

Headquarters: Alomone Labs Ltd. Har Hotzvim Hi-Tech Park P.O. Box 4287, Jerusalem 91042, Israel.

Tel: +972-2-587 2202 Fax: +972-2-587 1101 or +972-2-642 6975 email: alomone@netvision.net.il <http://www.alomone.com>

PRODUCT # G-450
LOT # GR100

CERTIFICATE OF ANALYSIS
 ω -Grammotoxin SIA
(*Grammostola spatulata*)

M.W.: 4113 daltons.¹

Sequence: DCVRF WGKCS QTSDC CPHLA CKSKW PRNIC VWDGS V

Purity: > 98% by HPLC

Solubility: Any aqueous buffer.

Preparation:

ω -Grammotoxin SIA is a natural peptide isolated from the venom of *Grammostola spatulata* spider.

Reconstitution:

The peptide concentration and identification were determined by amino acid analysis. Each vial contains 5 μ g of unbuffered peptide. Dissolving of 5 μ g in 2.3 ml of any conventional buffer gives a stock solution of 0.5 μ M.

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^{-6} M. After preparing the stock solution, it should be divided into aliquots and stored 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 two weeks at 4° C.
Three months at -20° C.



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Molecular Tools for the Neuroscience Community

DATA SHEETS

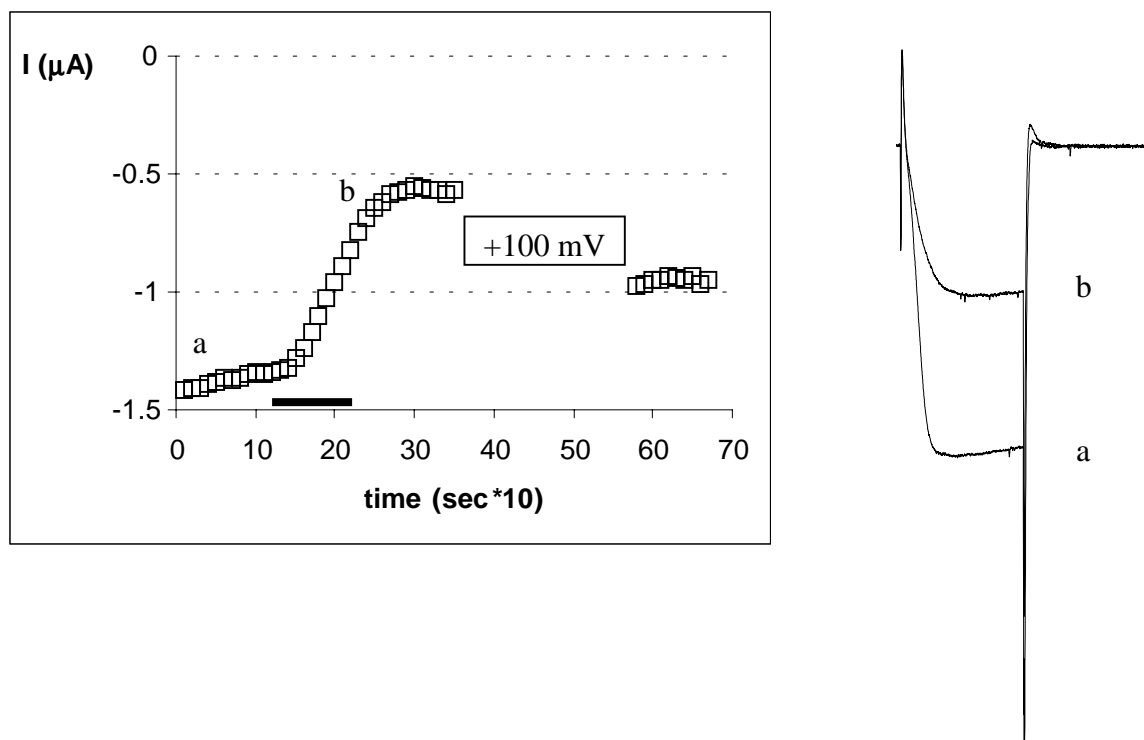
Certificate of Analysis

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Known action: ω -Grammotoxin SIA potently inhibits both $\text{Ca}_v2.1$ (P-type) and $\text{Ca}_v2.2$ (N-type) channels by altering the voltage-dependence of channel gating. ω -Grammotoxin SIA caused a concentration-dependent and virtually complete inhibition of K^+ -evoked influx of $^{45}\text{Ca}^{2+}$ into either rat or chick brain synaptosomes.¹ ω -Grammotoxin SIA at $1 \mu\text{M}$, a maximally effective concentration, blocked 52% of I_{Ba} in cultured rat hippocampal neurons.² $>50 \text{ nM}$ toxin completely inhibited calcium currents in Purkinje neurons (predominantly, P-type channels) and in sympathetic neurons (predominantly, N-type channels).³

Bioassay: ω -Grammotoxin SIA, was tested on cloned $\text{Ca}_v2.2$ channels expressed in *Xenopus* oocytes, were 500 nM caused about 60% inhibition (see Figure).



Legend: ω -Grammotoxin SIA effect on cloned N-type ($\text{Ca}_v2.2/\alpha2\delta1/\beta2a$) channels expressed in *Xenopus* oocytes using two electrode voltage clamp. The time course on the left shows current amplitude in response to 100 ms test pulse to 0 mV delivered every 10 seconds, before, during (bar) and after application of 500 nM of ω -Grammotoxin SIA. The box denoting +100 mV shows the period in which similar pulses to +100 mV were delivered in order to partially relieve the inhibition. The current traces on the right were taken at the indicated positions a and b.

References:

1. Lampe R. A., et al.,(1993) *Mol. Pharmacol.* **44**, 451.
2. Piser T. M., et al.,(1995) *Mol. Pharmacol.* **48**, 131.
3. McDonough S. I., et al.,(1997) *Mol. Pharmacol.* **52**, 1095.