



**Stably Transfected Cell Line - Product Data Sheet**  
**hP2X3-HEK**  
**Catalog Number CT6188**

**Related Services and Products**

FastPatch<sup>®</sup> 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

P2X3 is an ionotropic purinergic receptor that forms a cation-selective channel. Expressed in sensory neurons, P2X3 is a potential therapeutic target in treatment of inflammatory bowel disease, urinary incontinence, and pain.

### 1.2 Pore-forming subunit identifier: hP2X3

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

### 1.3 Sequence Information

The cDNA sequence of the P2RX3 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\_002559.2.

### 1.4 Expression System

HEK293 (human embryonic kidney) cells, constitutive expression.

### 1.5 Product Format

Cryopreserved cells,  $1 \times 10^6$  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

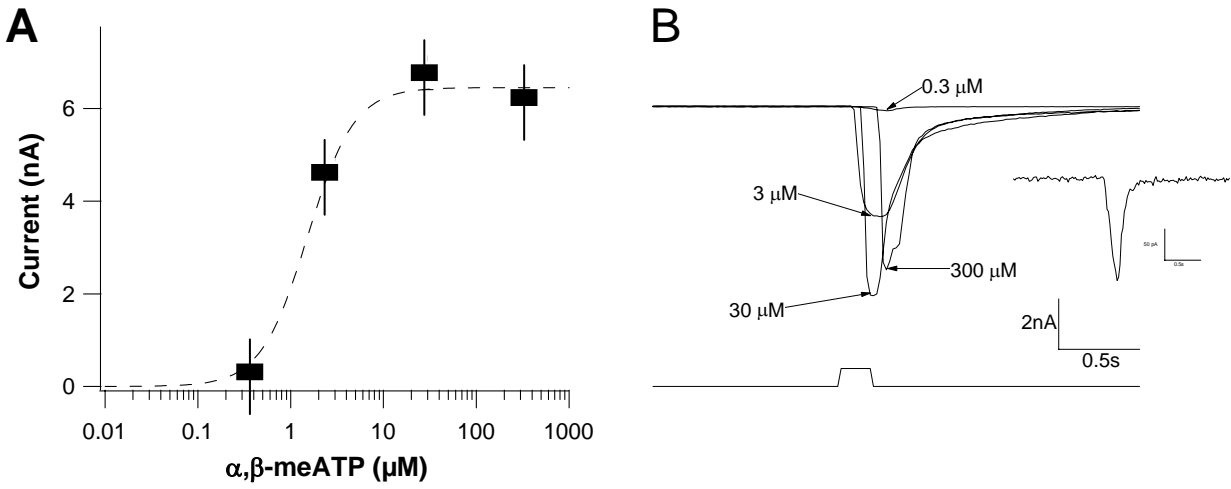
Channel expression has been shown to be stable for at least 35 passages.

## 2 Validated Test Platforms

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

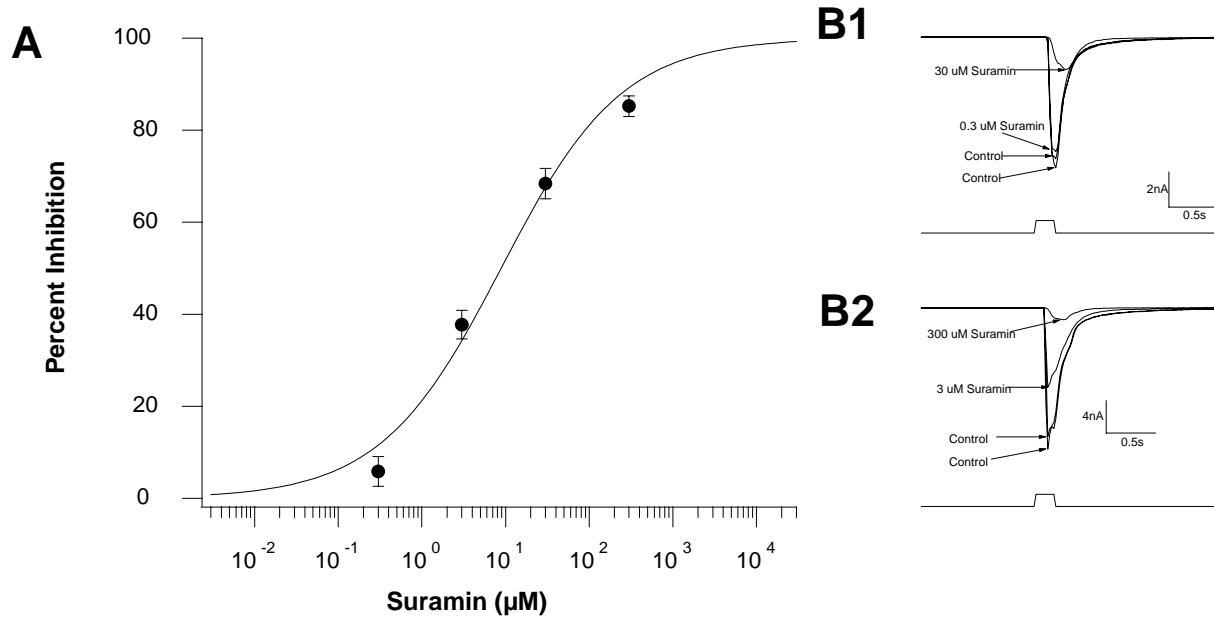
Manual Patch Clamp  
PatchXpress<sup>®</sup> (MDS-AT)  
FLIPR<sup>®</sup> (MDS-AT)

## 2.1 Manual Patch Clamp Representative Data



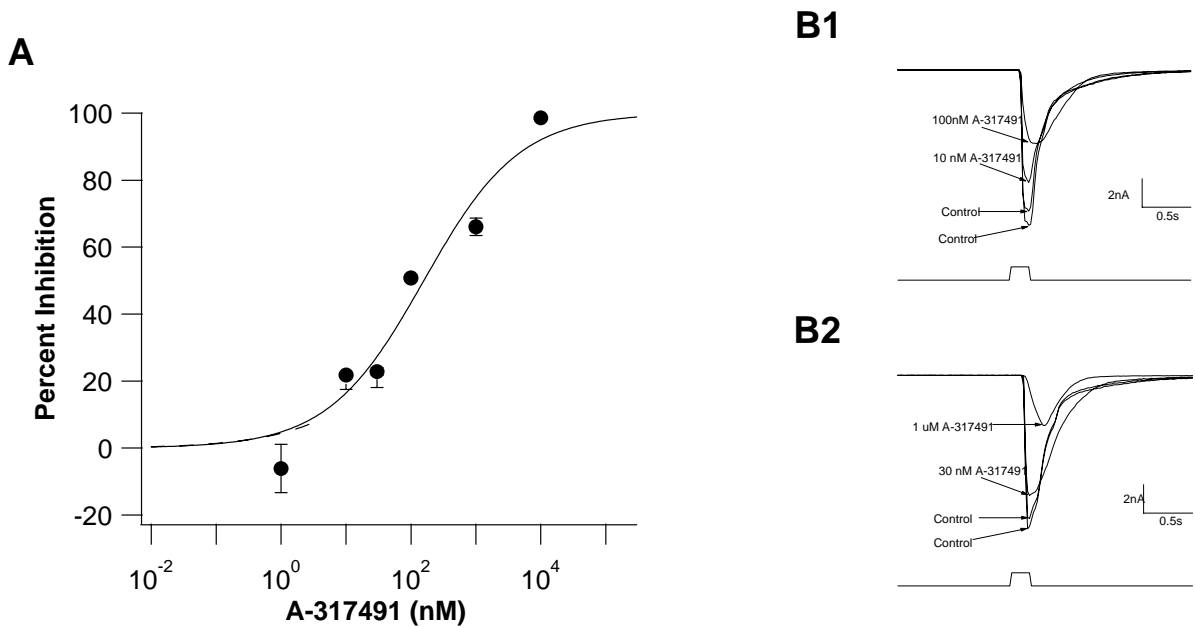
**Figure 1. Concentration-Dependent P2X3 Activation by the Agonist  $\alpha,\beta$ -meATP**

**A:** Concentration-response relationship. Mean  $\pm$  SD, n = 6 - 9 cells/concentration. Each cell was only exposed to one concentration of the agonist. The data are fitted to a Hill equation with  $EC_{50} = 6.5 \mu\text{M}$ . **B:** Typical current traces elicited by  $\alpha,\beta$ -meATP (superimposed traces from different cells). The inset shows a magnified version of the P2X3 receptor response to  $0.3 \mu\text{M}$   $\alpha,\beta$ -meATP.



**Figure 2. Suramin Inhibition of  $\alpha,\beta$ -meATP-Induced P2X3 Activation.**

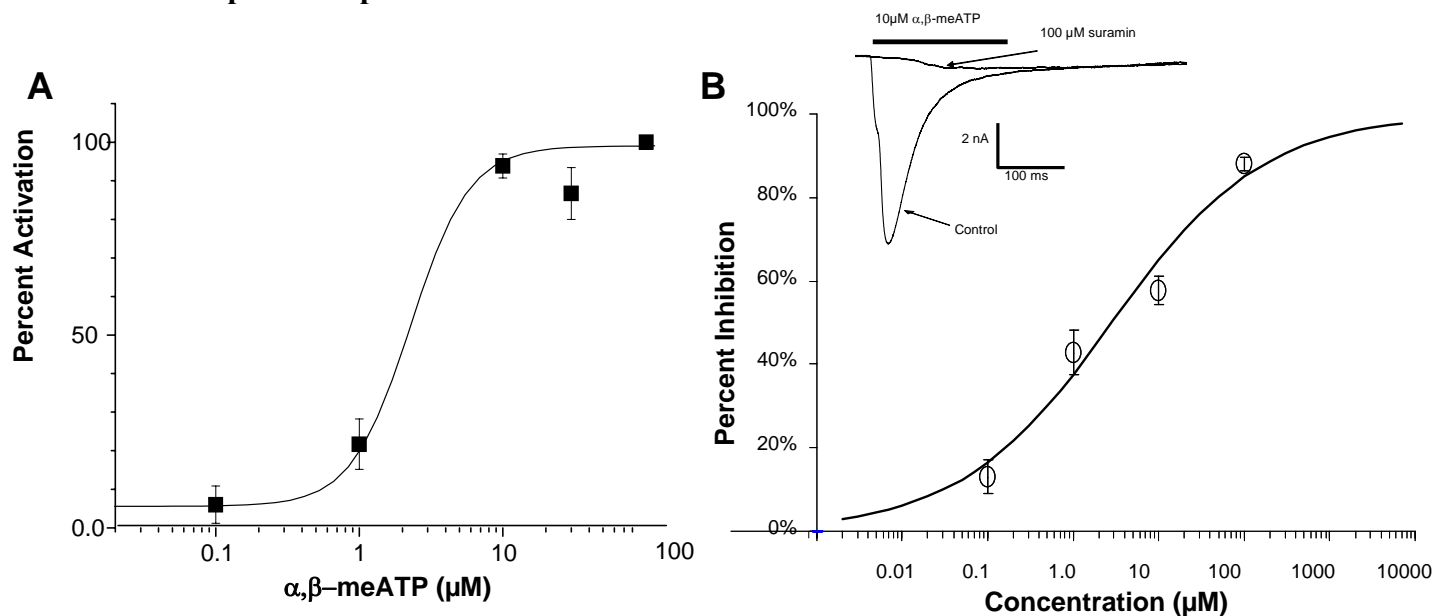
**A:** Suramin concentration-response relationship. Mean  $\pm$  SEM,  $n = 4$  cells/concentration.  $\text{IC}_{50} = 8.8 \mu\text{M}$ . **B1 - B2:** Current traces elicited by  $30 \mu\text{M}$   $\alpha,\beta$ -meATP before (control) and after two ascending suramin concentrations (either 0.3 and  $30 \mu\text{M}$  in **B1**, or 3 and  $300 \mu\text{M}$  in **B2**).



**Figure 3. A-317491 Inhibition of  $\alpha,\beta$ -meATP-Induced P2X3 Activation.**

**A:** Concentration-response relationship for A-317491, a non-nucleotide antagonist. Mean  $\pm$  SEM,  $n = 4$  cells/concentration.  $\text{IC}_{50} = 157 \text{ nM}$ . **B1 - B2:** Current traces elicited by  $30 \mu\text{M}$   $\alpha,\beta$ -meATP before (control) and after two ascending A-317491 concentrations (either 10 and  $100 \text{ nM}$  in **B1** or 30 and  $1000 \text{ nM}$  in **B2**).

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

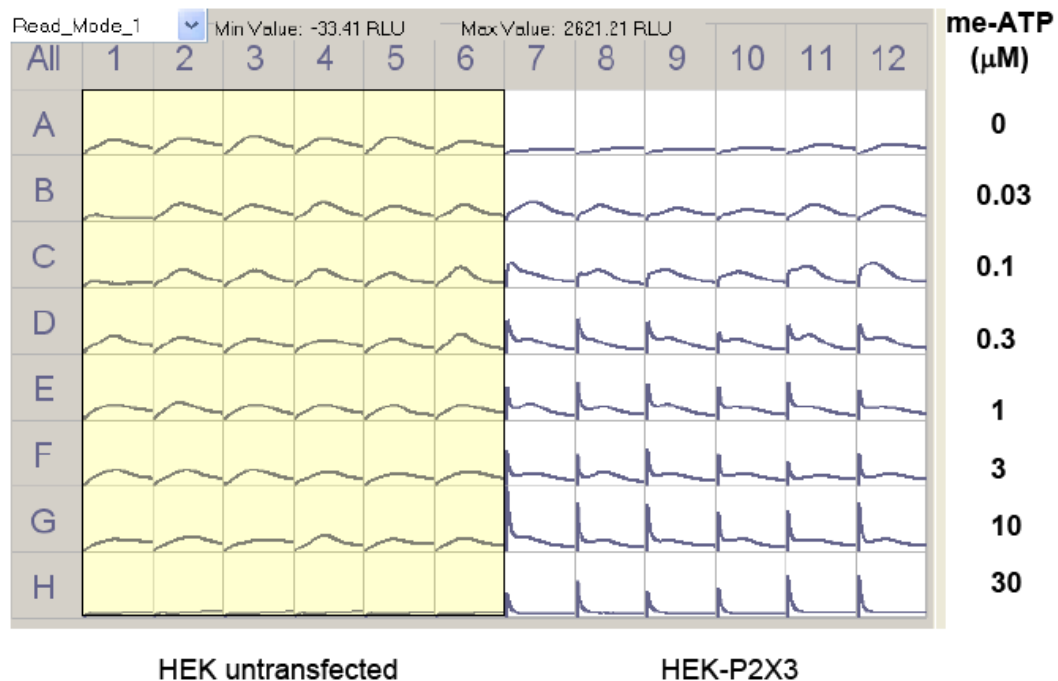


**Figure 4. P2X3 Activation and Inhibition in PatchXpress<sup>®</sup>**

**A:**  $\alpha,\beta$ -MeATP concentration-response relationship for activation. Mean  $\pm$  SEM, n = 7 cells/concentration.  $EC_{50}$  = 1.9  $\mu\text{M}$ . **B:** Suramin concentration-response relationship for inhibition of  $\alpha,\beta$ -meATP (10  $\mu\text{M}$ )-induced activation. Mean  $\pm$  SEM, n = 4 - 6 cells/concentration.  $IC_{50}$  = 2.8  $\mu\text{M}$ . The inset shows inward current traces elicited by 10  $\mu\text{M}$   $\alpha,\beta$ -meATP before (control) and after application of 100  $\mu\text{M}$  suramin.

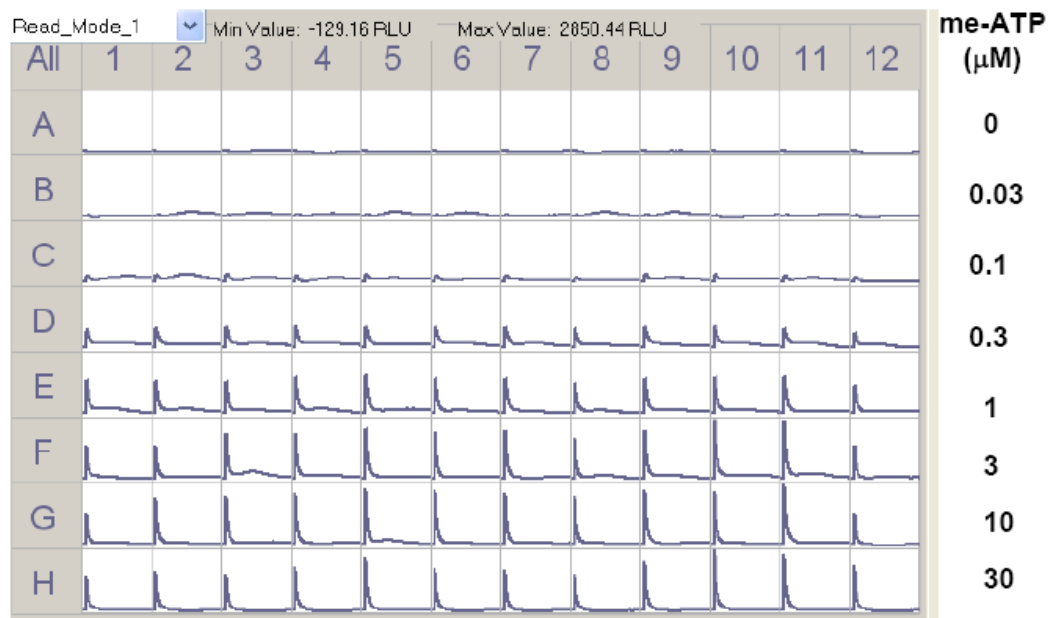
## 2.3 FLIPR® Representative Data

### 2.3.1 ATP Activation of P2X3



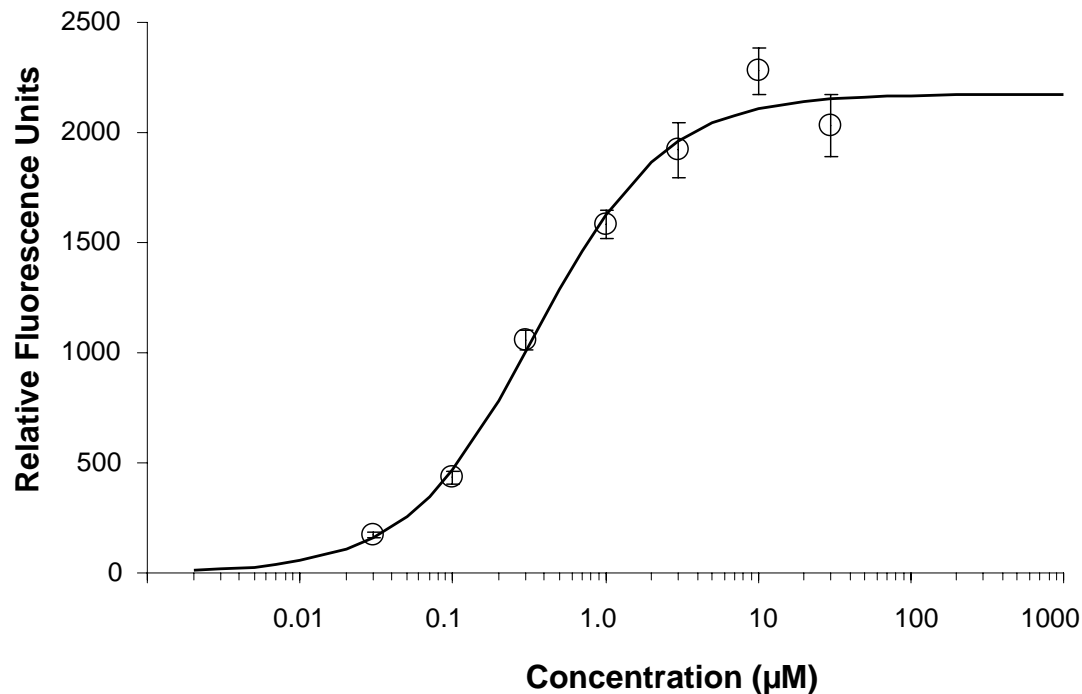
**Figure 5.  $\alpha,\beta$ -MeATP Activation of P2X3 in FLIPR®.**

$\alpha,\beta$ -MeATP evoked a concentration-dependent increase in  $[Ca^{2+}]_i$  in P2X3-HEK cells (columns 7 - 12) but had no significant effect in untransfected HEK cells (columns 1 - 6).



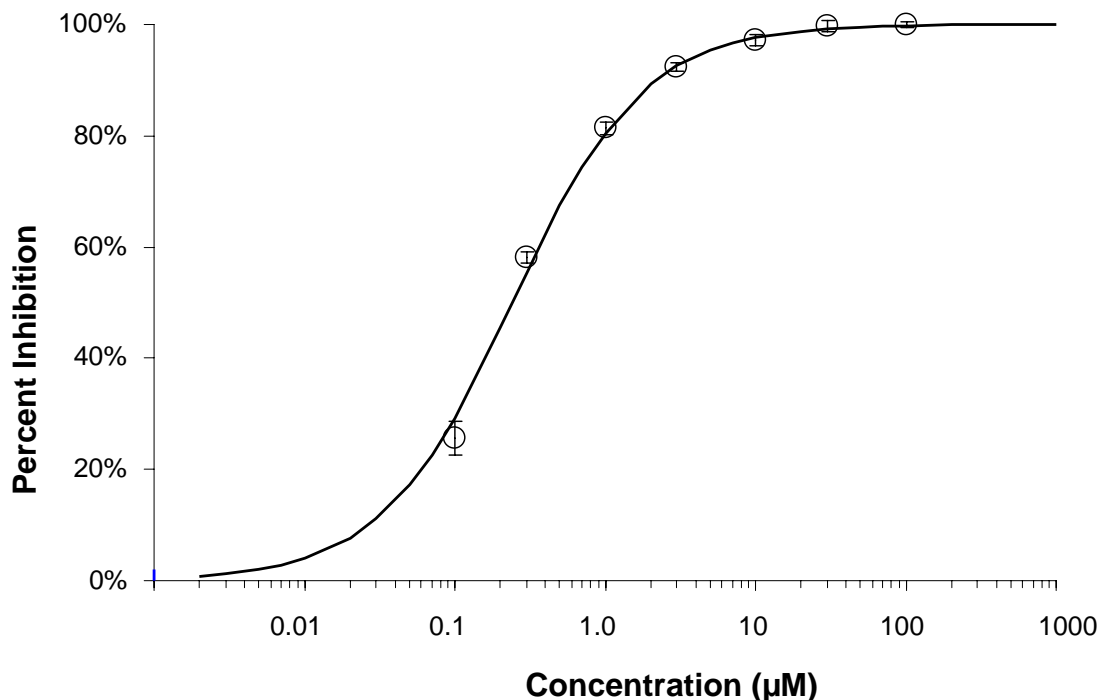
**Figure 6.  $\alpha,\beta$ -MeATP Concentration-Dependent Activation.**

$\alpha,\beta$ -MeATP evoked a concentration-dependent increase in  $[Ca^{2+}]_i$  in P2X3-HEK cells.



**Figure 7.  $\alpha,\beta$ -MeATP Concentration-Response Relationship.**  
Mean  $\pm$  SEM, n = 9 replicates/concentration. EC<sub>50</sub> = 0.35  $\mu$ M

### 2.3.2 Inhibition of $\alpha,\beta$ -meATP -induced activation of P2X3 by an antagonist



**Figure 8. PPADS Concentration-Response Relationship.**  
PPADS (0.01-100  $\mu$ M) inhibited  $\alpha,\beta$ -meATP (3  $\mu$ M)-induced  $[Ca^{2+}]_i$  responses in hP2X3-HEK cells in a concentration-dependent manner. Mean  $\pm$  SEM, n = 6 replicates/concentration. IC<sub>50</sub> = 0.24  $\mu$ M.

### **3 References**

Brown SG, et al. 2001. Actions of a series of PPADS analogs at P2X1 and P2X3 receptors. *Drug Dev Res* 53:281–291.

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.