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RABBIT ANTI-P2Y12 RECEPTOR AFFINITY PURIFIED POLYCLONAL ANTIBODY

CATALOG NUMBER: AB5479-200UL

LOT NUMBER:

QUANTITY: 200 µL

CONCENTRATION: 0.8 mg/mL (after reconstitution)

SPECIFICITY: Recognizes P2Y12 Receptor. The epitope specific for the P2Y12 receptor is not present in any other known proteins.

IMMUNOGEN: Highly purified peptide corresponding to residues 125-142 of human P2Y12 (Accession Q9H244).

APPLICATIONS: Western blotting: 1:200 using ECL on rat brain membranes and human platelets.
Immunohistochemistry on rat brain sections.
Dilutions should be made using a carrier protein such as BSA (1-3%).
Optimal working dilutions must be determined by the end user.

CONTROL ANTIGEN: Included free of charge with the antibody is XX µg of control antigen (lyophilized powder). The stock solution of the antigen can be made up using 100 µL of sterile distilled 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.

SPECIES REACTIVITIES: Human and rat. Reactivity with other species has not yet been tested. The immunogen sequence is conserved in mouse (16/18).

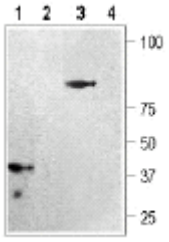
FORMAT: Affinity purified immunoglobulin.

PRESENTATION: 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).

STORAGE/HANDLING: Maintain lyophilized material at -20°C for up to 6 months after date of receipt. After reconstitution maintain at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

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:bisacrysmide) 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 Millipore 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 Millipore 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 (Chemilucent, Millipore Catalog Number 2600).



Western blotting of rat brain membranes (1,2) or human platelet (3,4) proteins

1,3. AB5479 1:200

2,4. AB5479 preincubated with control peptide.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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