Product Datasheet

P2Y2 Antibody
NB110-39032

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Publications: 5

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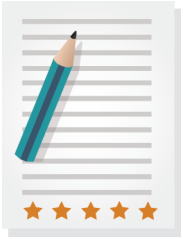

NB110-39032

P2Y2 Antibody

Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	42.3 kDa

Product Description	
Host	Rabbit
Gene ID	5029
Gene Symbol	P2RY2
Species	Human, Mouse, Rat
Immunogen	A synthetic peptide made to a C-terminal portion of the human P2Y2 protein (between animo acids 300-377) [UniProt P41231].

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:500-1:1000, Immunohistochemistry 1:100-1:200, Immunocytochemistry/ Immunofluorescence 1:50 - 1:100, Immunohistochemistry-Paraffin 1:100 - 1:200
Application Notes	The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Publications

Katagiri A, Tsubota K, Mikuzuki L et al. Tear secretion by Diquafosol suppresses the excitability of trigeminal brainstem nuclear complex neurons by reducing excessive P2Y2 expression in the trigeminal ganglion in dry eye rats Neuroscience research 2023-01-16 [PMID: 36657726] (IHC, Rat)

Details:
Dilution used in IHC 1:300

Jelcic M, Wang K, Hui KL et al. A Photo-clickable ATP-Mimetic Reveals Nucleotide Interactors in the Membrane Proteome Cell Chem Biol 2020-06-05 [PMID: 32521230]

Velazquez-Miranda E, Molina-Aguilar C, Gonzalez-Gallardo A et Al. Increased Purinergic Responses Dependent on P2Y2 Receptors in Hepatocytes from CCl4-Treated Fibrotic Mice Int J Mol Sci 2020-03-26 [PMID: 32225112] (WB, Mouse)

Lommen J, Stahr A, Ingenwerth M et al. Untersuchung tageszeitlicher Expressionsprofile purinerger Rezeptoren im Nucleus suprachiasmaticus der Maus Dissertation 2018-01-01 (IF/IHC, Mouse)

Lommen J, Stahr A, Ingenwerth M et al. Time-of-day-dependent expression of purinergic receptors in mouse suprachiasmatic nucleus Cell Tissue Res. 2017-05-26 [PMID: 28547658] (IF/IHC, Mouse)

Procedures

Western Blot Protocol for P2Y2 Antibody (NB110-39032)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute anti-P2Y2 primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin Protocol for P2Y2 Antibody (NB110-39032)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence Protocol for P2Y2 Antibody (NB110-39032)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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