

**RABBIT ANTI-P2X1 PURINERGIC RECEPTOR
AFFINITY PURIFIED
POLYCLONAL ANTIBODY**

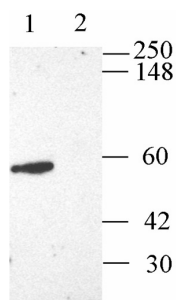
CATALOG NUMBER:	AB5224-200UL	QUANTITY:	200 µL
LOT NUMBER:		CONCENTRATION:	0.8 mg/mL (after reconstitution)
ALTERNATE NAMES:	ATP Receptor	EPITOPE:	C-terminus

BACKGROUND: The P2X1 purinergic receptor is a homo- or heterooligomeric integral membrane protein that functions as a ligand-gated ion channel with high calcium permeability. P2X1 mediates interneuronal and neuromuscular synaptic transmission upon binding to its ligand, ATP. P2X1 may be involved in the induction of apoptosis in the presence of ATP by increasing intracellular calcium concentration.

SPECIFICITY: Recognizes the full-length P2X1 purinergic receptor protein. Does not cross react with other known proteins tested so far.

IMMUNOGEN: A purified peptide from rat P2X1 protein (amino acids 382-399 of Accession P47824; Owens, 1991; Valera, 1994) .

APPLICATIONS: Western blot: 1:200 using ECL on human platelet lysates.
Immunohistochemistry on rat heart paraffin embedded tissue sections.
Immunoprecipitation
Dilutions should be made using a carrier protein such as BSA (1-3%)
Optimal working dilutions must be determined by the end user.



Western blotting of human platelets lysate
1. AB5224 (1:200)
2. AB5224 preincubated with the control peptide antigen

SPECIES REACTIVITY: Reacts with Human and Rat. Reactivity with other species has not been determined.

CONTROL: Included free of charge with the antibody is 40 µg of control antigen (lyophilized powder). The stock solution of the antigen can be made up using 100 µL of sterile deionized water. For negative control, preincubate 1 µg of purified peptide with 1 µg of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.

- FORMAT:** Affinity purified immunoglobulin.
- PRESENTATION:** Lyophilized from phosphate buffered saline, pH 7.4, containing 1% BSA, 5% sucrose as a stabilizer and 0.05% sodium azide as a preservative. Reconstitute lyophilized material with 200 μ L of sterile deionized water. Centrifuge antibody preparation before use (10,000 xg for 5 min).
- STORAGE/HANDLING:** Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.
- REFERENCES:** Valera, S. *et al.* (1994). A new class of ligand-gated ion channel defined by P2x receptor for extracellular ATP. *Nature* **371**:516-519.
- Owens, G.P. *et al.* (1991). Identification of mRNAs associated with programmed cell death in immature thymocytes. *Mol. Cell. Biol.* **11**:4177-4188.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μ L or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

For research use only; not for use as a diagnostic.

Western Blotting

Suggested Protocol

1. Mix the samples (organ membranes: 50 µg/lane; transfected cells: 500,000 cells/lane) with sample-buffer X 2, and heat 10 min at 70°C.
2. 5-50 µL applied to Minigel lane (0.75-1.5 mm width) and run at standard conditions. (60 mA for 2 1.5 mm Minigel gels, 1.4 h). It is suggested that you run 5-15% acrylamide (37.5:1 acrylamide:bisacrylamide) minigel (1.5 mm width) at 30 mA/gel ~1-1.5 hours.
3. Transfer in semi-dry system under standard conditions (3 h 100 mA for two minigel gels)
4. Stain the transferred bands with Chemicon BLOT-*FastStain* (Catalog Number 2076).
5. Destain with deionized water.
6. Block with 5% non-fat milk (Marvel or Carnation) in PBS, and 0.025 % sodium azide, overnight at 2-8°C. The non-fat milk should be dissolved freshly, centrifuged 10,000 rpm for 10 min, and filtered through glass filter (Gelman Acrodisc).
7. Incubation with first antibody 2 h at room temperature or overnight at 4°C in blocking solution. The antibody preparation should be centrifuged before use (10,000 g 5 min.). Optimal working dilutions and incubation time will need to be determined by the end user.
8. Wash 4 x 10 min. with PBS-0.1% tween 20. From this stage, azide should be omitted.
9. Incubation with the secondary antibody (HRP-conjugated goat anti-rabbit antibody, for example Chemicon Catalog Number AP132P, diluted appropriately) 1 h at room temperature.
10. Wash 4 x 10 min. with PBS-0.1% tween 20.
11. Perform ECL with commercial kits (Chemilucifer, Chemicon Catalog Number 2600).

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