



Molecular Tools for the Life Science Community

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Certificate of Analysis

PRODUCT # RTK-350

LOT # RK-001

rKurtoxin (*Parabuthus transvaalicus*)

M.W.: 7386 daltons.¹

Sequence: KIDGYVDYW NCKRICWYNN KYCNDLCKGL KADSGYCWGW TLSCYCOGLP
DNARIKRSGRCRA

Purity: > 98% by HPLC.

Solubility: Any aqueous buffer.

Preparation:

rKurtoxin is a recombinant peptide expressed in and extracted from *E. coli* and purified to homogeneity. Originally native Kurtoxin was isolated from the South African scorpion *Parabuthus transvaalicus*.

Reconstitution:

The peptide identity was confirmed by amino acid and mass spectrometry analysis and its concentration was determined by UV spectroscopy. Each vial contains 5 µg, 10 µg, or 0.1 mg of unbuffered protein. Before dissolving the toxin, the tube should be centrifuged. A stock solution of 1 µM can be prepared by dissolving 10 µg of toxin in 1.35 ml of any conventional buffer. The stock solution should be aliquotted and stored for up to three months at -20° C.

Storage and Stability:

Lyophilized form: 2-3 weeks at room temperature.
Two years at -20° C.
Liquid form: Up to four weeks at 4° C.
Three months at -20° C.

Known action:

Kurtoxin binds to the low voltage activated T-type ($Ca_v3.1$ and $Ca_v3.2$) Ca^{2+} channels with high affinity and inhibits the channels by modifying voltage-dependent gating with a K_d of 15nM. A concentration of 200nM inhibits the current completely.¹ It does not affect high voltage activated $Ca_v2.1$ (P/Q-type), $Ca_v2.2$ (N-type), $Ca_v1.2$ (L-type) and $Ca_v2.3$ (R-type) Ca^{2+} channels at 350nM.¹ On the other hand, a recent report has shown that although Kurtoxin potently inhibits T-type Ca^{2+} channels, it also partially inhibited both L-, P/Q- and N-type currents between 250-500nM.² Kurtoxin was also shown to block Na^+ channel inactivation in a voltage-dependent manner, by binding to the Na^+ channel at site 3.^{1,3}

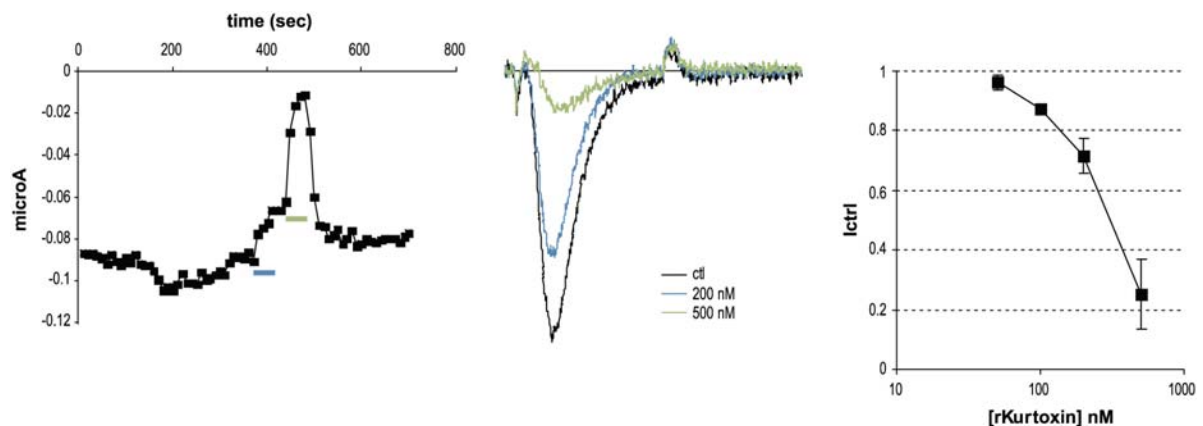
Kurtoxin was used to study the involvement of T-type channels in developing arterial smooth muscle cell proliferation in the *ductus arteriosus*.⁴ Kurtoxin was also shown to protect neurons from delayed ischemia-induced

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damage, when using rat organotypic hippocampal slice cultures for delayed cell death in *in vitro* assays.⁵ Furthermore, Kurtoxin blocked T-type currents in lung microvascular endothelial cells with IC₅₀ of about 100 nM.⁶

Bioassay:

The activity of this lot was tested using two-electrode voltage clamp recording from *Xenopus* oocytes expressing Ca_v3.1 T-type Ca²⁺ channel.



Inhibition of Ca_v3.1 channels expressed in *Xenopus* oocytes by rKurtoxin.

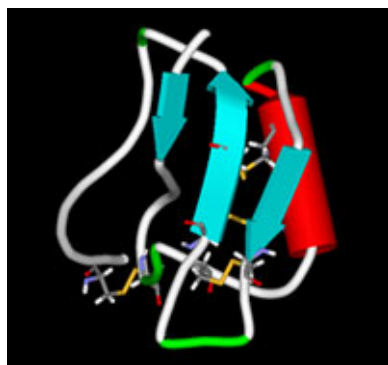
Left: Time course of Ca_v3.1 channel current amplitudes upon increasing toxin concentrations and wash. Horizontal bars below the plot indicate periods of toxin perfusion (blue 200 nM; green 500 nM). Currents were elicited from holding potential of -100 mV by a 50 ms test pulse to -30 mV delivered every 10 seconds.

Center: Ca_v3.1 channel current traces before (black) and during application of rKurtoxin (see inset).

Right: Dose response curve plotting the fraction of current inhibited for each toxin concentration (mean and SD n=2).

References:

1. Chuang, R.S. *et al.* (1998) *Nat. Neurosci.* **1**, 668.
2. Sidach, S.S. and Mintz, I.M. (2002) *J. Neurosci.* **22**, 2023.
3. Olamendi-Portugal, T. *et al.* (2002) *Biochem. Biophys. Res. Commun.* **299**, 562.
4. Yokoyama, U. *et al.* (2006) *Am. J. Physiol. Heart Circ. Physiol.* **290**, H1660.
5. Nikonenko, I. *et al.* (2005) *Mol. Pharmacol.* **68**, 84.
6. Wu, S. *et al.* (2003) *Circ. Res.* **93**, 346.



NMR structure of Kurtoxin (1T1T).