

Datasheet for ABIN2856876  
**anti-ENTPD5 antibody (Center)**



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## Overview

Quantity:	100 µL
Target:	ENTPD5
Binding Specificity:	Center
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Application:	Western Blotting (WB), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p))

## Product Details

Immunogen:	Recombinant protein encompassing a sequence within the center region of human ENTPD5.
Characteristics:	Rabbit polyclonal antibody to ENTPD5(CD39L4) (ectonucleoside triphosphate diphosphohydrolase 5) ENTPD5 antibody [N1N3]
Purification:	Purified by antigen-affinity chromatography.

## Target Details

Target:	ENTPD5
Alternative Name:	ENTPD5 ( <a href="#">ENTPD5 Products</a> )
Gene ID:	957

## Application Details

Application Notes: WB: 1:500-1:3000, IHC-P: 1:100-1:1000.  
Not yet tested in other applications.  
Optimal working dilutions should be determined experimentally by the end user.

Restrictions: For Research Use only

## Handling

Format: Liquid

Concentration: 1 mg/mL

Buffer: 0.1M Tris, 0.1M Glycine, 10 % Glycerol (pH 7).  
0.01 % Thimerosal was added as a preservative.

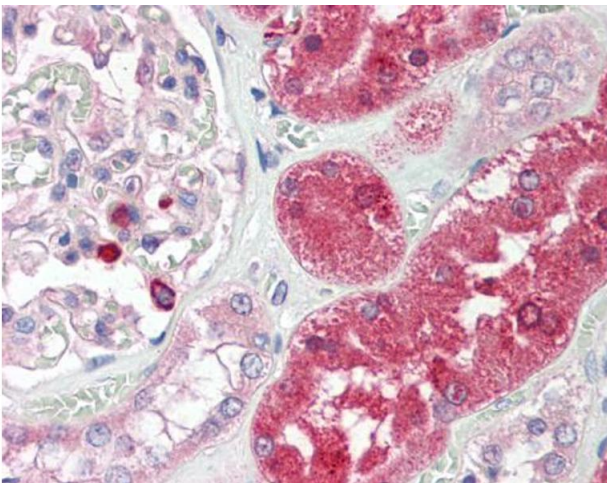
Preservative: Thimerosal (Merthiolate)

Precaution of Use: This product contains Thimerosal (Merthiolate): a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Storage: -20 °C

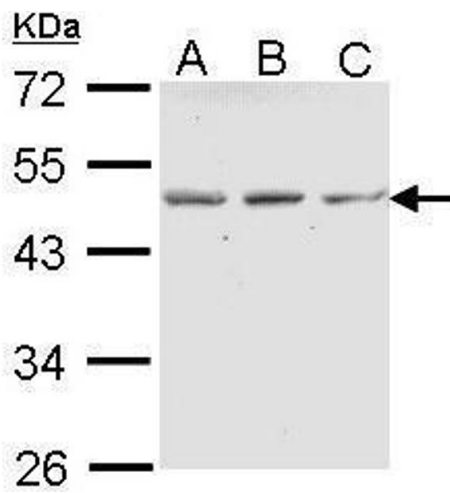
Storage Comment: Keep as concentrated solution. Aliquot and store at -20°C or below.  
Avoid multiple freeze-thaw cycles.

## Images



### Immunohistochemistry

**Image 1.** IHC-P Image ENTPD5 antibody [N1N3] detects ENTPD5(CD39L4) protein at cytoplasm on human placenta by immunohistochemical analysis. Sample: Paraffin-embedded placenta. ENTPD5 antibody [N1N3] , dilution: 1:100.



### Western Blotting

**Image 2.** WB Image Sample (30 ug of whole cell lysate) A: A431 , B: H1299 C: HeLa 10% SDS PAGE antibody diluted at 1:1000



**Successfully validated (Western Blotting (WB))**

by [Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago](#)

Report Number: 101762

Date: Nov 02 2017

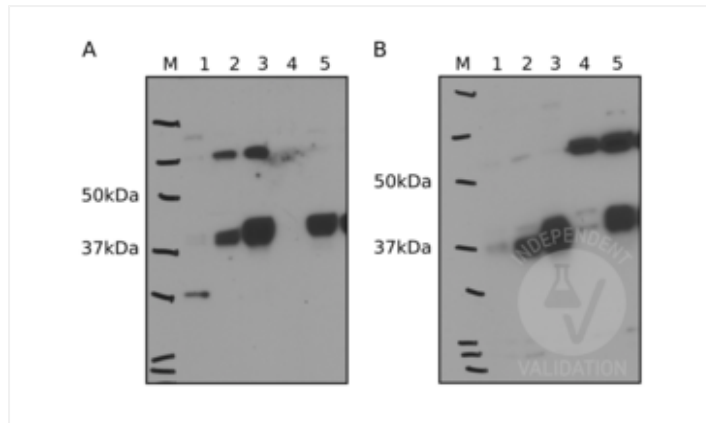
Target:	ENTPD5
Lot Number:	40128
Method validated:	Western Blotting (WB)
Positive Control:	Tet-inducible ENTPD5 LNCaP cells, Tet-inducible ENTPD5 BPH1 cells
Negative Control:	BPH1 cells
Notes:	Passed. ABIN2856876 specifically recognizes ENTPD5 in Tet-inducible ENTPD5 LNCaP cells and Tet-inducible ENTPD5 BPH1 cells.
Primary Antibody:	ABIN2856876
Secondary Antibody:	HRP-conjugated goat-anti rabbit antibody (ThermoFisher, A16104, lot 42-28-042114)
Protocol:	<ul style="list-style-type: none"><li>• Grow<ul style="list-style-type: none"><li>◦ LNCaP cells in RPMI 1640 1x with L-glutamine medium (Corning Cellgro, 10-040-CV, lot 11717002) supplemented with 10% FBS (GE Healthcare, SH30910.03, lot AC10225544) and penicillin-streptomycin (Mediatech, MT30002CI, lot 30002292) at 37°C and 5% CO<sub>2</sub> in 5ml on a tissue culture dish to 1.5x10<sup>6</sup> cells/ml.</li><li>◦ Tet-inducible GFP-LNCaP cells in RPMI 1640 1x with L-glutamine medium supplemented with 10% FBS, penicillin-streptomycin, and 1µg/ml Doxycycline hyclate (Sigma Aldrich, D9891, 056M4029V) at 37°C and 5% CO<sub>2</sub> in 5ml on a tissue culture dish to 1.5x10<sup>6</sup> cells/ml.</li><li>◦ Tet-inducible ENTPD5-LNCaP cells in RPMI 1640 1x with L-glutamine medium and 1µg/mL Doxycycline hyclate at 37°C and 5% CO<sub>2</sub> in 5ml on a tissue culture dish to 1.5x10<sup>6</sup> cells/ml.</li><li>◦ BPH1 cells in RPMI 1640 1x with L-glutamine medium supplemented with 10% FBS and penicillin-streptomycin at 37°C and 5% CO<sub>2</sub> in 5ml on a tissue culture dish to 1.5x10<sup>6</sup> cells/ml.</li><li>◦ Tet-inducible ENTPD5-BPH1 cells in RPMI 1640 1x with L-glutamine medium and 1µg/ml Doxycycline hyclate (Sigma Aldrich, D9891) at 37°C and 5% CO<sub>2</sub> in 5ml on a tissue culture dish to 1.5x10<sup>6</sup> cells/ml.</li></ul></li><li>• Collect and resuspend cells in 50µl lysis buffer (20mM HEPES pH7.4, 1% Triton X-100, 150mM NaCl, 1mM EGTA, 1mM EDTA, 10mM sodium pyrophosphate, 100mM NaF, 5mM iodoacetic acid, 20nM okadaic acid, 0.2mM PMSF, and 1 tablet/10mL Pierce Protease Inhibitor mini Tablet (ThermoFisher, 88665, Lot RC2161121)).</li></ul>

- Lyse cells by 5 rounds of freeze/thaw cycles: 60sec in LN2, 30sec in a 37°C water bath, 15sec vortex, 60sec on ice.
- Spin lysed cells at 10000xg for 5min at 4°C.
- Recover the supernatant and measure protein concentration with Bradford reagent (Bio-Rad, 5000006).
- Denature 25µg total proteins of each sample for 5min at 95°C in Laemmli SDS sample buffer and subsequently separate them on two separate 4-12% Express Plus Page Gels (GenScript, M41215, Lot:C30251704) for 40min at 160V in an electrophoresis chamber (BioRad).
- Transfer proteins onto PVDF membrane (Millipore, IPFL00010, Lot K0MA0558B) with a wet blotting system (Bio-Rad) for 75min at 115V at 4°C.
- Block the membrane with 5% non-fat dry milk in TBSTween (0.1% v/v) for 1h at RT.
- Incubation the membranes with primary
  - rabbit anti-ENTPD5 antibody (antibodies-online, ABIN2856876, Lot: 40128) or
  - rabbit anti-ENTPD5 antibody (Sigma, HPA002927 Lot: A40077)
- diluted 1:500 in 5% non-fat dry milk in TBST at for 16h at 4°C.
- Wash the membrane 5x for 5min in TBST.
- Incubate the membrane with secondary HRP-conjugated goat-anti rabbit antibody (ThermoFisher, A16104, lot 42-28-042114) diluted 1:3000 in 5% non-fat dry milk in TBST for 1h at RT.
- Wash membrane 5x for 5min with PBST.
- Visualize protein bands using SuperSignal West Pico Chemiluminescent Substrate (ThermoScientific, 34087, Lot SC246874) on CL-X Posure Film (ThermoScientific, 34091).

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Experimental Notes:

- The ENTPD5 antibody ABIN2856876 detects a single band around expected molecular weight (47kDa). The intensity of this band becomes more intense upon ENTPD5 overexpression and disappears in the negative control cell line.
  - ABIN2856876 also reveals fewer non-specific bands than the control antibody. We recommend this antibody for detecting ENTPD5. The positive control antibody performed as predicted with showing increased expression in the overexpression cells and no expression in the negative control BPH1 cell line.
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**Validation image no. 1 for anti-Ectonucleoside Triphosphate diphosphohydrolase 5 (ENTPD5) (Center) antibody (ABIN2856876)**

A Western blot detection of ENTPD5 in lysates of untransfected LNCaP (1) and BPH1 (4) cells, doxycycline-induced GFP-LNCaP (2), ENTP5-LNCaP (3), and BPH1-ENTPD5 cells (5) using ABIN2856876 (A) or a reference antibody (B).