

## RABBIT ANTI-P2X7 AFFINITY PURIFIED POLYCLONAL ANTIBODY

<b>CATALOG NUMBER:</b>	AB5581-200UL
<b>LOT NUMBER:</b>	xxxxxxx
<b>QUANTITY:</b>	200 µL
<b>CONCENTRATION:</b>	0.7 mg/mL (after reconstitution)
<b>SPECIFICITY:</b>	Recognizes P2X7, extracellular domain.
<b>IMMUNOGEN:</b>	Highly purified peptide corresponding to residues 136-152 of mouse P2X7 (Accession Q9Z1M0).
<b>APPLICATIONS:</b>	Western blotting: 1:200 using ECL on rat brain membrane. Flow cytometry, indirect: 5-10 µg antibody per 1x10 <sup>6</sup> cells. Dilutions should be made using a carrier protein such as BSA (1-3%). Optimal working dilutions must be determined by the end user.
<b>CONTROL ANTIGEN:</b>	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.
<b>SPECIES REACTIVITIES:</b>	Rat and human. Reactivity with other species has not yet been tested.
<b>FORMAT:</b>	Affinity purified immunoglobulin.
<b>PRESENTATION:</b>	Lyophilized from PBS, pH 7.4, containing 1% BSA and 0.05% sodium azide. Reconstitute with 200 µL of sterile distilled water. Centrifuge antibody preparation before use (10,000 x g for 5 min).
<b>STORAGE/HANDLING:</b>	Maintain lyophilized material at -20°C for up to 6 months after date of receipt. After reconstitution maintain antibody and control peptide at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

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## SUGGESTED WESTERN BLOT 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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