



alomone labs

Molecular Tools for the Neuroscience Community

DATA SHEETS

Certificate of Analysis

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PRODUCT # S-500

LOT # SX-01

CERTIFICATE OF ANALYSIS

SNX-482

*(Hysteroocrates gigas)*¹

M.W.: 4495 daltons

Sequence: GVDKA GCRYM FGGCS VNDDC CPRLG CHSLF SYCAW DLTF S D-OH¹

Purity: >98% by HPLC.

Solubility: Any aqueous buffer.

Preparation:

SNX-482 is a synthetic peptide, originated from *Hysteroocrates gigas*.

Reconstitution:

The peptide concentration and identification were determined by amino acid analysis. Each vial contains 5 µg of unbuffered protein. Dissolving of 5 µg in 1.15 ml of any conventional buffer gives a stock solution of 1 µM.

Before dissolving the toxin, the tube should first be centrifuged, to concentrate the lyophilized toxin in the bottom of the tube. After centrifuging, the toxin must be dissolved into a stock solution using distilled water, or an appropriate buffer (see below), to a concentration of 10⁻⁵-10⁻⁶M. After preparing the stock solution, it should be divided into aliquots and can be stored this way for up to three months at -20°C.

Storage and Stability:

Lyophilized form: 2-3 weeks at room temperature.
One year at -20° C.

Liquid form: Up to four weeks at 4° C.
Three months at -20° C.

Known action:

Blocks specifically Ca_v2.3 (α1E, R-type) channels¹ in a voltage dependent manner. The block is reversible only upon application of strong voltage to facilitate unbinding² (see below).

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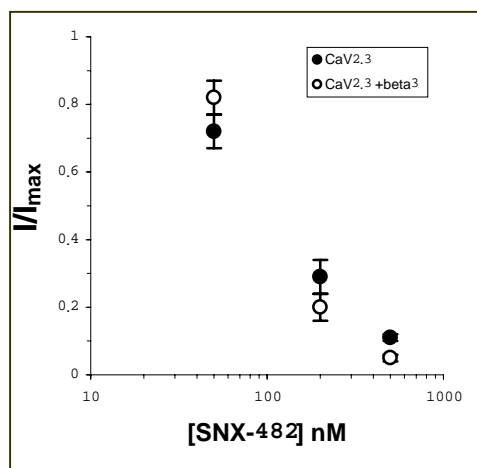
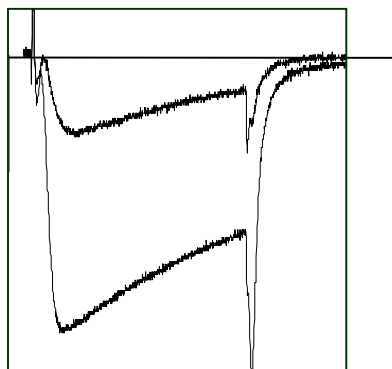
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Bioassay:

SNX-482 inhibits human $Ca_v2.3$ channels stably expressed in a mammalian cell line. An IC_{50} of 15-30 nM was obtained for block of $Ca_v2.3$ channel, using either patch clamp electrophysiology or K^+ -evoked Ca^{2+} flux. At low nanomolar concentrations, SNX-482 also blocked a native R-type Ca^{2+} current in rat neurohypophyseal nerve terminals, but concentrations of 200-500 nM had no effect on R-type Ca^{2+} currents in several types of rat central neurons¹. SNX-482 was also used to demonstrate the contribution of $Ca_v2.3$ channels to transmitter release.³

In *Xenopus* oocytes expressing $Ca_v2.3$, SNX-482 blocked the currents with an IC_{50} of about 100 nM (Figure 1, right).



Legend:

Left: Block of cloned $Ca_v2.3$ channel currents (with 10 mM Ba^{2+} as charge carrier) co expressed with $Ca_v\beta3$ in *Xenopus* oocytes. Currents were elicited by 100 ms pulse from holding potential of -100 mV to 0 mV, every 15 s. Traces show response before and during application of 200 nM SNX-482. The vertical bar represents $1\mu A$.

Right: Dose response curves for $Ca_v2.3$ channels with ($n=4$) or without ($n=4$) the auxiliary $\beta3$ subunit. For these experiments we used 40 mM Ba^{2+} as charge carrier.

References:

1. Newcomb, R. *et al.* (1998) *Biochemistry* **37**, 15353.
2. Bourniet, E. *et al.* (2001) *Biophys. J.* **81**, 79.
3. Wang, G. *et al.* (1999) *J. Neurosci.* **19**, 9235.

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