

Size	Price
100 µl of serum	\$200 (CAN)

Polyclonal rabbit anti-rat ecto-5'-Nucleotidase/CD73 antibodies

Name: rNu-8_L(I₄,I₅); rNu-9_L(I₄,I₅); rNu-16_L(I₄,I₅)

Applications¹

	Yes	Dilution	No	Not tested
Western blot (non-reduced)	+	1:4000		
Western blot (reduced) [¶]	+	1:500		
Immunohistochemistry*				
Frozen section	+	1:2000		
Paraffin	+	1:500		
Flow cytometry	+	1:100		
ELISA				×
Immunoprecipitation				×

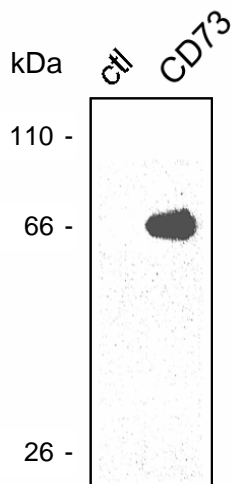
¶ Note that these antibodies work better in non-reduced conditions (i.e. in absence of DTT, mercaptoethanol...)

* All of these antibodies work on cryosection and acetone fixation. rNu-9_L(I₄, I₅) work also on paraffin section using proteinase K (20 µg/mL) as antigen retrieval technic.

Cross-reactivity

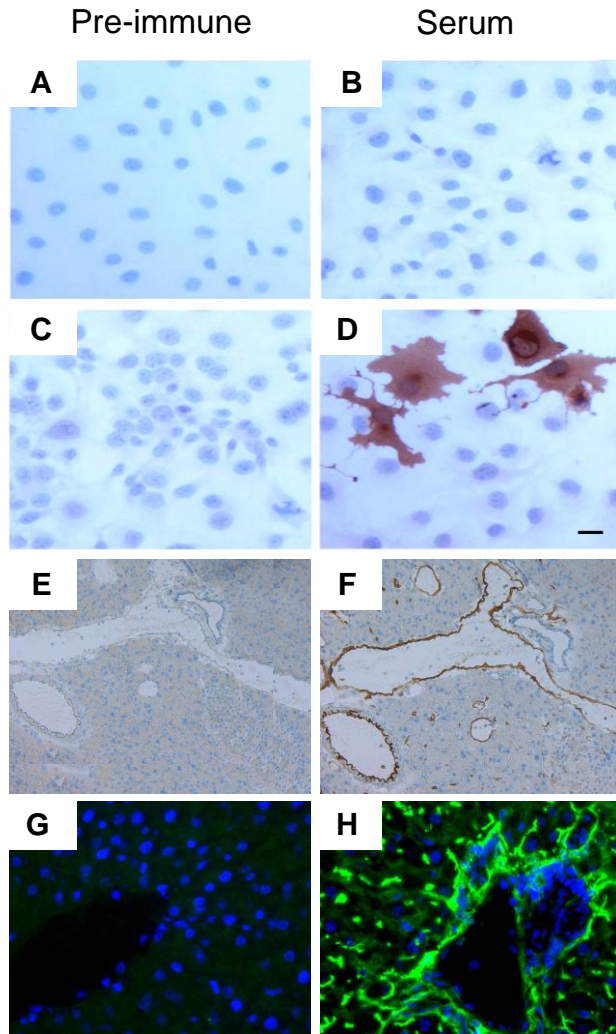
In Western blot and Immunohistochemistry, rNu-9_LI₅ cross-reacts with mouse ecto-5'-nucleotidase. In Western blot, rNu-9_LI₅ cross-reacts weakly with human ecto-5'-nucleotidase. In Western blot, rNu-9_LI₄ does not cross-react with human ecto-5'-nucleotidase.

Western blot¹



Protein samples (3 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-9_LI₅ (1:4000). A 66-kDa band is detected only in samples from cells expressing rat CD73.

Immuno(cyto/histo)chemistry¹



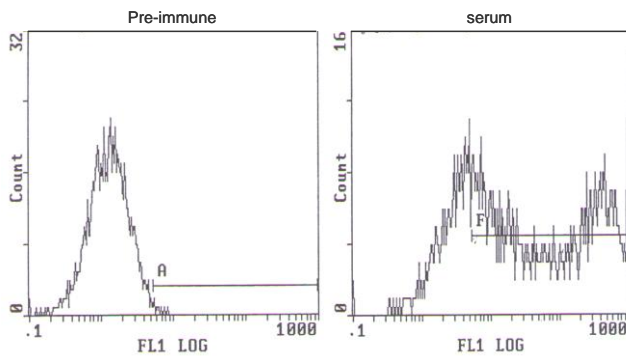
A-D: Immunocytochemistry of untransfected COS-7 cells (A, B) or transfected (C, D) with a plasmid encoding rat ecto-5'-nucleotidase (CD73) both incubated with rNu-9_LI₅ (B, D) or preimmune serum (A, C). A strong signal is observed only with the antiserum in cells expressing rat CD73 (D). No signal is detected in any of the control cells (A-C).

E-F: A mouse pancreas section incubated with either rNu-9_LI₅ (1:2000) (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 was stained by immunofluorescence with rNu-9_LI₅ (1:2000) (H) or preimmune serum (G). A strong reaction is observed in the canaliculi and a weaker reaction on the basolateral surface of hepatocytes and sinusoids only with the antiserum (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).

Flow cytometry¹



COS-7 cells transfected with rat ecto-5'-nucleotidase cDNA vector. Cells were incubated with rNu-9_LI₅ (1:80) or preimmune serum at the same dilution. Transfected cells incubated with the antiserum show a rightward shift.

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

- Martín-Satué M, Lavoie EG, Fausther M, Lecka J, Aliagas E, Kukulski F, Sévigny J. High expression and activity of ecto-5'-nucleotidase/CD73 in the male murine reproductive tract. *Histochem Cell Biol.* 2010; 133(6):659-668.
- Aliagas E, Torrejón-Escribano B, Lavoie EG, de Aranda IG, Sévigny J, Solsona C, Martín-Satué M. Changes in expression and activity levels of ecto-5'-nucleotidase/CD73 along the mouse female estrous cycle. *Acta Physiol.* 2010; 199(2):191-197.
- 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.
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- Vongtau HO, Lavoie EG, Sévigny J, Molliver DC. Distribution of ecto-nucleotidases in mouse sensory circuits suggests roles for nucleoside triphosphate diphosphohydrolase-3 in nociception and mechanoreception. *Neuroscience.* 2011; 193:387-398.
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- Fausther M, Sheung N, Saiman Y, Bansal MB, Dranoff JA. Activated hepatic stellate cells upregulate transcription of ecto-5'-nucleotidase/CD73 via specific SP1 and SMAD promoter elements. *Am J Physiol Gastrointest Liver Physiol.* 2012; 303(8):G904-914.
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- Zanin RF, Bergamin LS, Braganhol E, Sévigny J, de Souza Wyse AT, Battastini AM. Homocysteine modifies extracellular ATP availability in macrophages. *Toxicol In Vitro.* 2013; 27(8):2273-2278.
- Augusto E, Matos M, Sévigny J, El-Tayeb A, Bynoe MS, Muller CE, Cunha RA, Chen JF. Ecto-5'-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A2A receptor functions. *J Neurosci.* 2013; 33(28):11390-11399.

- Vieira C, Magalhaes-Cardoso MT, Ferreirinha F, Silva I, Dias AS, Pelletier J, Sévigny J, Correia-de-Sa P. Feed-forward inhibition of CD73 and upregulation of adenosine deaminase contribute to the loss of adenosine neuromodulation in postinflammatory ileitis. *Mediators Inflamm.* 2014; 254640.
- Rockenbach L, Braganhol E, Dietrich F, Figueiro F, Pugliese M, Edelweiss MI, Morrone FB, Sévigny J, Battastini AM. NTPDase3 and ecto-5'-nucleotidase/CD73 are differentially expressed during mouse bladder cancer progression. *Purinergic signalling.* 2014; 10(3):421-430.
- Gonzalez DA, Egado P, Balcarcel NB, Hattab C, Barbieri van Haaster MM, Pelletier J, Sévigny J, Ostuni MA. Rat submandibular glands secrete nanovesicles with NTPDase and 5'-nucleotidase activities. *Purinergic signalling.* 2015; 11(1):107-116.
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