OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for CH23031-100

GAD67 (GAD1) Chicken Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	IF, IHC, WB
Recommend Dilution:	**Immunocytochemistry:** 1:1000 -1:2000. **Immunohistochemistry**: 1:1000-1:2000. **Western Blots:** 1:2000-1:5000.
Reactivity:	Human, Mouse, Rat
Host:	Chicken
Clonality:	Polyclonal
Immunogen:	Synthetic peptide (keyhole limpet hemocyanin -KLH conjugate) corresponding to a region near the C-terminus of this gene product; 100% conserved between human (Q99259, NCBI), mouse (P48318, NCBI) and rat (NP_058703, NCBI)
Formulation:	State: Aff - Purified State: 100 ul Liquid PBS (pH 7.2; 10 mM; isotonic 0.9%, w/v) with sodium azide (0.02%, w/v).
Purification:	Affinity Chromatography
Gene Name:	glutamate decarboxylase 1
Database Link:	Entrez Gene 2571 Human
Background:	Glutamic acid decarboxylase (GAD) catalyzes the conversion of glutamic acid to gamma-aminobutyric acid (GABA); which is a major inhibitory neurotransmitter in the higher brain regions and a putative paracrine hormone in pancreatic islets. There are two forms of GAD; GAD65 and GAD67 (65 kDa and 67kDa respectively, with 64% aa identity between forms). GAD67 is a rate limiting enzyme and is responsible for >90% of GABA production in the central nervous system. GAD67 is cytoplasmic and encoded on chromosome 2. GAD67 expression is plastic and can change in response to experimental manipulation or disease progression. GAD67 levels are altered in multiple neuropsychiatric disorders including schizophrenia.
Synonyms:	Glutamate decarboxylase 1, GAD-67
Note:	Protocol: **Immunostaining Cell Cultures** 1. Draw of culture medium with aspirator and add 1 ml of 3.7 % formalin in PBS solution to the dish. (make up from 10mls Fisher 37% formalin plus 90mls PBS, the Fisher formalin contains 37% formaldehyde plus about 1% methanol which may be relevant sometimes). Let

sit at room temp for 1 minute. (can add 0.1% Tween 20 to PBS used here and all subsequent steps to reduce background; probably best not to do this first time round though as it may extract your antigen or help wash your cells off the dish).
2. Take off the formalin/PBS and add 1ml of cold methanol (-20°C, kept in well sealed bottle in fridge). Let sit for no more than 1 minute.
3. Take off methanol and add 1ml of PBS, not letting the specimen dry out. To block nonspecific antibody binding can add ~10ml (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Can then add antibody reagents. Typically 100ml of hybridoma tissue culture supernatent or 1ml of mouse ascites fluid or crude serum. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight, exact time not too critical). Can do very gentle shaking for well adherent cell lines (3T3, Hek293 etc.).
4. Remove primary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.
5. Add 0.5 mls of secondary antibody. These are fluorescently labeled Goat anti mouse or rabbit antibodies and are conjugated to ALEXA dyes and are from Molecular probes (Eugene Oregon, the ALEXA dyes are sulphonated rhodamine compounds and are much more stable to UV than FITC, TRITC, Texas red etc.). Typically make 1:2,000 dilutions of these secondaries in PBS plus 1% goat serum, BSA or non fat milk carrier. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight). Can do gentle shaking for well adherent cell lines (3T3, HEK293 etc.).
6. Remove secondary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.
7. Drop on one drop of Fisher mounting medium onto dish and apply 22mm square coverslip. View in the microscope!

Immunostaining Tissue

Solutions
PBS - sodium phosphate-buffered (100 mM; pH 7.2) isotonic (0.9% NaCl, w/v) saline Antibody dilution buffer (PBS with 0.1% non-ionic detergent, such as Triton X-100 or Tween-20). For anti-fading, use Neuromics' i-BRITE Plus –Catalog#: SF40000 or make your own fluorescein anti-fading reagent -- Make up a 2 mg/ml phenylene diamine solution in PBS (phenylene diamine requires extensive vortexing to put it into solution). Once the phenylene diamine is completely dissolved, add an equal volume of glycerol and mix. This reagent will last about a week at -20OC. Discard this reagent when it starts to turn dark brown.
Other Reagents
Fluorescein-labeled goat anti-chicken IgY
1. Prepare your tissue sections or cultured cells as you normally would. Wash your sections or cells for 1 min with PBS at room temperature.
2. Incubate your sections or cells with your chicken primary antibodies (diluted in "antibody dilution buffer") for at least 1 hour at room temperature. The concentration of your antibody may be anywhere from 1:50-1:150 depending on the titre of the antibody and the concentration of your antigen.
3. Wash your sections or cells over a 10 minute period at room temperature (with two

changes of PBS).
4. Incubate your sections or cells with fluorescein-labeled goat anti-chicken IgY (1:500 dilution in "antibody dilution buffer" for 1 hour at room temperature. Be sure to keep these slides or culture dishes in subdued light (e.g., in a drawer) to avoid bleaching of the fluorescein dye.
5. Repeat step #4
6. Add a drop of "fluorescence anti-fading reagent" (i-BRITE Plus) to your sections or cells. Place a coverslip over the section. If you want to reduce messiness, you may also seal the coverslip by painting the edges with nail polish.
7. Store the slides or culture dishes in the refrigerator (in the dark).

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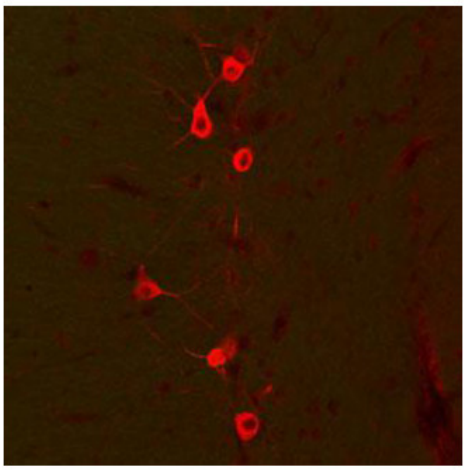
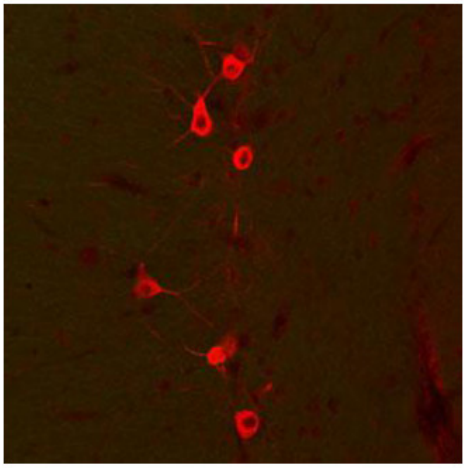
Product images:



A tissue section through an adult mouse brain showing GAD-67 (red staining) (cat # [CH23031]) in basket cells of the hippocampal formation. Green staining is autofluorescence from green florescent protein (GFP) expressed in this transgenic mouse. Photo courtesy of Dr. Felix Eckenstein, University of Vermont.



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