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Product datasheet for AP00654SU-N

PDE3B Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	IHC, IP, WB
Recommended Dilution:	**Immunohistochemistry.** **Immunoprecipitation.** **Western blot** (See picture below).
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Clonality:	Polyclonal
Immunogen:	Designed from the C-terminal region common to the 3B isoform.
Specificity:	The antibody selectively detects proteins corresponding to PDE3B.
Formulation:	PBS containing 0.05% Sodium Azide as preservative State: Serum State: Liquid Diluted Serum
Conjugation:	Unconjugated
Storage:	Store the antiserum at 2-8°C undiluted for one month or (in small aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	phosphodiesterase 3B
Database Link:	Entrez Gene 5140 Human Q13370
Background:	Cyclic nucleotide phosphodiesterases (PDEs) catalyse the hydrolytic inactivation of the common intracellular second messengers cyclic adenosine and guanosine 3', 5'-monophosphate (cAMP and cGMP). Thus, these enzymes play a critical role in the regulation of a wide range of physiological processes modulated by cyclic nucleotide signalling. The PDE3 enzyme belongs to a family of PDEs known as cGMP-inhibited PDEs. The enzymes bind both cGMP and cAMP with different affinities. The PDE3 family is comprised of two genes, PDE3A and PDE3B.

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Synonyms: Phosphodiesterase 3B, Phosphodiesterase-3B cGMP-inhibited, HcGIP1, CGIPDE1, CGI-PDE B, CGIP, CGIP1

Note: Protocol: **Immunofluorescence protocol - Formaldehyde fixation**
1. Collect cells from T.c.unit and remove media from petri dish using suction.
2. Wash with 1x PBS and remove.
3. Incubate cells in pre-warm (37°C) Para-Formaldehyde for 12 minutes at room temperature on an orbital shaker.
4. Remove PFA and incubate in 0.5% Triton X-IOO in 1x PBS for 5 minutes at room temperature.
5. Prepare blocking reagent, this is also the antibody diluent.
6. Wash cells 2x with 1x PBS at room temperature, for 4 minutes/wash on an orbital shaker.
7. Block with 1 % NCS and 1x PBS for 30 minutes at room temperature.
8. Prepare primary antibodies (50?l/coverslip) and moist staining chambers.
9. Wash cells 2x with lx PBS at room temperature and air dry briefly.
10. Incubate with primary antibody for 1 hr at room temperature in the dark in staining chambers. During this time prepare the secondary antibody.
11. Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)
12. Incubate with secondary antibody for 1 hour at room temperature in the dark in staining chambers.
13. Wash cells 5x with 1x PBS.
14. Mount in Dapi.

Solutions (prepare fresh the same day of staining).
* 1x Phosphate buffered saline.
* Blocking reagent: 1% NCS in 1x PBS (use fresh l0x PBS).
* Fixation solution: 3.5% Para formaldehyde.

1.75g PFA in 20 ml d.H20 plus 5 drops 1M NaOH. Stir on a hot plate at 50-60°C until dissolved. Add 4 drops IN HCI and check pH indicator strip. PH should be 7.4. Complete volume with d.H20 to 25ml and add 25ml 2xPBS. Check pH before adding to cover slips.

Immunofluorescence protocol - Methanol/acetone fixation
1. Collect cells from T.C.unit and remove media from petri dish using suction.
2. Wash with 1x PBS and remove.
3. Fix cells with cold methanol: acetone 1:1 for 10 minutes on ice.
4. Prepare blocking reagent, this is also the diluent for the antibodies.
5. Remove fixative and wash cells 3x with Ix PBS at RT, for 4 minutes/wash on orbital shaker.
6. Block with 1% NCS and Ix PBS for 30 minutes at RT.
7. Prepare primary antibodies (50?l/coverslip) and moist staining chambers.
8. Wash cells 2x with 1 x PBS at RT and air dry for approximately 7 minutes.
9. Incubate with primary antibody for 1 hr at RT in the dark in staining chambers. During this time prepare secondary antibody.
10. Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)

11. Incubate with secondary antibody for 1 hr at R T in the dark in staining chambers.
12. Wash cells 5x with 1x PBS.
13. Mount in Dapi.

Solutions (prepare fresh the same day of staining)
* 1x Phosphate buffered saline.
* Blocking reagent: 1% NCS in 1x PBS (use fresh 10x PBS).
* Fixation solution: methanol:acetone 1: 1 ice cold.

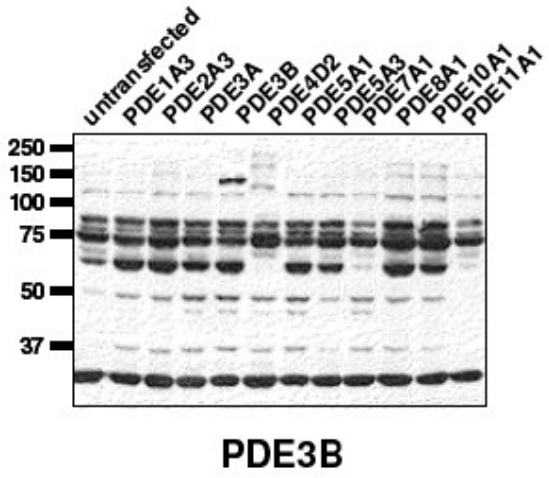
Western Blotting Protocol
1. Transfer gel to PDVF or nitrocellulose membrane
2. Place membrane in plastic tray in blocking buffer for one hour with agitation
3. Rinse in wash buffer
4. Incubate in wash buffer plus primary antibody for one hour
5. Wash 6 X 5 minutes with wash buffer
6. Incubate in wash buffer plus secondary antibody for one hour
7. Wash 6X 5 minutes with wash buffer
8. Detect (e.g. ECL, Amersham according to manufacturers instructions)

Wash buffer
PBS + 0.1% Tween 20

Blocking buffer
Wash buffer + 5% dried milk powder

The concentration of antibodies used depends on each antibody, the amount of antigen and the detection method used. Generally, dilution is in the range of a few hundred times dilution to a few thousand times dilution, but usually has to be determined empirically.

Product images:



Western blot using AP00654SU-N (1:20,000) against lysate from COS cells transfected with the indicated human PDE isoform.