

Size	Price
50 µl of serum	\$200 (CAN)

Polyclonal guinea pig anti-rat ecto-5'-Nucleotidase/CD73 antibodies

Name: rNu-4_cI₄; rNu-5_c(I₄,I₅); rNu-6_c(I₄,I₅)

Applications¹

	Yes	Dilution	No	Not tested
Western blot (non-reduced)	+	1:500-1:2000		
Western blot (reduced) [¶]	+	1:500		
Immunohistochemistry [*]	+	1:1000		
Flow cytometry				×
ELISA				×
Immunoprecipitation				×

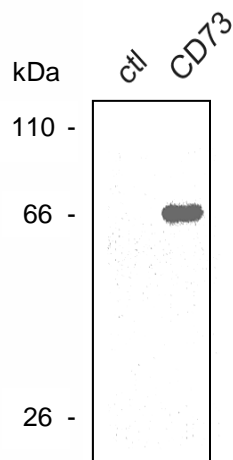
[¶] Note that these antibodies work better in non-reduced conditions (i.e. in absence of DTT, mercaptoethanol...)

^{*} Cryosection and acetone fixation

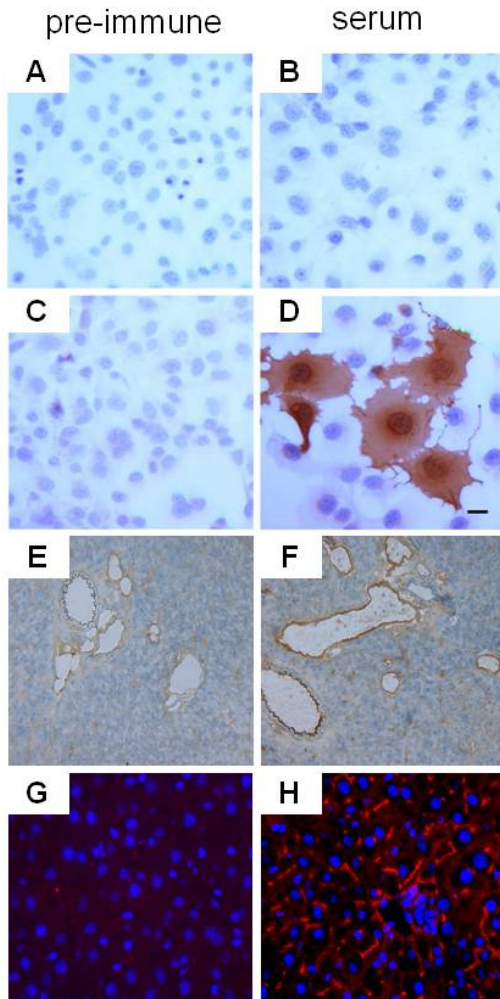
Cross-reactivity

In Immunohistochemistry, rNu-4_cI₄ cross-reacts with mouse CD73. In Western blot, all these antibodies cross-reacts weakly with human ecto-5'-nucleotidase.

Western Blot¹



Protein samples (6 µg) from a lysate from COS-7 cells (ctl) or from COS-7 transiently transfected with a plasmid encoding for rat ecto-5'-nucleotidase (CD73) were loaded on a NuPAGE® Novex® Bis-Tris 4-12% gel under non-reducing conditions, transferred to an Immobilon-P membrane and incubated with rNu-4_cI₄ (1:2000). A 66-kDa band is detected only in sample from cells expressing rat CD73.

Immuno(cyto/histo)chemistry¹

A-D: Immunocytochemistry of untransfected COS-7 cells (A, B) or transfected (C, D) with a plasmid encoding rat ecto-5'-nucleotidase (CD73) both incubated with rNu-4cI₄ (B, D) or preimmune serum (A, C). A strong signal is observed only with the antiserum in cells expressing rat CD73 (D). No signal is detected in any of the controls (A-C).

E-F: A mouse pancreas section incubated with either the antiserum (F) or preimmune serum (E) displays a positive reaction in blood vessels and capillaries only with the antiserum (F).

G-H: A rat liver section stained by immunofluorescence with rNu-4cI₄ (H) or preimmune serum (G) displays a strong reaction in the canaliculi only with the antisera (H).

The preimmune serum is devoid of any reactions (A, C, E, G). Nuclei are stained blue with either hematoxylin (A-F) or DAPI (G-H).

Storage

To avoid excessive freeze-thaw cycles, a small amount can be kept at 4°C for generally up to one year. A better method consists to dilute the antibody 10 times in one part of 145 mM NaCl, 1% BSA, 10 mM Tris (pH 7.4), and one part of glycerol (for a final concentration of 50% v/v) and to keep it at -20°C (note that 50% glycerol solutions freeze at about -30°C). For long-term storage, freeze samples directly at -80°C.

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

This antibody was obtained from ectonucleotidases-ab.com and its specificity was characterized in:

Fausther M, Lecka J, Soliman E, Kauffenstein G, Pelletier J, Sheung N, Dranoff JA, Sévigny J. Co-expression of ecto-5'-nucleotidase/CD73 with specific NTPDases differentially regulates adenosine formation in the rat liver. *Am J Physiol Gastrointest Liver Physiol.* 2012; 302(4):G447-459.

Few other references where these antibodies were used

- 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 Ca²⁺ mobilization and cell proliferation. *J Biol Chem.* 2013; 288(38):27571-27583.