

## Product Information

### Anti-GABA<sub>A</sub> $\alpha$ 4, N-Terminus

Developed in Rabbit, Affinity Isolated Antibody

Product Number **G 0170**

#### Product Description

Anti-phospho-GABA<sub>A</sub>  $\alpha$ 4 N-terminus antibody is developed in rabbit using a synthetic peptide from the N-terminus of the rat GABA<sub>A</sub> receptor  $\alpha$ 4 subunit coupled to KLH as immunogen. The antiserum is affinity purified with peptide coupled to thiopropylsepharose.

The antibody specifically detects GABA<sub>A</sub> receptor  $\alpha$ 4 subunit (protein with apparent molecular mass of 64 kDa) in rat brain membrane fractions. It has been used in immunoblotting and immunoprecipitation applications.

*Gamma*-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system, causing a hyperpolarization of the membrane through the opening of a Cl<sup>-</sup> channel associated with the GABA<sub>A</sub>-Receptor (GABA<sub>A</sub>-R) subtype. GABA<sub>A</sub>-Rs are important therapeutic targets for a range of sedative, anxiolytic, and hypnotic agents and are implicated in several diseases including epilepsy, anxiety, depression, and substance abuse. The GABA<sub>A</sub>-R is a multimeric subunit complex. To date six  $\alpha$ s, four  $\beta$ s and four  $\gamma$ s, plus alternative splicing variants of some of these subunits, have been identified. Injection in oocytes or mammalian cell lines of cRNA coding for  $\alpha$  and  $\beta$  subunits results in the expression of functional GABA<sub>A</sub>-Rs sensitive to GABA. However, coexpression of a  $\gamma$  subunit is required for benzodiazepine modulation. The various effects of the benzodiazepines in brain may also be mediated via different  $\alpha$  subunits of the receptor. Lastly, phosphorylation of  $\beta$  subunits of the receptor has been shown to modulate GABA<sub>A</sub>-R function.

#### Reagent

The antibody is supplied in 100  $\mu$ l in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA and 50% glycerol.

#### Storage/Stability

Store at -20 °C. Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. The antibody is stable for at least 24 months when stored appropriately. Defrosted aliquots in use should be stored at 4 °C, refreeze always with liquid nitrogen or similar. Avoid repeated freezing and thawing.

#### Product Profile

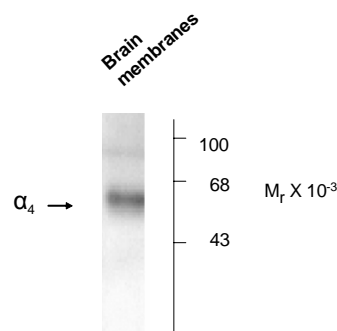
The supplied reagent is sufficient for 10 immunoblots.

A recommended working dilution of 1:1000 – 1:2000 is determined by immunoblotting specific for the 64kD GABA<sub>A</sub> Receptor  $\alpha$ 4-Subunit in rat brain lysate.

**Note:** In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

#### Results

Anti-  $\alpha$ 4 subunit of the GABA<sub>A</sub>-R



Immunoblot analysis of rat brain (hippocampal) lysates using approximately 5 – 7  $\mu$ g of tissue per slot. Blots were incubated with anti-GABA<sub>A</sub>-R,  $\alpha$ 4-Subunit, N-Terminus diluted 1:1000 overnight at 4°C. The antibody labelled the ~64k  $\alpha$ 4 subunit of the GABA<sub>A</sub>-R.

## References

1. Zezula, J., et al., Separation of  $\alpha_1$ ,  $\alpha_2$  and  $\alpha_3$  subunits of the GABA<sub>A</sub>-benzodiazepine receptor complex by immunoaffinity chromatography. Brain Res, **563**, 325-8 (1991).
2. Bencsits, E., et al., A significant part of native  $\gamma$ -aminobutyric acid<sub>A</sub> receptors containing  $\alpha_4$  subunits do not contain  $\gamma$  or  $\delta$  subunits. J. Biol. Chem. **274**, 19613-19616 (1999).
3. Kittler, J.T., et.al., Mechanisms of GABA<sub>A</sub> receptor assembly and trafficking - Implications for the modulation of inhibitory neurotransmission. Mol. Neurobiol. **26**, 2251–268 (2002).

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