

PRODUCT DATA SHEET

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Product name(s):	Guinea pig polyclonal antiserum to γ-aminobutyric acid (GABA)			
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Catalogue number:	GZ 1081	Batch number:	Ztemp	Expiry date:	12 months from receipt
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Introduction:

GABA is a type I neurotransmitter which has been recorded from many animal species. In mammalian CNS GABA has long been regarded as the major inhibitory neurotransmitter, its rôle being to regulate neuronal excitability. Certain areas of the brain, notably the suprachiasmatic nucleus (SCN) are rich in GABA-containing cell bodies, processes and terminals¹ and this led to the proposal that GABA is the principal neurotransmitter in circadian time-keeping, which is known to be regulated by the firing rate of SCN neurons^{2,3}. Recently, GABA has been found to act as an inhibitory transmitter at night, decreases the firing frequency, but as an excitatory transmitter during the day, increasing the firing frequency, the mechanism being driven by changes in intracellular chloride ion concentration⁴.

Product information:

The GABA antiserum was raised by immunising guinea pigs with a GABA glutaraldehyde-haemocyanin complex. The antiserum appears to be specific for GABA without significant cross-reactivity with glutamate, aspartate, β -alanine or taurine. Immunocytochemical staining was completely blocked by pre-incubation of the diluted antiserum with 10mM conjugated GABA.

Application data:
Test tissues: Tissues from many animal species can be used.
Fixatives: Fixation by transcardial perfusion is best.
For optimal tissue preservation and maintenance of antigenicity, use 4% buffered paraformaldehyde (4g paraformaldehyde heated to 70°C in 100mL 0.1M sodium phosphate buffer pH 7.2, allowed to cool). A short pre-wash (50-100mL) to remove blood is advised. Following dissection small pieces of tissue should be post-fixed for 30-60min in the same fixative at room temperature. Glutaraldehyde (up to 0.2%) can be added if required. Tissues unsuitable for perfusion should be fixed by immersion in the above fixative, but care should be taken to avoid over-fixation (small pieces of tissue do not require more than 60min in general).
Processing: Tissue treatment should be carried out as suggested:
Vibratome slices
Tissues should be rinsed in buffer (1h to 24h) before sectioning.
Cryostat sections
After washing in buffer, place tissue in 10-30% buffered sucrose overnight or until the tissue sinks.
Paraffin wax sections
Wash tissue in buffer and dehydrate in the following sequences:
50% ethanol (2-3h), 70% ethanol (overnight), 95% ethanol (1-2h, 2 changes), 100% ethanol (1-2h, 2 changes), xylene: absolute ethanol (1:1; 15 min), xylene (30 min or until clear), paraffin wax (2 20-30 minute changes under vacuum). A brief (10min) pre-treatment in trypsin solution (1mg/mL in PBS) dramatically enhances immunostaining.
Dilution range: 1:1000 or greater, using overnight incubation (at 4°C) and PAP / ABC-peroxidase procedure. 1:200 when using an overnight incubation at 4°C with the indirect immunofluorescence method. The use of high quality second-layer (anti-guinea pig Ig) reagents is strongly advised.

Vial contents, Storage and Use:

See vial label for contents.
Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes prior to freezing if appropriate. Store diluted antibody at 2-4°C and use within 1 month.
Dilute to working strength with phosphate-buffered saline (PBS) containing 0.1% w/v sodium azide.

References:

1. Decavel C. *et al.* GABA: A dominant neurotransmitter in the hypothalamus. *J. Comp. Neurol.*, **302**: 1019-1037, 1990.
2. Ralph M. R. *et al.* GABA regulation of circadian responses to light. Involvement of GABA_A-benzodiazepine and GABA_B receptors. *J. Neuroscience*, **9**: 2858-2865, 1989.
3. Moore R. Y. *et al.* GABA is the principle neurotransmitter of the circadian system. *Neurosci. Lett.*, **150**: 112-116, 1993.
4. Wagner S. *et al.* GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. *Nature*, **387**: 598-603, 1997.

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