbio.com/products/synaptotagmin-1-antibody\\_nb100-1938](https://www.novusbio.com/products/synaptotagmin-1-antibody_nb100-1938)

Citrate Buffer Antigen Retrieval Protocol

**Background:** Formaldehyde fixation (2% or 4%, or as a component of 10% formalin) produces protein cross-links in tissues that tends to interfere with antibody penetration. This seems to be particularly true of paraffin- embedded formaldehyde-fixed tissue. Since chicken IgY antibodies are larger than rabbit or mouse IgG's, "extra steps" may be necessary to compensate for their larger size.

The citrate-based "antigen retrieval" protocol outlined below has been shown to improve chicken IgY antibody penetration into 4% formaldehyde-fixed paraffin-embedded sections, and can increase the degree and intensity of immunoreactivity and immunostaining.

**Reagents** (NOTE: You can use either the Sodium Citrate or Citric Acid Buffers in step #3, below)

"Sodium Citrate Buffer" (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0)

Weigh out 2.94 grams of trisodium citrate (dihydrate). Dissolve in approximately 900 mls of deionized, distilled water. Adjust the pH to 6.00 with 1.0 N HCl. Add 0.5 ml of Tween-20. Mix. Bring up the volume to 1.0 litres with water. Store this solution at room temperature for 3 months or at 4C for longer periods.

"Citric Acid Buffer" (10mM Citric Acid, 0.05% Tween 20, pH 6.0)

Weigh out 1.92 grams of citric acid (anhydrous). Dissolve in approximately 900 mls of deionized, distilled water. Adjust the pH to 6.0 with 1.0 N NaOH. Add 0.5 ml of Tween-20. Mix. Bring up the volume to 1.0 litres with water. Store this solution at room temperature for 3 months or at 4C for longer periods.

"Phosphate-Buffered Saline" [PBS, 10 mM Sodium phosphate-buffered (pH 7.2) isotonic (0.9%, w/v) saline solution] PBS Tween (0.05% Tween 20 in PBS)  
Ethanol (80%, 90%, 95%, 100%) diluted with water

Xylene

**Procedure** (for use with paraffin-embedded sections):

- 1 Deparaffinize tissue sections in 2 changes of xylene (5 minutes each).
2. Hydrate in 2 changes of 100% ethanol (3 minutes each), 95% ethanol (1 minute), 90% ethanol (1 minute), 80% ethanol (1 minute). Rinse in distilled water.
3. Pre-heat steamer or water bath with staining dish containing either Sodium Citrate Buffer or Citrate Buffer. Wait until temperature reaches 95-100 degrees C.

NOTE: Microwave or pressure cooker can be used as an alternative as a heating source.

4. Immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20-40 minutes (optimal incubation times will vary).
5. Remove the staining dish, and allow it to cool to room temperature (for 20 minutes or so).

6. Rinse sections in PBS Tween twice for 2 minutes each time.

NOTE: The remainder of this protocol is meant to be a suggestion, and can be substituted with your regular immunostaining protocol.

7. Block sections for 30 minutes with Blocking buffer diluted 1:10 with water.

8. Incubate sections with primary antibody at appropriate dilution in antibody dilution buffer overnight at 4 degrees C. Since chicken IgY antibodies are larger than mammalian IgG's, this overnight incubation allows more time for antibody penetration into tissue sections.

9. Rinse sections with PBS Tween 20 twice for 5 minutes each time.

10. Incubate sections with labeled secondary antibody (see NOTE, below) at appropriate dilution (for one hour at room temperature) in a 1:100 dilution of blocking buffer (diluted in PBS).

11. Rinse with PBS Tween 20 for three times for 5 minutes each time.

NOTE: This protocol may use HRP- or fluorescently-labeled secondary antibodies produced in goats or rabbits.

#### References:

1. Shi SR, Chaiwun B, Young L, Cote RJ, Taylor CR. (1993). Antigen retrieval technique utilizing citrate buffer or urea solution for immunohistochemical demonstration of androgen receptor in formalin-fixed paraffin sections. *J Histochem Cytochem* 41 (11): 1599-1604.
2. Kanai K, Nunoya T, Shibuya K, Nakamura T, Tajima M (1998). Variations in effectiveness of antigen retrieval pretreatments for diagnostic immunohistochemistry. *Res Vet Sci* 64 (1): 57-61.
3. Brown RW, Chirala R. (1995). Utility of microwave-citrate antigen retrieval in diagnostic immunohistochemistry. *Mod Pathol* 8 (5): 515-20.
4. Morgan JM, Navabi H, Schmid KW, Jasani B (1994). Possible role of tissue-bound calcium ions in citrate-mediated high-temperature antigen retrieval. *J Pathol* 174 (4): 301-7.
5. Pellicer EM, Sundblad A (1994). Antigen retrieval by microwave oven with buffer of citric acid. *Medicina (B Aires)*. 54 (2): 129-32.
6. Shi SR, Chaiwun B, Young L, Cote RJ, Taylor CR (1993). Antigen retrieval technique utilizing citrate buffer or urea solution for immunohistochemical demonstration of androgen receptor in formalin-fixed paraffin sections. *J Histochem Cytochem* 41 (11): 1599-604.





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