



Stably Transfected Cell Line - Product Data Sheet
hP2X1-HEK
Catalog Number CT6186

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

P2X1 is an ionotropic purinergic receptor that forms a cation-selective channel. Expressed in sensory neurons, P2X1 is a potential therapeutic target in treatment of urinary incontinence and pain.

1.2 Pore-forming subunit identifier: hP2X1

Class: Ionotropic purinergic receptor
Species: Human
Gene name: P2RX1

1.3 Sequence Information

The cDNA sequence of the P2RX1 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_002558.2.

1.4 Expression System

HEK293 (human embryonic kidney) cells, constitutive 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 33 passages.

2 Validated Test Platforms

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

Manual Patch Clamp
PatchXpress[®] (MDS-AT)
FLIPR[®] (MDS-AT)

2.1 Manual Patch Clamp Representative Data

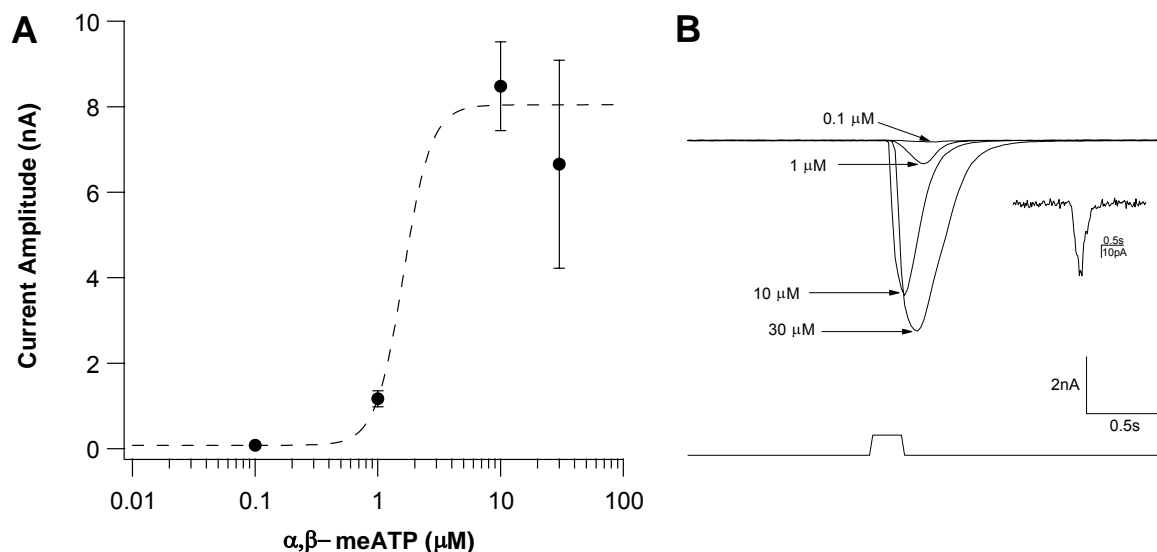


Figure 1. Concentration-Dependent P2X1 Activation by the Agonist α,β -meATP.
A: Concentration-response relationship. Mean \pm SD, $n = 4 - 7$ cells/concentration. Each cell was only exposed to one concentration of the agonist. The data are fitted to a Hill equation with $EC_{50} = 3.8 \mu\text{M}$. **B:** Typical current traces elicited by α,β -meATP (superimposed traces from different cells). The inset shows a magnified version of the P2X1 receptor response to $0.1 \mu\text{M}$ α,β -meATP.

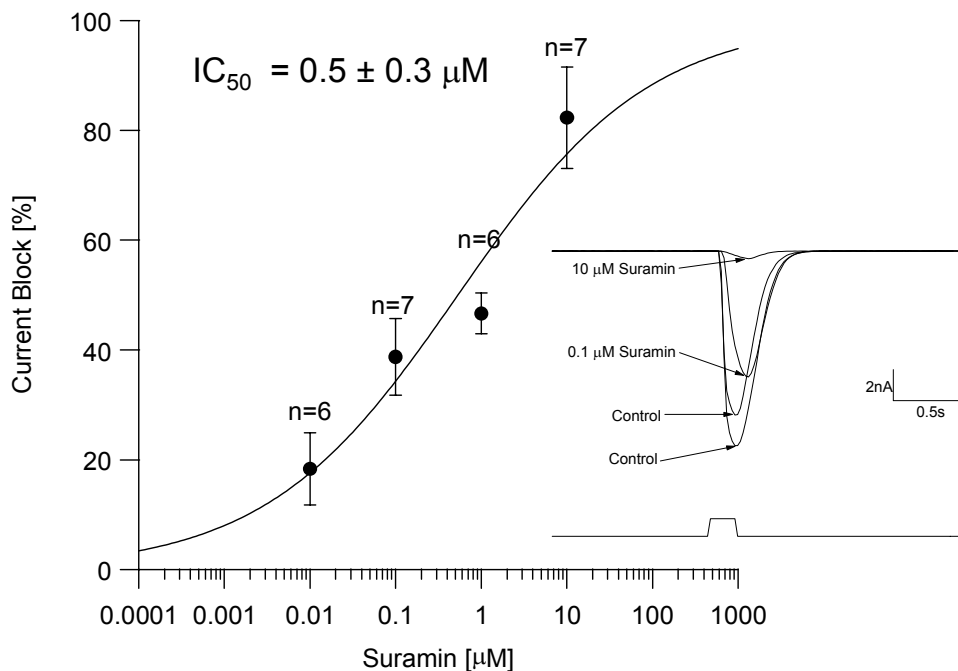


Figure 2. Concentration-dependent P2X1 inhibition by the antagonist suramin. Currents elicited by $10 \mu\text{M}$ α,β -meATP. Mean percent block \pm SEM, $n = 6 - 7$ cells/concentration. $IC_{50} = 0.5 \mu\text{M}$.

2.2 PatchXpress[®] Representative Data

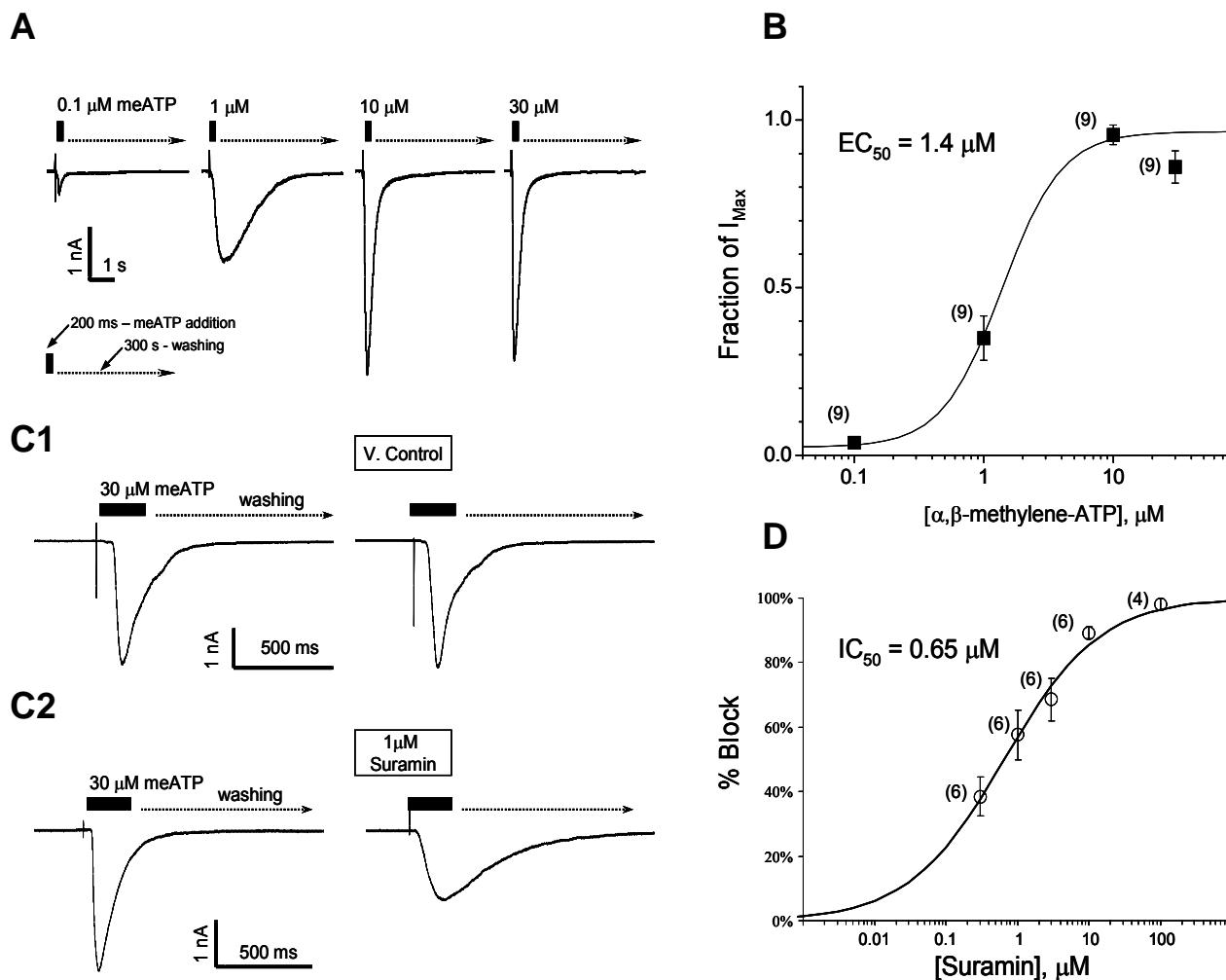


Figure 3. Electrophysiological and Pharmacological Characteristics of P2X1 in PatchXpress[®]

A: P2X1 currents elicited by ascending concentrations of the agonist, α,β -methylene-ATP. **B:** α,β -Methylene-ATP concentration-response. Mean \pm SEM, n = 9 cells/concentration. $EC_{50} = 1.4 \mu\text{M}$. **C1- C2:** Suramin inhibition of α,β -methylene-ATP-activated currents. Channels were activated by a saturating concentration of agonist (30 μM α,β -methylene-ATP) followed by washout. In vehicle controls (C1) multiple agonist applications yielded constant activation levels. In the presence of suramin (C2) at 1 μM current activation was suppressed. **D:** Suramin concentration-response relationship. Mean \pm SEM, n = 4 - 6 cells/concentration. $IC_{50} = 0.65 \mu\text{M}$.

2.3 FLIPR[®] Representative Data

2.3.1 ATP Activation of P2X1

α,β -meATP produced a concentration-dependent increase in $[Ca^{2+}]_i$ in hP2X1-HEK cells but had no significant effect on untransfected HEK cells (Figure 4). Another agonist, Bz-ATP, showed a similar profile of concentration-dependent activation (Figure 5).

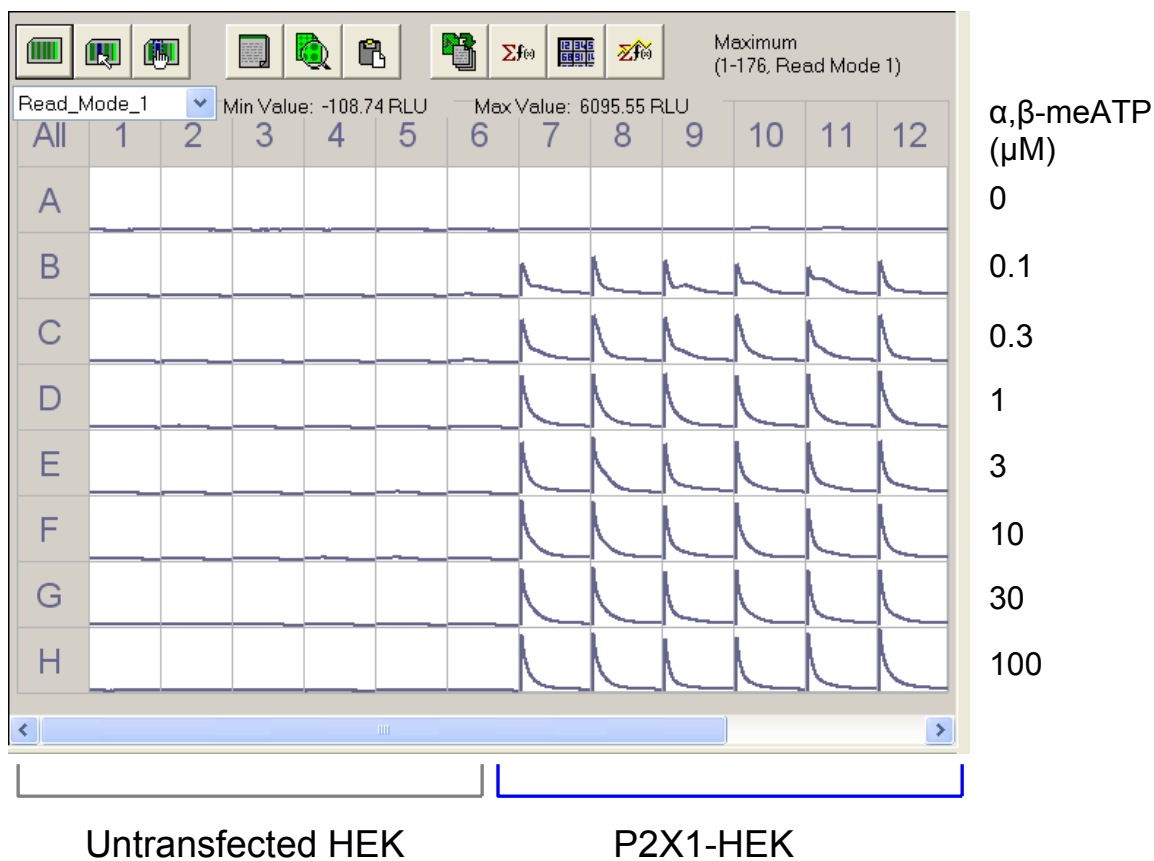


Figure 4. Comparison of effects of α,β -meATP on untransfected HEK cells (columns 1-6) and stably transfected hP2X1-HEK293 cells (columns 7-12).

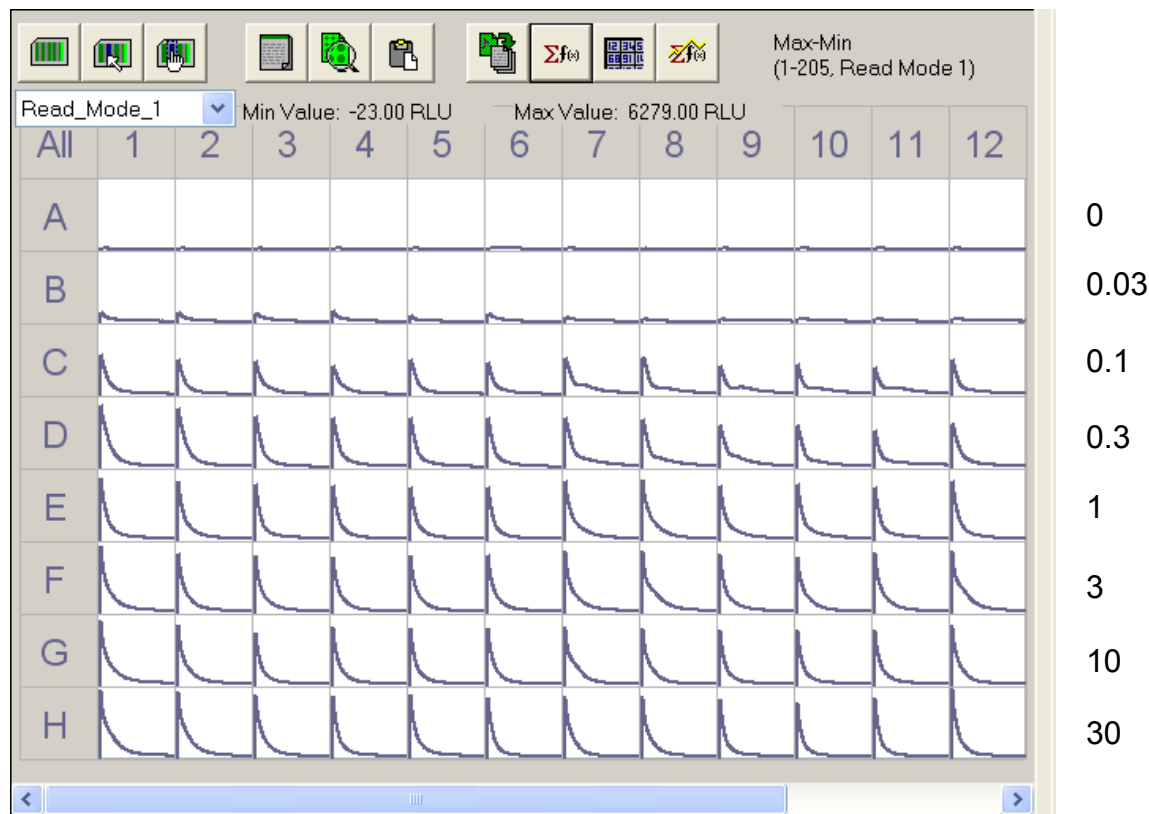


Figure 5. Concentration-Dependent Activation of P2X1 by the Agonists Bz-ATP and α,β -meATP

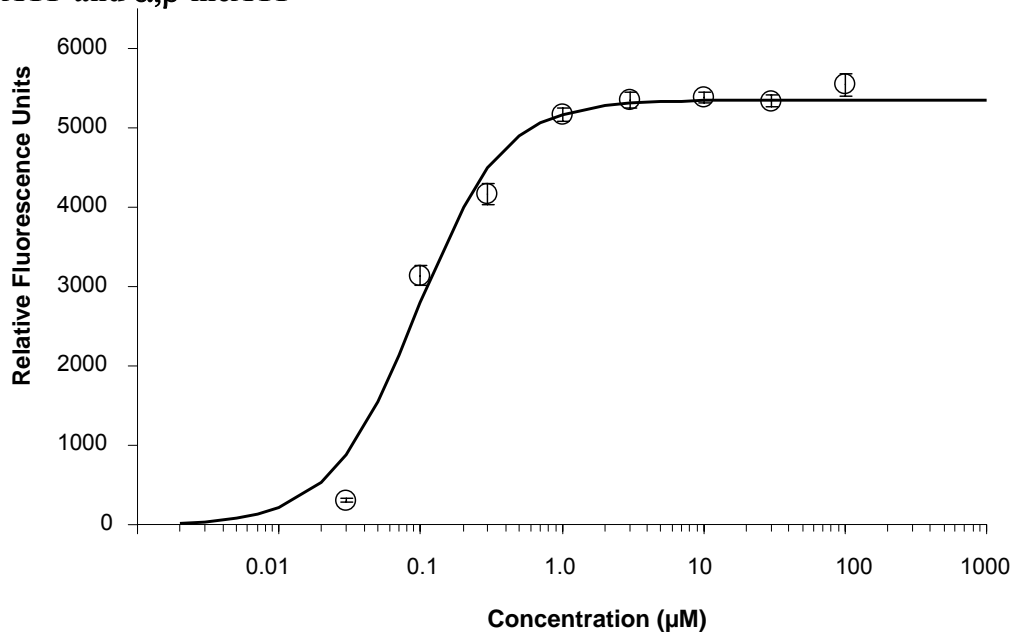


Figure 6. α,β -MeATP Concentration-Response Relationship.
Mean \pm SEM, n = 6 - 12 replicates/concentration. EC_{50} = 0.094 μM .

2.3.2 Antagonist-Induced Inhibition of P2X1

NF449 (0.01-10 μM) inhibited Bz-ATP (1 μM) or $\alpha\beta$ -meATP (1 μM)-induced Ca^{2+} responses in hP2X1-HEK cells in a concentration-dependent manner (Figure 7).

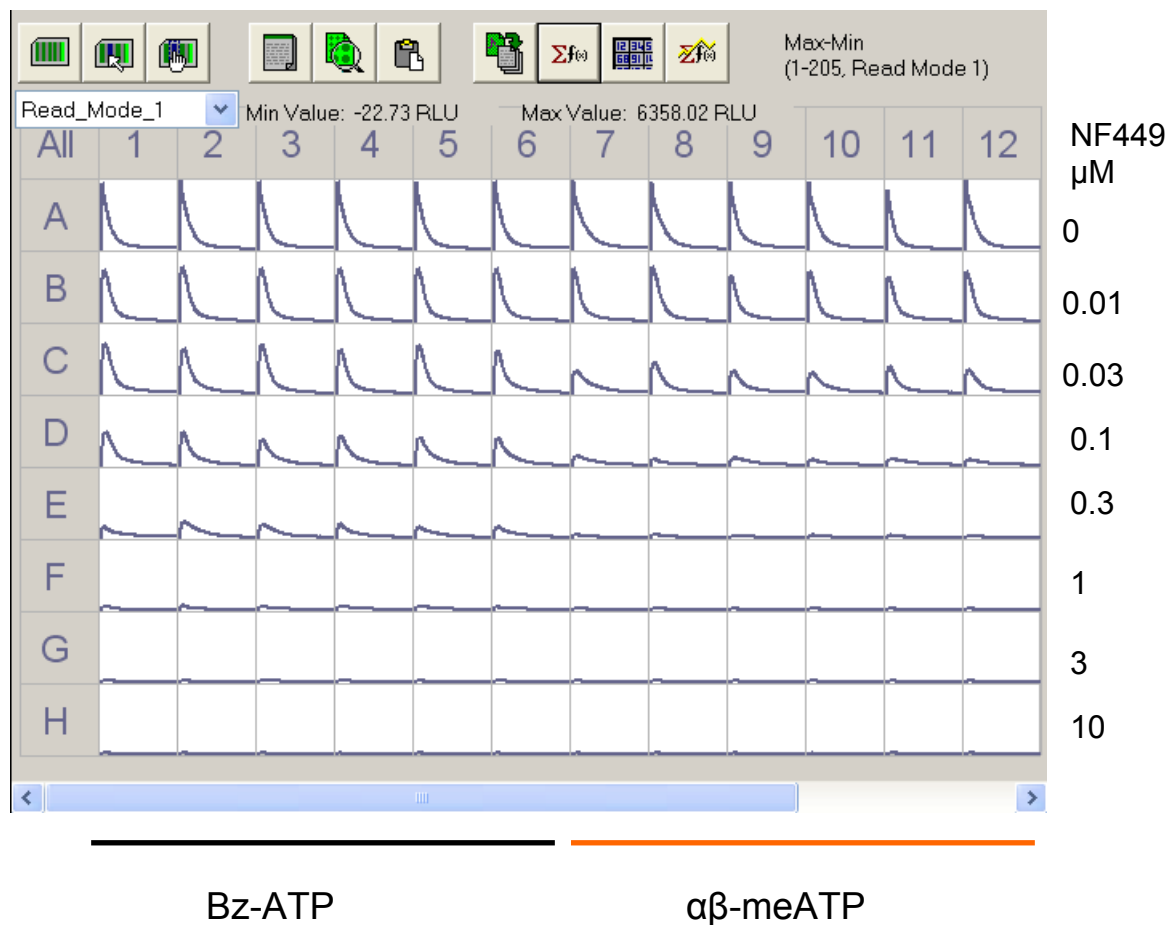


Figure 7. Concentration-Dependent Inhibition of Bz-ATP-Induced (1 μM , columns 1 - 6) or $\alpha\beta$ -meATP -Induced (1 μM , columns 7 - 12) Ca^{2+} responses in hP2X1-HEK cells by NF449

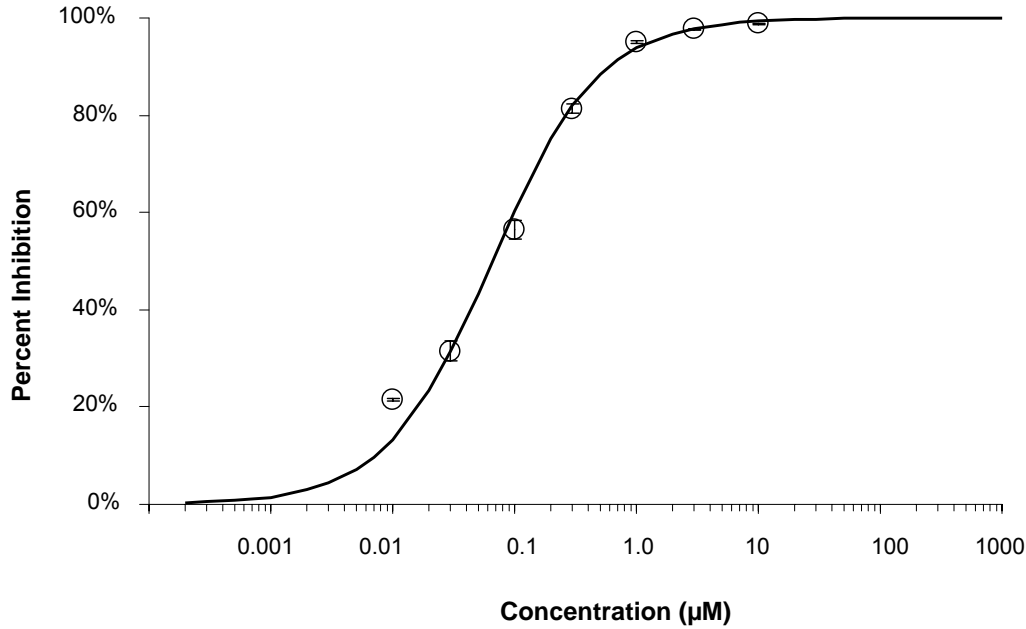


Figure 8. NF449 Concentration-Response Relationship for Bz-ATP (1 μM) - Induced Ca²⁺ Responses.
Mean ± SEM, n = 6 replicates/concentration. IC₅₀ = 66 nM.

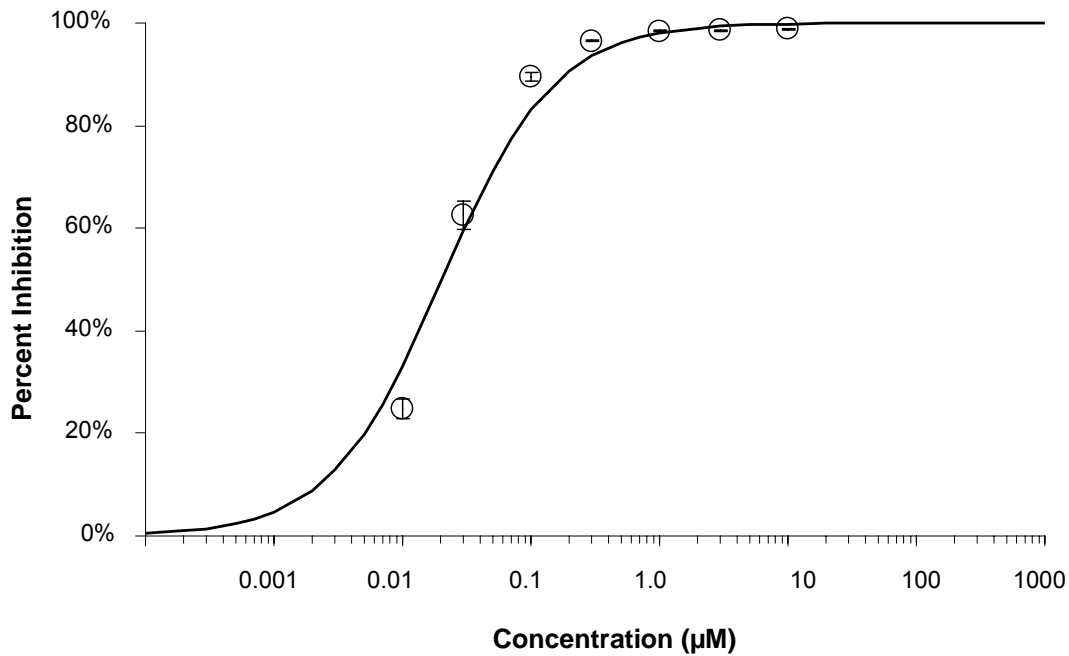


Figure 9. NF449 Concentration-Response Relationship for αβ-meATP (1 μM) - Induced Ca²⁺ Responses. Mean ± SEM, n = 6 replicates/ concentration. IC₅₀ = 20 nM.

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.

North RA. 2002. Molecular physiology of P2X receptors. *Physiol Rev* 82:1013-1067.

Rettinger J, et al. 2005. Profiling at recombinant homomeric and heteromeric rat P2X receptors identifies the suramin analogue NF449 as a highly potent P2X1 receptor antagonist. *Neuropharmacology* 48:461-468.