

# Product Datasheet

## GAD1/GAD67 Antibody - BSA Free NBP1-02161

Unit Size: 0.5 ml

Store at 4C in the dark.

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**NBP1-02161**

GAD1/GAD67 Antibody - BSA Free

Product Information	
Unit Size	0.5 ml
Concentration	0.1 mg/ml
Storage	Store at 4C in the dark.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgY
Purity	Immunogen affinity purified
Buffer	10mM PBS (0.9% isotonic, w/v, pH 7.2)
Target Molecular Weight	67 kDa

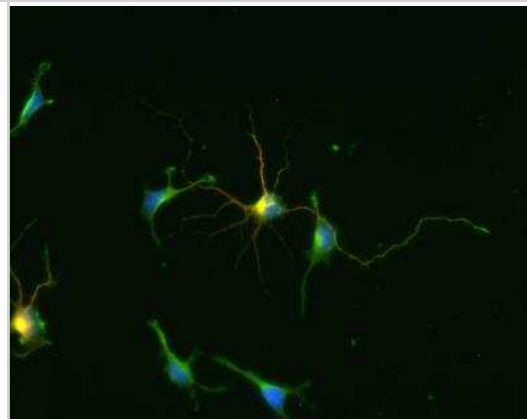
Product Description	
Host	Chicken
Gene ID	2571
Gene Symbol	GAD1
Species	Human, Mouse, Rat
Marker	Neuron Cell Marker
Immunogen	Chickens were immunized with a synthetic peptide/keyhole limpet hemocyanin (KLH) conjugate. This synthetic peptide corresponded to a region near the C-terminus of GAD1/GAD67, and was 100% conserved between the human (Q99259, NCBI), mouse (P48318, NCBI) and rat (NP_058703, NCBI) gene products.
Notes	<p>Chicken products cannot be exported to Canada.</p> <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, the concentrations of the eluate adjusted to 100 ug/ml, and the preparation was filter-sterilized through a 0.45 um filter.</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>

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1:1000-1:2000, Immunohistochemistry 1:500-1:1000, Immunocytochemistry/ Immunofluorescence 1:500-1:1000

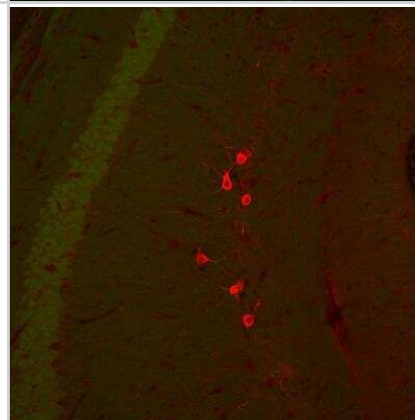


## Images

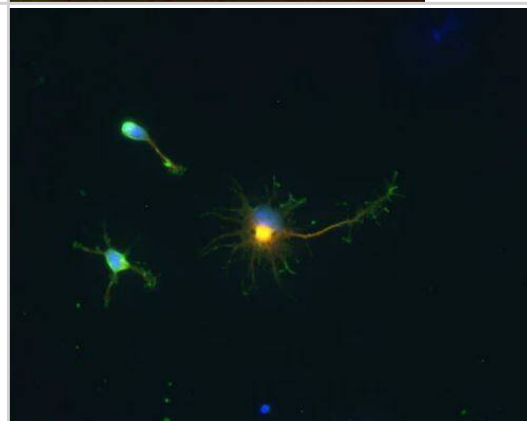
Immunocytochemistry/Immunofluorescence: GAD1/GAD67 Antibody [NBP1-02161] - Immunocytochemical staining of GAD1/GAD67 (RED/YELLOW) in cultured cortical neurons from a neonatal mouse (C57/Black6). GAD1/GAD67 staining was detected using Texas Red goat anti-chicken IgY. MAP2 staining (GREEN) used a rabbit antibody.



Immunocytochemistry/Immunofluorescence: GAD1/GAD67 Antibody [NBP1-02161] - Immunohistochemical staining of inhibitory neurons (RED) within the hippocampal formation of a transgenic, adult male Black6 mouse brain. The brains were fixed with 4.0% paraformaldehyde. The green staining is low level GFP fluorescence expressed off the actin promoter in this mouse transgenic strain.



Immunocytochemical staining of GAD1/GAD67 (red/yellow) in cultured cortical neurons from a neonatal mouse (C57/Black6) using Texas Red goat anti-chicken IgY as a secondary (1:500).



## Publications

Scheuer T, Endesfelder S, Auf dem Brinke E et al. Neonatal Oxidative Stress Impairs Cortical Synapse Formation and GABA Homeostasis in Parvalbumin-Expressing Interneurons Oxidative Medicine and Cellular Longevity 2022-05-25 [PMID: 35663195] (Western Blot)

Guerrero-Bautista R, Franco-Garcia A, Hidalgo J, et al. Distinct regulation of dopamine D3 receptor in the basolateral amygdala and dentate gyrus during the reinstatement of cocaine CPP induced by drug priming and social stress Int J Mol Sci 2021-04-03 [PMID: 33803578]

Franco-Garcia A, Fernandez-Gomez FJ, Gomez-Murcia V et al. Molecular Mechanisms Underlying the Retrieval and Extinction of Morphine Withdrawal-Associated Memories in the Basolateral Amygdala and Dentate Gyrus Biomedicines 2022-03-02 [PMID: 35327388] (IF/IHC, Rat)

Guerrero-Bautista R, Franco-Garcia A, Hidalgo JM et al. Blockade of D3 receptor prevents changes in DAT and D3R expression in the mesolimbic dopaminergic circuit produced by social stress- and cocaine prime-induced reinstatement of cocaine-CPP J. Psychopharmacol. (Oxford) 2020-07-10 [PMID: 32648812]

Arcidiacono B, Chiefari E, Foryst-Ludwig A et al. Obesity-related hypoxia via miR-128 decreases insulin-receptor expression in human and mouse adipose tissue promoting systemic insulin resistance EBioMedicine 2020-07-27 [PMID: 32739259] (WB, Human)

Valladolid-Acebes I, Merino B, Principato A et al. High fat diets induce changes in hippocampal glutamate metabolism and neurotransmission. American Journal of Physiology. Endocrinology and Metabolism. 2011-11-22 [PMID: 22114023]



## Procedures

### Immunohistochemistry Chicken IgY Protocol (NBP1-02161)

Immunohistochemistry Chicken IgY Protocol (NBP1-02161):

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% formalde- hyde-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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### **Products Related to NBP1-02161**

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BAF010	Goat anti-Chicken IgY Secondary Antibody [Biotin]
NB7276	Goat anti-Chicken IgM Heavy Chain Secondary Antibody
NBP2-51655-0.05mg	Recombinant Human GAD1/GAD67 His Protein
DBD00	BDNF [HRP]

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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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