



**Human P2X2/P2X3-HEK Product Data Sheet**  
**Stably Transfected Cell Line**  
**Catalog Number CT6179**

**Related Services and Products**

FastPatch<sup>®</sup> automated patch clamp and FLIPR<sup>®</sup> screening services  
Additional information available at [www.chantest.com](http://www.chantest.com)

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## 1 Cell Line Description

### 1.1 Background

P2X2/P2X3 is an ionotropic purinergic receptor that forms a cation-selective channel. Expressed in the central and peripheral nervous systems, P2X2/P2X3 is a potential therapeutic target in pain treatment.

### 1.2 Pore-forming subunit identifier: P2X2

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

### 1.3 Pore-forming subunit identifier: P2X3

Class: Ionotropic purinergic receptor  
Species: Human  
Gene name: P2RX3

### 1.4 Sequence Information

The cDNA sequences of the P2RX2 and P2RX3 genes used to create this cell line were confirmed prior to transfection. The P2X2 and P2X3 amino acid sequences encoded by the transfected cDNAs are identical to the translated sequence for GenBank accession numbers: NM\_170682.2 and NM\_002559.2, respectively.

### 1.5 Expression System

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

### 1.6 Product Format

Cryopreserved cells, 1 x10<sup>6</sup> cells/vial.

### 1.7 Mycoplasma Status: Negative

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

### 1.8 Cell Line Stability

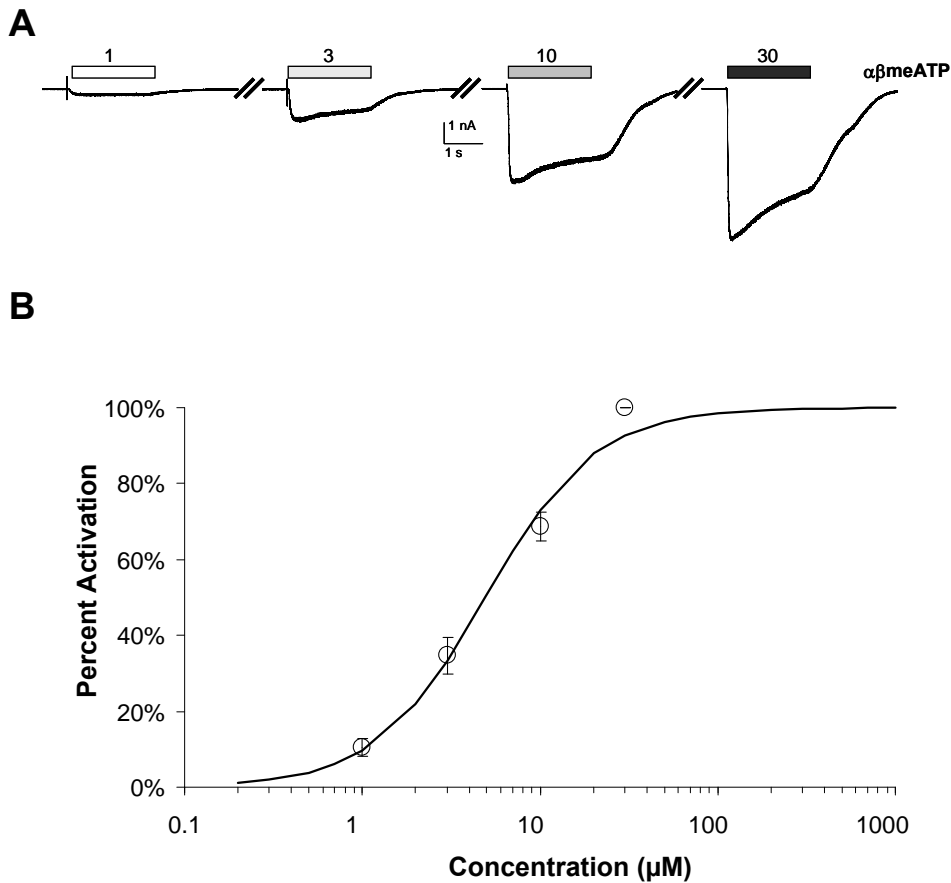
Channel expression in this cell line has been shown to be stable for at least 63 passages.

## 2 Validated Test Platforms

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

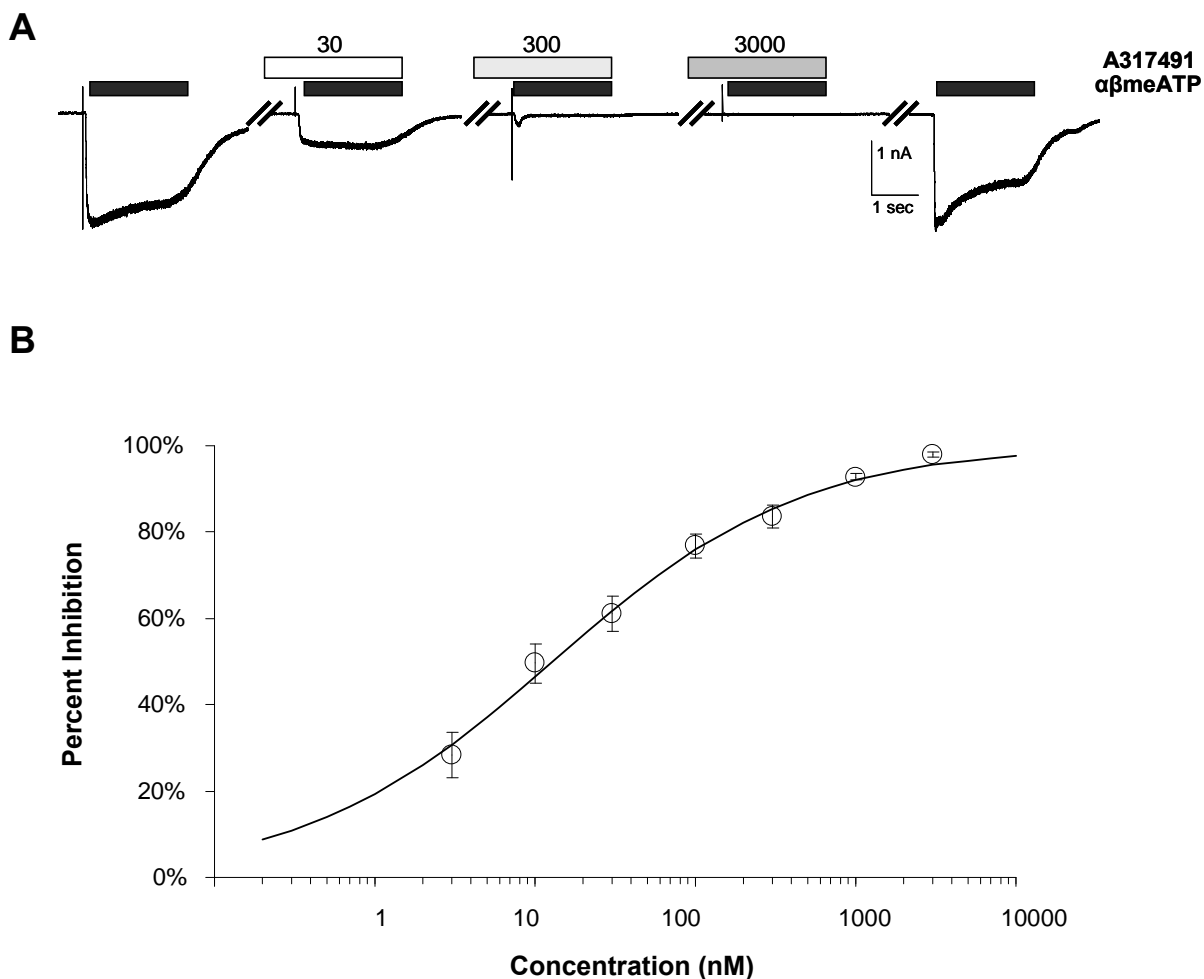
PatchXpress®  
FLIPR® (MDS-AT)

## 2.1 PatchXpress<sup>®</sup> Representative Data



### Figure 1. Electrophysiological Characterization in PatchXpress<sup>®</sup>.

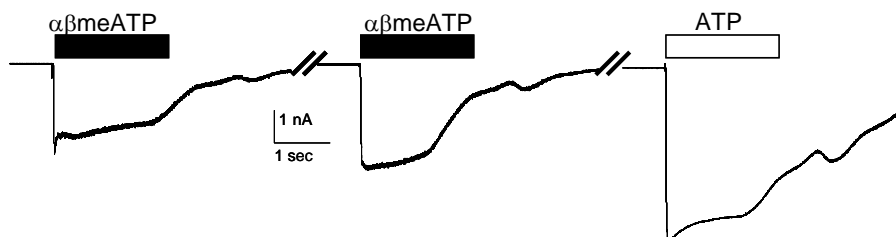
**A:** P2X2/P2X3 activation with  $\alpha,\beta$ -methylene-ATP ( $\alpha\beta\text{-meATP}$ ). Currents elicited upon exposure to increasing  $\alpha\beta\text{-meATP}$  concentrations (1 to 30  $\mu\text{M}$ ). The agonist was applied for two seconds followed by a 3 minute wash with buffer (denoted by the break symbol, //). **B:** Concentration-response relationship.  $\text{EC}_{50} = 4.9 \mu\text{M}$ .



**Figure 2. A317491 Concentration-Dependent Inhibition in PatchXpress®**

**A:** P2X2/P2X3 currents were elicited by applying 10 μM αβ-meATP for 2 seconds. After the first αβ-meATP application, the cell was washed with ATP-free buffer for 3 minutes followed by a 1-minute application of A317491 during the incubation period denoted by the break symbol (//), after which 10 μM αβ-meATP was applied for 2 seconds. Ascending concentrations of A317491 were applied followed by a final wash and application of 10 μM αβ-meATP alone. **B:** A317491 concentration-response relationship. IC<sub>50</sub> = 13 nM.

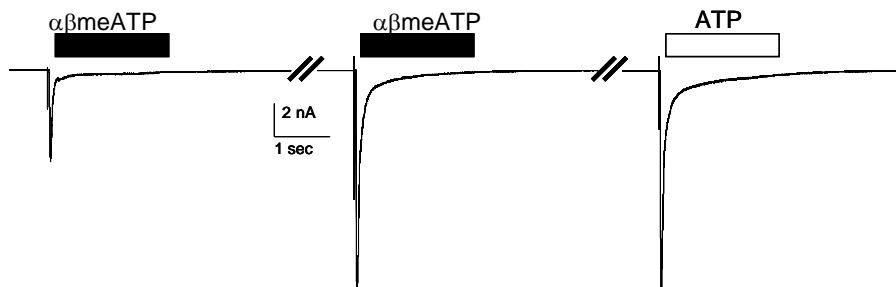
### A P2X2/P2X3



### B P2X2



### C P2X3



### Figure 3. P2X2/P2X3 Gating Characteristics Distinguished from Homomeric P2X2 and P2X3

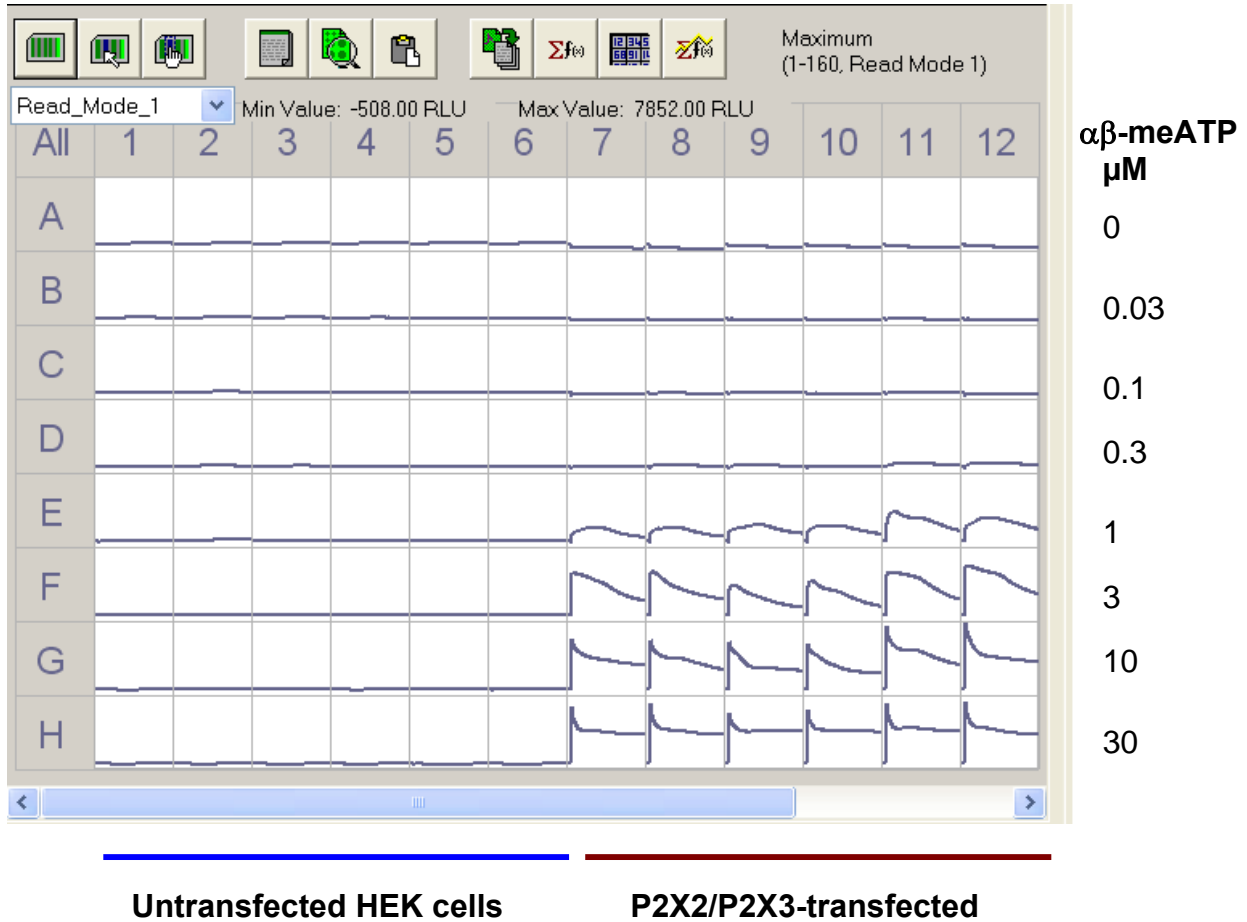
**A:** Slow desensitization is characteristic of heteromeric P2X2/P2X3 currents elicited by application of  $\alpha\beta\text{-meATP}$  (10  $\mu\text{M}$ , black bar) or ATP (10  $\mu\text{M}$  white bar).

**B:** Homomeric P2X2 channels were insensitive to 10  $\mu\text{M}$   $\alpha\beta\text{meATP}$  (black bar). ATP at 10  $\mu\text{M}$  (white bar) evoked a slowly desensitizing current.

**C:**  $\alpha\beta\text{-MeATP}$  at 10  $\mu\text{M}$  (black bar) elicited a rapidly desensitizing P2X3 current.

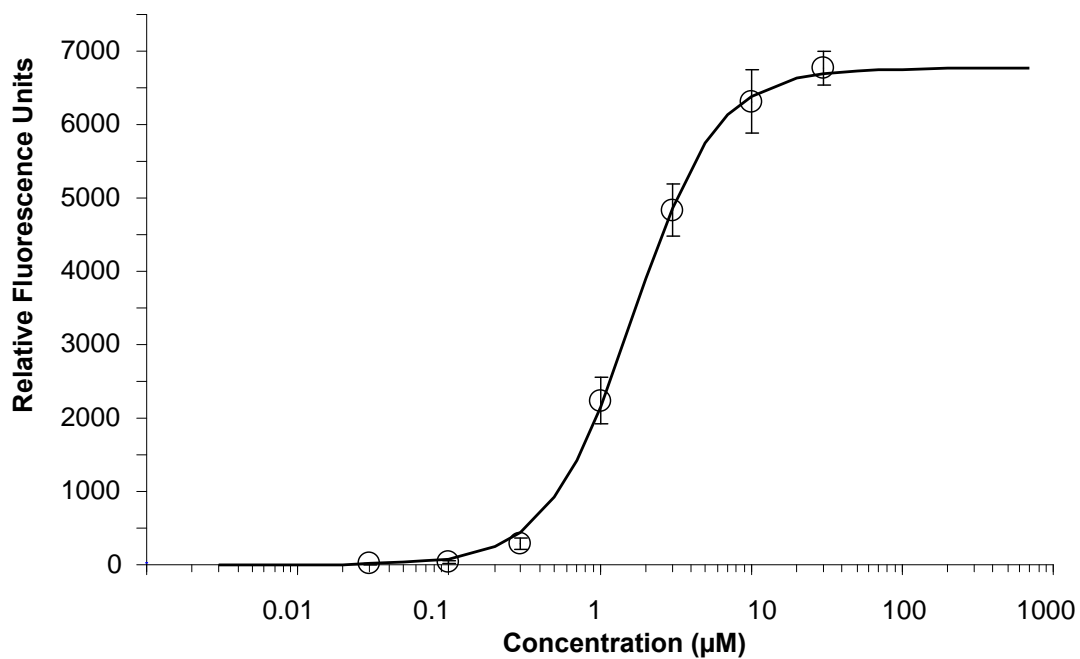
## 2.2 FLIPR® Representative Data

### 2.2.1 $\alpha\beta$ -meATP Activation



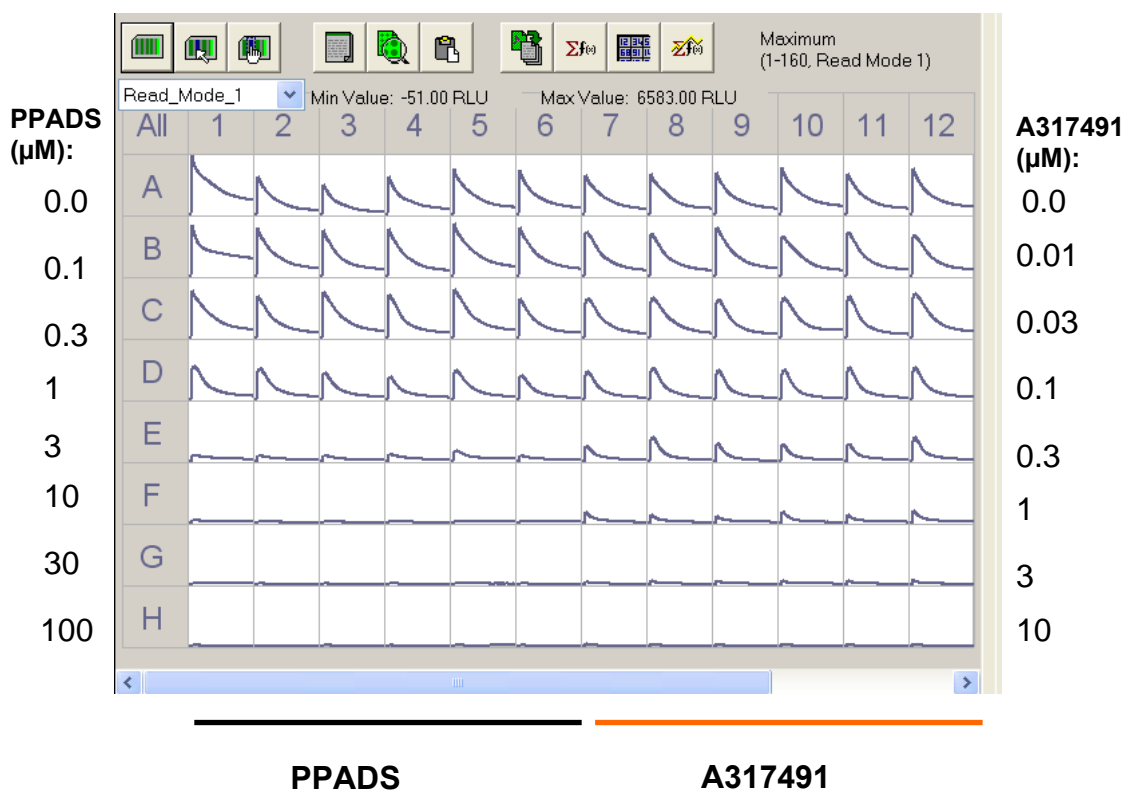
**Figure 4. Concentration-Dependent Activation of P2X2/P2X3 Receptors in FLIPR®**

$\alpha\beta$ -meATP-induced activation elicited increase intracellular  $\text{Ca}^{2+}$  in a concentration-dependent manner. The effect was specific to P2X2/P2X3-transfected cells.



**Figure 5.  $\alpha\beta$ -meATP Concentration-Response Relationship.**  
Mean  $\pm$  SD, n = 6 replicates/concentration.  $EC_{50} = 1.64 \mu\text{M}$ .

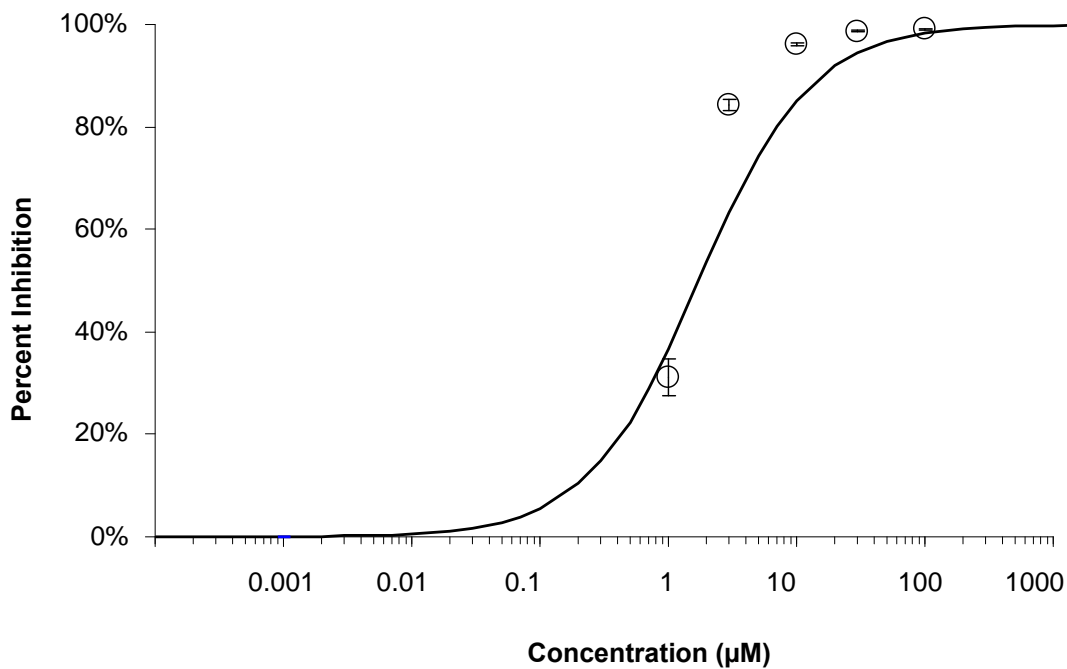
## 2.2.2 Effects of Antagonists on P2X2/P2X3



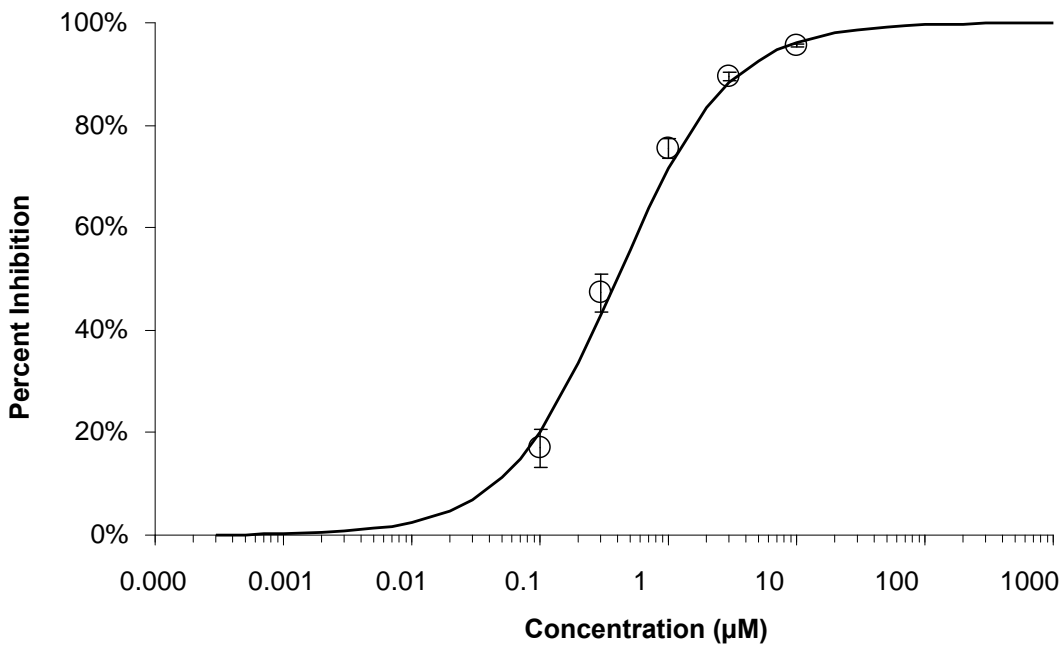
**Figure 6. PPADS and A317491 Inhibition of P2X2/P2X3 in FLIPR®**

Row A shows the maximal  $[Ca^{2+}]_i$  level achieved by application of  $3 \mu M$   $\alpha\beta$ -meATP in the absence of inhibitors. Rows B - H show replicate measurements of the decrease in  $[Ca^{2+}]_i$  with increasing concentrations of pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (PPADS, 0.1 to  $100 \mu M$ , columns 1 - 6) or A317491 (0.01 -  $10 \mu M$ , columns 7 - 12).

### A PPADS



### B A317491



#### Figure 7. Antagonist Concentration-Response Relationships

**A:** PPADS. Mean  $\pm$  SD, n = 20 replicates/concentrations. IC<sub>50</sub> = 1.7  $\mu$ M.

**B:** A317491. Mean  $\pm$  SD, n = 12 replicates/concentrations. IC<sub>50</sub> = 0.4  $\mu$ M.

### **3 References**

Jarvis MF, et al. 2002. A-317491, a novel potent and selective non-nucleotide antagonist of P2X3 and P2X2/3 receptors, reduces chronic inflammatory and neuropathic pain in the rat. *Proc Natl Acad Sci USA* 99:17179-1784.

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