



Stably Transfected Cell Line - Product Data Sheet
hP2X2-HEK
Catalog Number CT6187

Related Services and Products

FastPatch[®] and FLIPR[®] screening services
Additional information available at www.chantest.com

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1 Cell Line Description

1.1 Background

P2X2 is an ionotropic purinergic receptor that forms a cation-selective channel. Expressed in sensory neurons, P2X2 is a potential therapeutic target in treatment of pain.

1.2 Pore-forming subunit identifier: hP2X2

Class: Ionotropic purinergic receptor
Species: Human
Gene name: P2RX2

1.3 Sequence Information

The cDNA sequence of the P2RX2 gene used to create this cell line was confirmed prior to transfection. The amino acid sequence encoded by the transfected cDNA is identical to the translated sequence for GenBank accession number NM_170682.2.

1.4 Expression System

HEK293 (human embryonic kidney) cells, tetracycline-inducible expression.

1.5 Product Format

Cryopreserved cells, 1×10^6 cells/vial.

1.6 Mycoplasma Status: Negative

The absence of mycoplasma species in this cell line was confirmed with the MycoAlert Kit (Lonza Rockland, Inc.).

1.7 Cell Line Stability

Channel expression has been shown to be stable for at least 44 passages.

2 Validated Test Platforms

Electrophysiological and pharmacological verification of the functional properties of the cloned channels was assessed in the following test platforms:

QPatch (Sophion Bioscience)
FLIPR[®] (MDS-AT)

2.1 QPatch™ Representative Data

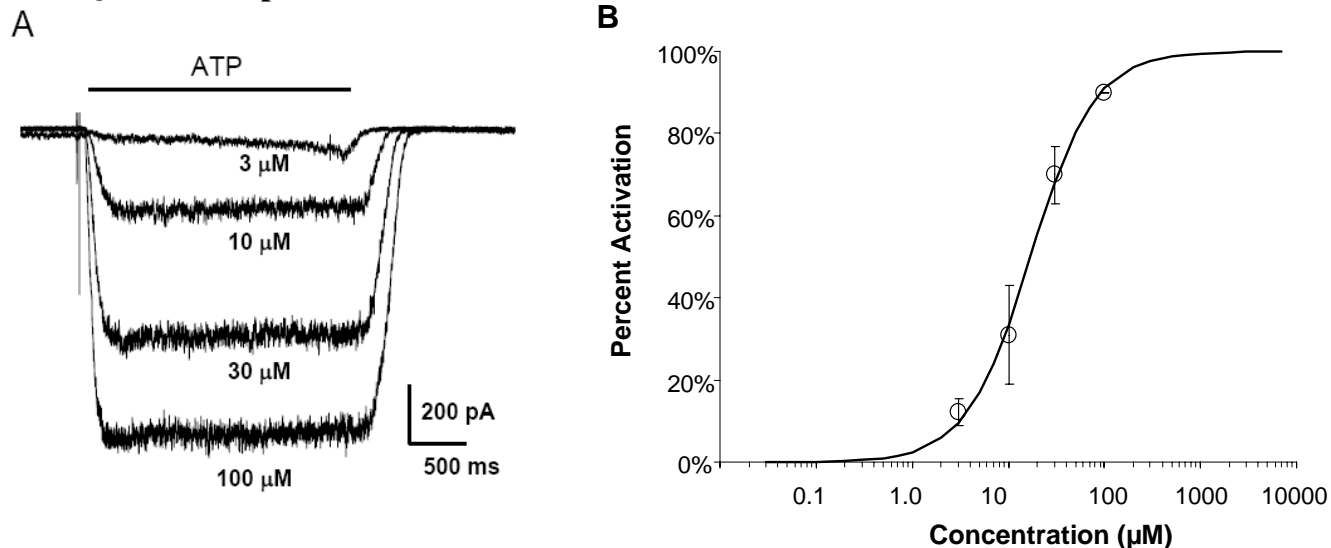


Figure 1. ATP-Dependent Activation of P2X2 Inward Current in QPatch™.

A: P2X2 currents elicited by application of increasing concentrations of ATP. Holding potential -60 mV. **B:** ATP concentration-response relationship. Mean \pm SEM, $n = 3$ cells/concentration. $EC_{50} = 16.9 \mu\text{M}$.

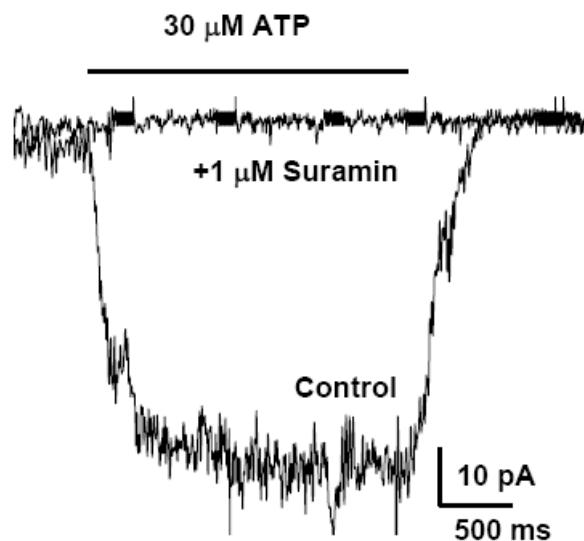


Figure 2. Suramin Inhibition of ATP-Activated Currents.

Current traces elicited by 30 μM ATP before (control) and after application of 1 μM suramin.

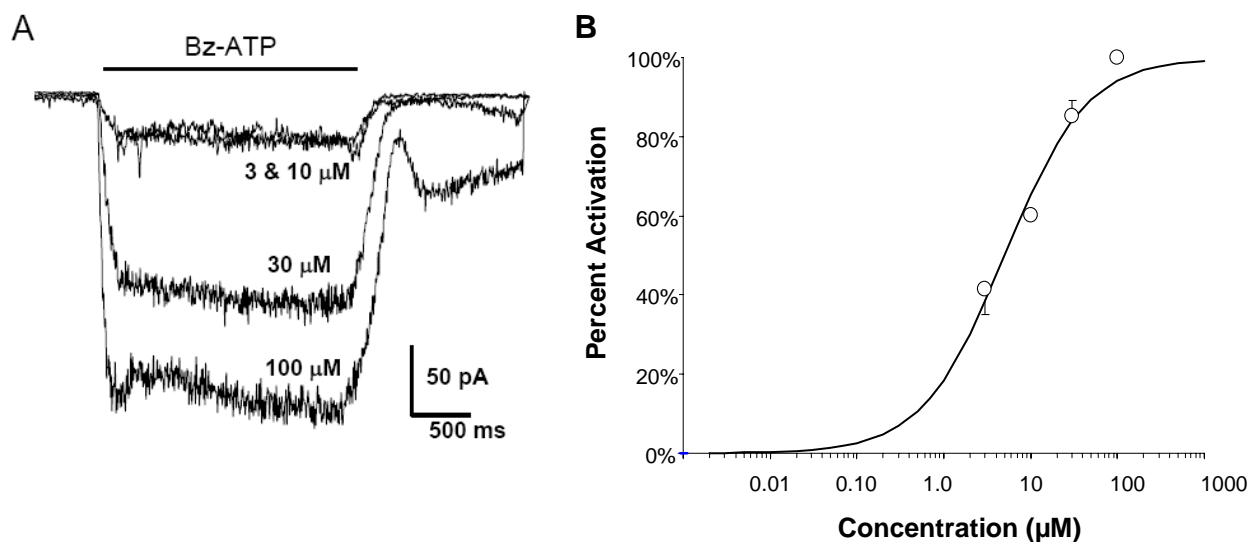


Figure 3. Benzoylbenzoyl-ATP (BzATP)-Induced Activation of P2X2 in QPatch™
A: Current traces elicited by ascending concentrations of BzATP. **B:** BzATP concentration-response relationship. Mean \pm SEM, $n = 3$ cells/concentration. $EC_{50} = 5.0 \mu\text{M}$.

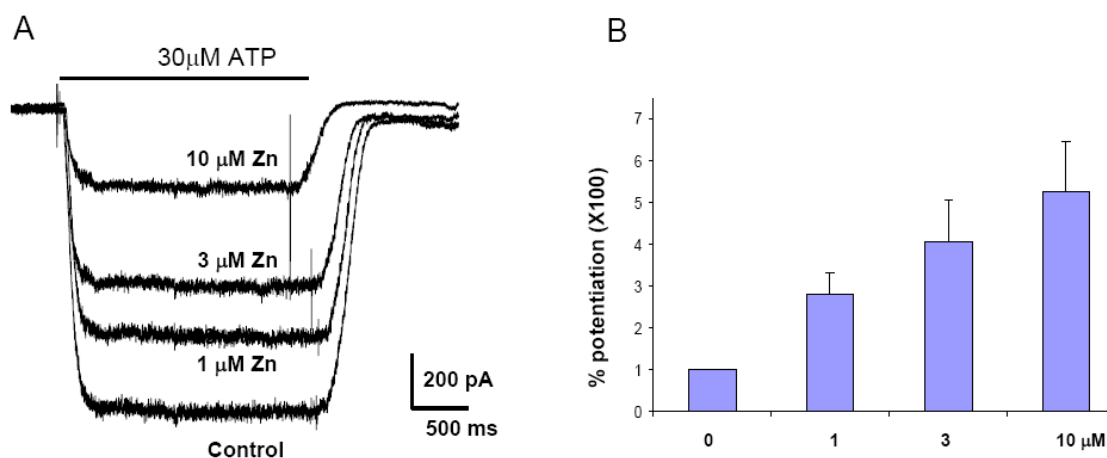


Figure 4. Zinc Potentiation of P2X2 Current in QPatch™
A: Current traces elicited by 30 μM Bz-ATP before and after application of Zn^{2+} in ascending concentration. **B:** Zn^{2+} concentration-response relationship. Mean \pm SEM, $n = 3$ cells/concentration.

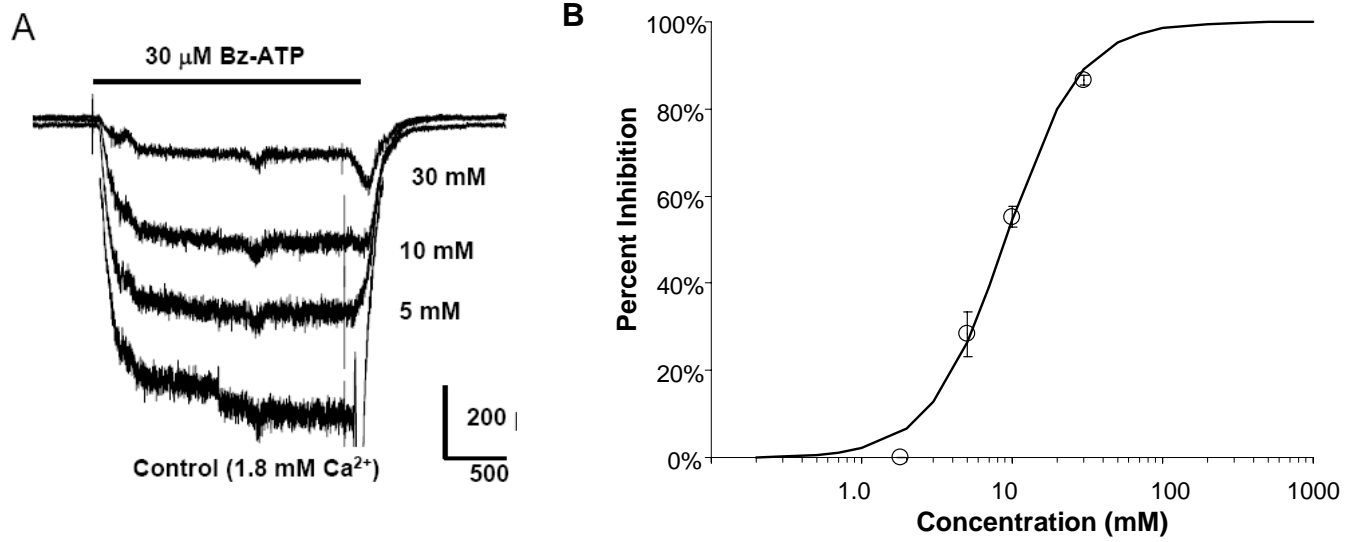


Figure 5. Ca²⁺ Inhibition of BzATP-Induced Current

A: Current traces elicited by 30 μM BzATP in control (1.8 mM Ca²⁺) and after application of ascending Ca²⁺ concentrations (5 - 30 mM). **B:** Ca²⁺ concentration-response relationship. Mean ± SEM, n = 4 cells/concentration. IC₅₀ = 9.0mM.

2.2 FLIPR® Representative Data

2.2.1 Activation of P2X2

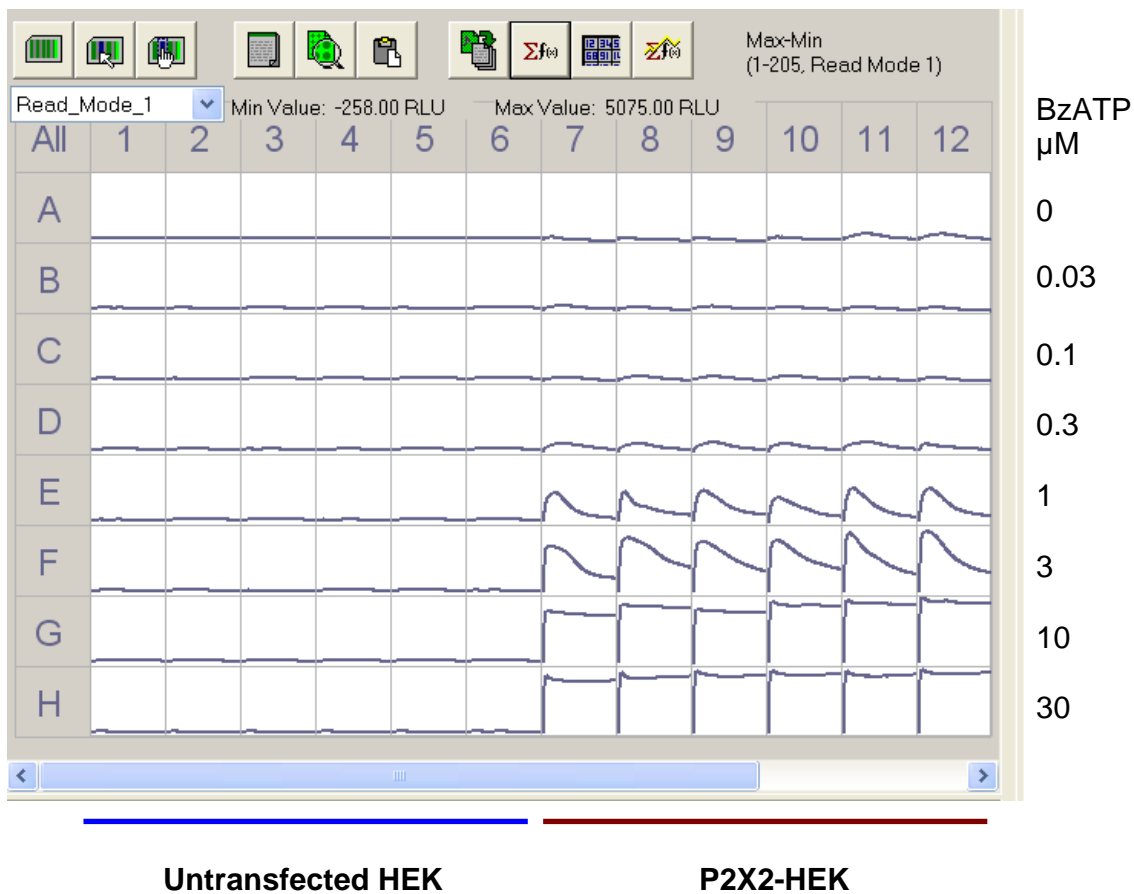


Figure 6. BzATP Activation of P2X2-HEK in FLIPR®

BzATP evoked a concentration-dependent increase in $[Ca^{2+}]_i$ in hP2X2-HEK cells (columns 7 - 12) cells but had no significant effect in untransfected HEK cells (columns 1 - 6).

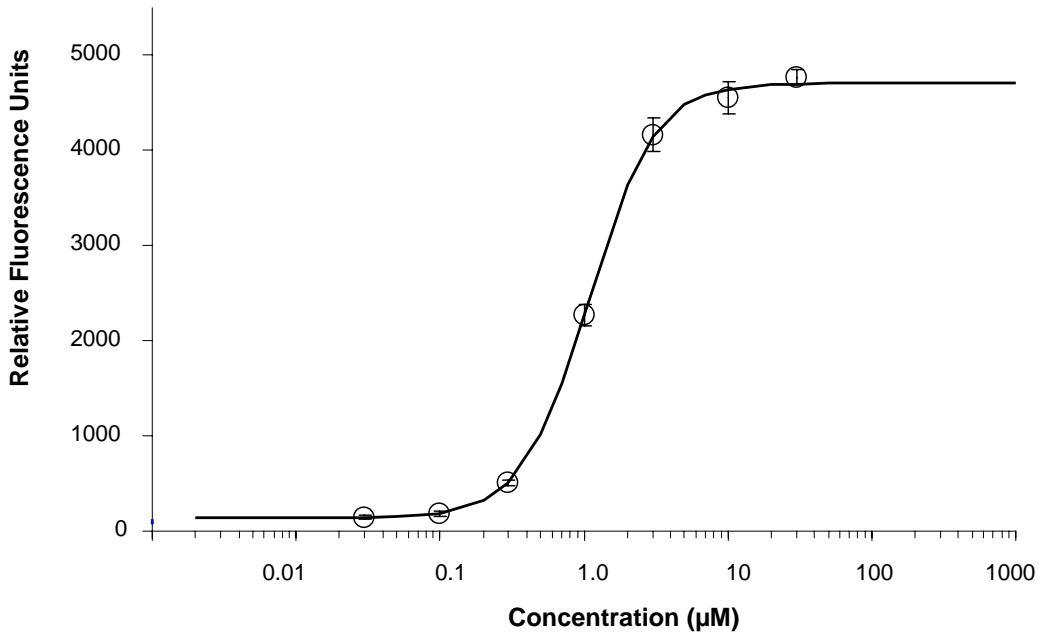


Figure 7. BzATP Concentration-Response Relationship.
Mean \pm SEM, n = 6 replicates/concentration. EC₅₀ = 1.1 μ M.

2.2.2 Inhibition of BzATP-Induced Activation of P2X2 by Antagonists

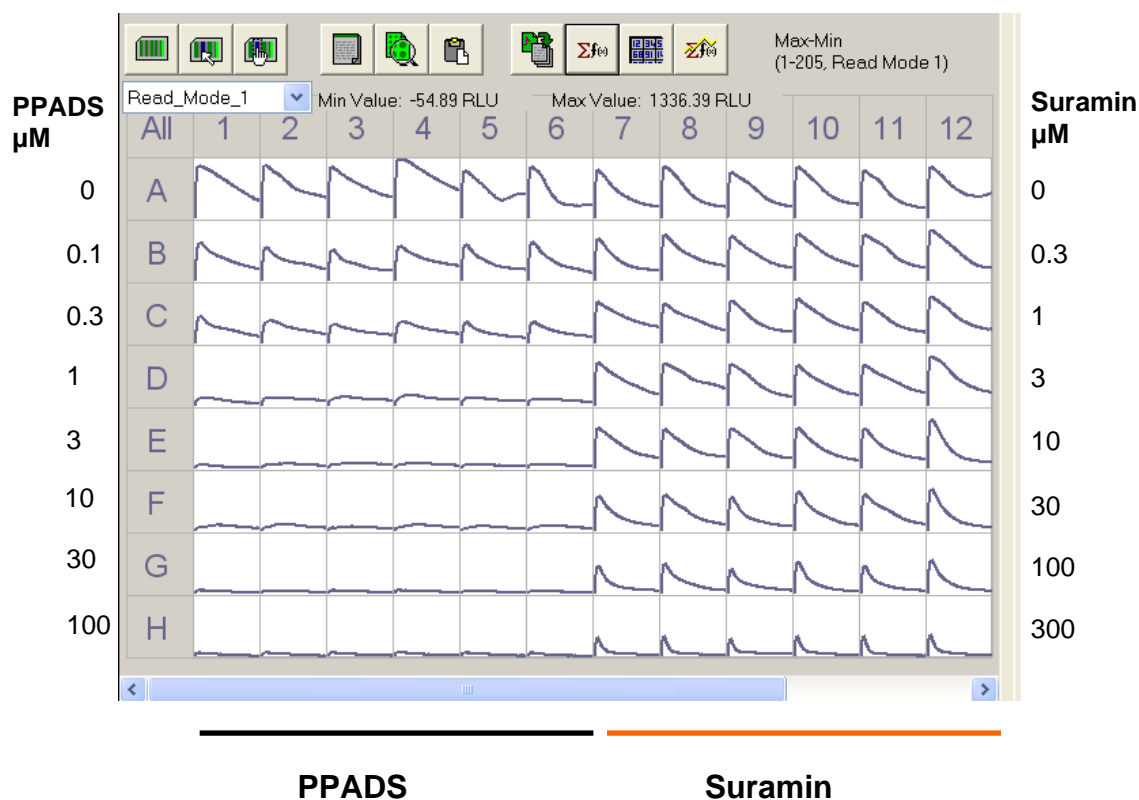


Figure 8. Concentration-Dependent Inhibition of BzATP (3 μM)-Induced Ca²⁺ Responses in hP2X2-HEK.

PPADS (0.1-100 μM, columns 1 - 6) and suramin (0.3-300 μM, columns 7 - 12) inhibited BzATP (3 μM)-induced [Ca²⁺]_i responses in P2X2-HEK cells in a concentration-dependent manner.

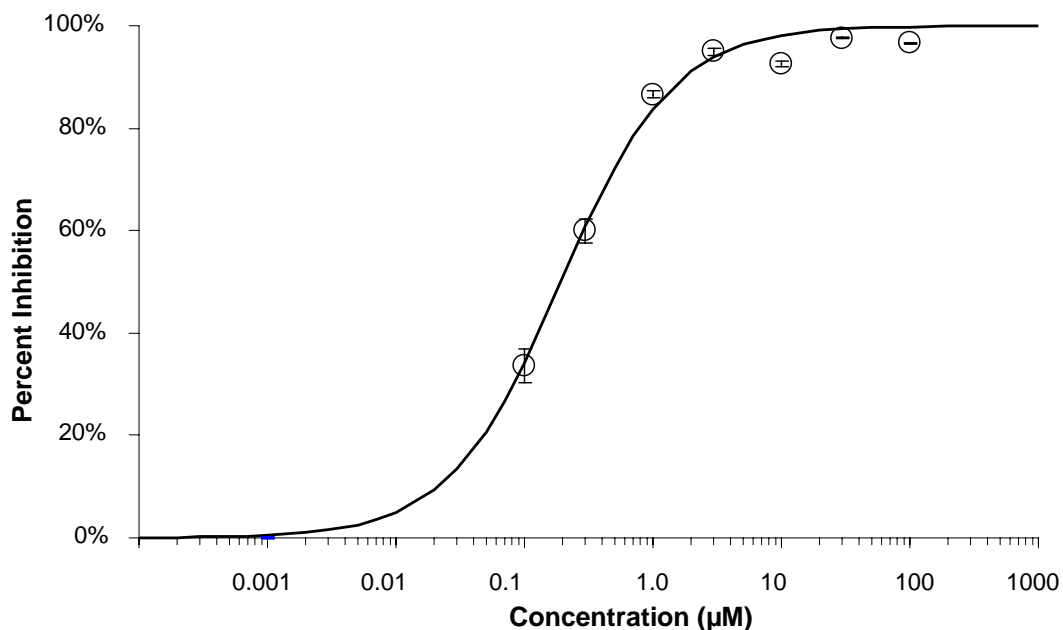


Figure 9. PPADS Concentration-Response Relationship.
Mean \pm SEM, n = 6 replicates/concentration. IC₅₀ for PPADS inhibition of BzATP (30 μ M)-activated P2X2 channels was determined to be 0.19 μ M.

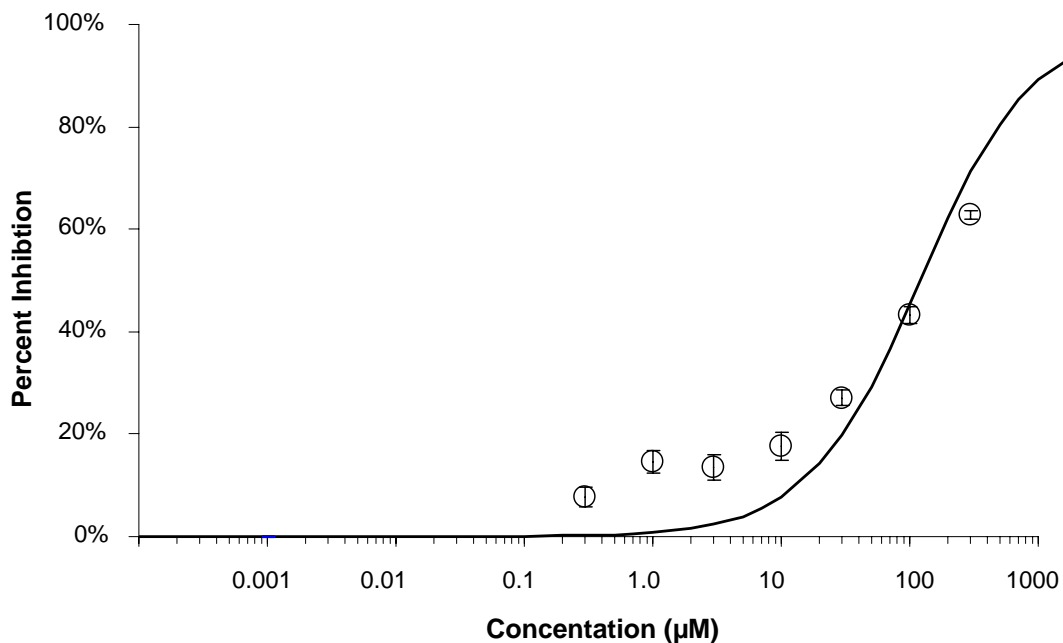


Figure 10. Suramin Concentration-Response Relationship for Inhibition of BzATP (3 μ M) -Induced Ca²⁺ Responses in P2X2-HEK Cells.
Mean \pm SEM, n = 6 replicates/concentration. IC₅₀ = 120.6 μ M.

3 References

Khakh BS, et al. 2001. International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. *Pharmacol Rev* 3:107-118.

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Virginio C, et al. 1998. Calcium permeability and block at homomeric and heteromeric P2X2 and P2X3 receptors, and P2X receptors in rat nodose neurons. *J Physiol* 510:27-35.

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