

Size	Price
50 µg purified IgG2b	\$300 (CAD)

## Monoclonal mouse anti-human NTPDase3 antibodies

**Name:** hN3-B3<sub>s</sub>

Note: epitope sequencing revealed that the antibody hN3-H10<sub>s</sub> previously offered was identical to hN3-B3<sub>s</sub>

**Isotype:** Mouse IgG2b

### Applications<sup>1</sup>

	Yes	Working concentration	No	Not tested
Western blot (non-reduced) <sup>§</sup>	+	0.5-1 µg/mL (purified)		
Western blot (reduced)			×	
Immunohistochemistry <sup>*</sup>	+	0.5-1 µg/mL (purified)		
Flow Cytometry	+	1-5 µg/mL (purified)		
ELISA <sup>†</sup>	+	0.5-1 µg/mL (purified)		
Immunoprecipitation				×
Inhibition assay	+	0.5 µg/mL (purified)		

§ Thiol-reactive reagents (e.g. β-mercaptoethanol, DTT) must be avoided as they destroy the epitope recognized by the antibody.

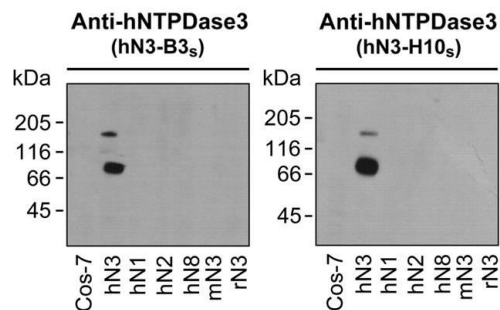
\* Cryosections were fixed with acetone.

† Indirect ELISA where the antibody is added to a well previously coated with the antigen.

### Cross-reactivity

In Western blot, hN3-B3<sub>s</sub> and hN3-H10<sub>s</sub> do not cross-react with rat and mouse NTPDase3 or with human NTPDase1, -2 and -8.

### Western blot<sup>1</sup>



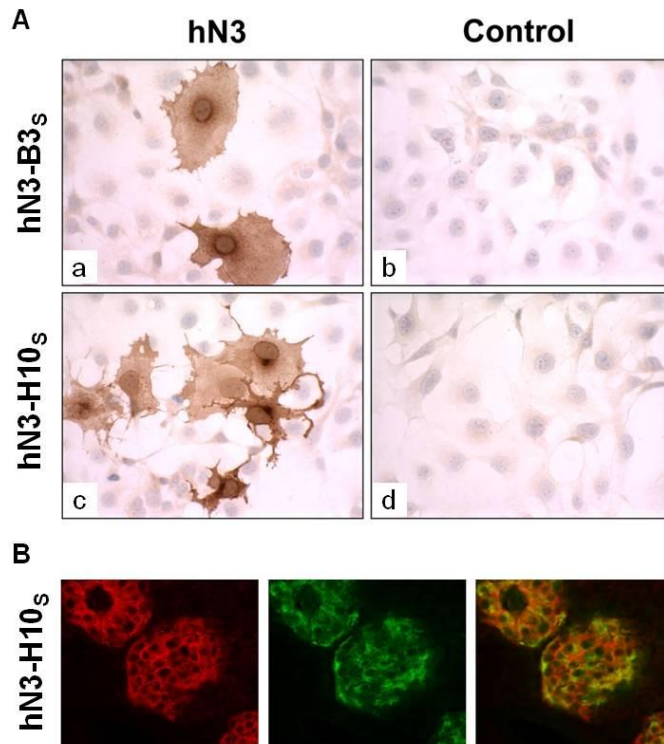
Protein samples from a lysate from COS-7 cells (negative control; COS-7) or from COS-7 transiently transfected with a plasmid encoding for the indicated NTPDases were loaded on a NuPAGE® Novex® Bis-Tris 4-12% gel under non-reducing conditions, transferred to an Immobilon-P membrane and incubated with either hN3-B3<sub>s</sub> or hN3-H10<sub>s</sub>. The monoclonal antibodies recognize specifically the monomeric (~75 kDa) and dimeric (150 kDa) form of human NTPDase3.

h: human; m: mouse; r: rat.

N1, N2, N3, N8: NTPDase1, -2, -3, -8, respectively.

Modified figure taken from Munkonda et al. (2009) *FEBS J*, 276(2): 479-496.

## Immuno(cyto/histo)chemistry<sup>1</sup>



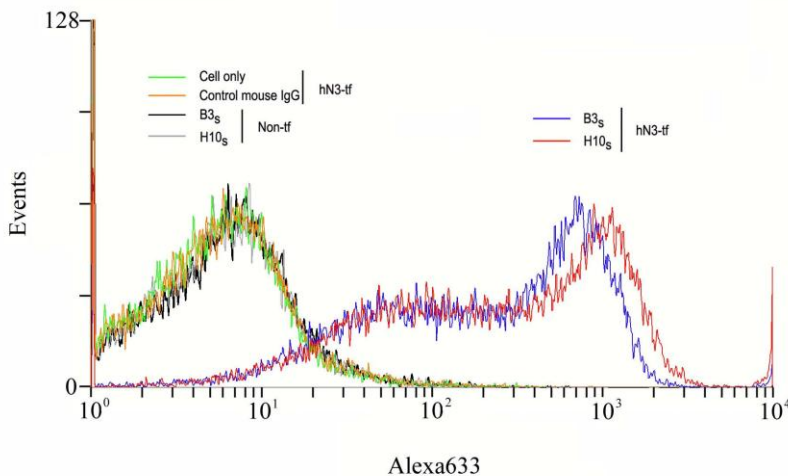
**A:** Immunocytochemistry of untransfected COS-7 cells (control: b,d) or transfected with a plasmid encoding human NTPDase3 (hN3; a,c) both incubated with the indicated monoclonal antibodies. A strong signal is observed only with the monoclonal antibodies in cells expressing human NTPDase3 (a,c). No signal is detected in any of the control cells (b,d). Nuclei are stained with hematoxylin (blue).

Figure taken from Munkonda et al. (2009) *FEBS J*, 276(2): 479-496.

**B:** A human pancreas serial section stained by immunofluorescence with hN3-H10<sub>s</sub> shows a positive reaction for NTPDase3 (red) on insulin-producing cells (green).

Figure taken from Lavoie et al. (2010) *Am J Physiol Endocrinol Metab*, 299(4):E647-656.

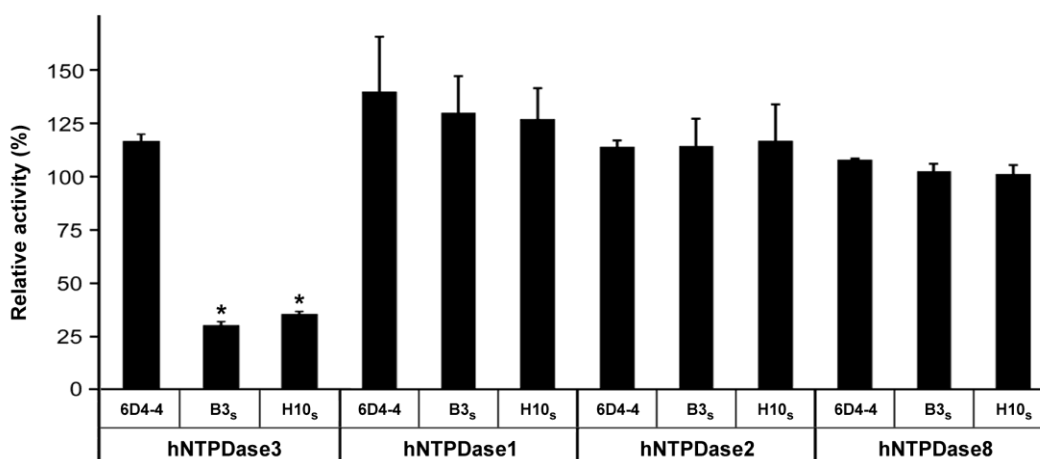
## Flow cytometry<sup>1</sup>



HEK 293T cells transfected with human NTPDase3 (hN3-tf) or untransfected (non-tf) were incubated with either one of the monoclonal antibodies, or with irrelevant mouse IgGs. The detection was carried out with an Alexa-conjugated secondary antibody.

A rightward shift is observed exclusively with cells expressing human NTPDase3 in the presence of either hN3-B3<sub>s</sub> or hN3-H10<sub>s</sub> monoclonal antibody, demonstrating the specificity of the antibodies to human NTPDase3.

## Inhibition of the enzymatic activity



ATPase activity was tested with cell lysates from COS-7 cells transfected with human (h) NTPDase3, or other NTPDases, as indicated, in presence or absence of the monoclonal antibodies hN3-B3<sub>s</sub>, hN3-H10<sub>s</sub> and 6D4-4 (anti-cytohesin, as a negative irrelevant control) at a concentration of 500 ng/mL. A specific inhibition of human NTPDase3 ATPase activity is observed with the anti-NTPDase3 monoclonal antibodies.

Figure taken from Munkonda et al. (2009) *FEBS J*, 276(2): 479-496.

N.B. Inhibition by enzyme histochemistry on human pancreas has also been reported in this article.

## Storage

Purified antibodies can be kept at 4°C for generally up to a year.

## Reference to cite in your publication (paper where these antibodies were characterized)

This antibody was obtained from ectonucleotidases-ab, Université Laval, Quebec, QC, Canada, and the specificity of the antibody was characterized in: Munkonda MN, Pelletier J, Ivanenkov VV, Fausther M, Tremblay A, Künzli B, Kirley TL, Sévigny J. Characterization of a monoclonal antibody as the first specific inhibitor of human NTP diphosphohydrolase-3 : partial characterization of the inhibitory epitope and potential applications. *FEBS J*. 2009; 276(2): 479-496.

## Other references where these antibodies were used

- Ivanenkov VV, Crawford PA, Toyama A, Sévigny J, Kirley TL. Epitope mapping in cell surface proteins by site-directed masking: defining the structural elements of NTPDase3 inhibition by a monoclonal antibody. *Protein Eng Des Sel*. 2010; 23(7):579-588.
- Fausther M, Pelletier J, Ribeiro CM, Sévigny J, Picher M. Cystic fibrosis remodels the regulation of purinergic signaling by NTPDase1 (CD39) and NTPDase3. *Am J Physiol Lung Cell Mol Physiol*. 2010; 298(6):L804-818.
- Lavoie EG, Fausther M, Kauffenstein G, Kukulski F, Künzli BM, Friess H, Sévigny J. Identification of the ectonucleotidases expressed in mouse, rat, and human Langerhans islets: potential role of NTPDase3 in insulin secretion. *Am J Physiol Endocrinol Metab*. 2010; 299(4):E647-656.

- Pinheiro AR, Paramos-de-Carvalho D, Certal M, Costa MA, Costa C, Magalhaes-Cardoso MT, Ferreirinha F, Sévigny J, Correia-de-Sa P. Histamine induces ATP release from human subcutaneous fibroblasts, via pannexin-1 hemichannels, leading to Ca<sup>2+</sup> mobilization and cell proliferation. *J Biol Chem.* 2013; 288(38):27571-27583.
- Saunders DC, Brissova M, Phillips N, Shrestha S, Walker JT, Aramandla R, Poffenberger G, Flaherty DK, Weller KP, Pelletier J, Cooper T, Goff MT, Virostko J, Shostak A, Dean ED, Greiner DL, Shultz LD, Prasad N, Levy SE, Carnahan RH, Dai C, Sévigny J, Powers AC. Ectonucleoside triphosphate diphosphohydrolase-3 antibody targets adult human pancreatic beta cells for *in vitro* and *in vivo* analysis. *Cell Metab.* 2018.