Polyclonal guinea pig anti-mouse NTPDase1 (CD39)

Name: mN1-2c(I4,I5)

Applications

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>Dilution</th>
<th>No</th>
<th>Not tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western blot (non-reduced)§</td>
<td>+</td>
<td>1:6000</td>
<td></td>
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<tr>
<td>Western blot (reduced)</td>
<td></td>
<td></td>
<td>×</td>
<td></td>
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<tr>
<td>Immunohistochemistry*</td>
<td></td>
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<tr>
<td>Frozen section</td>
<td>+</td>
<td>1:2000</td>
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<tr>
<td>Paraffin</td>
<td>+</td>
<td>1:500</td>
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<tr>
<td>Flow cytometry</td>
<td>+</td>
<td>1:400</td>
<td></td>
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<tr>
<td>ELISA</td>
<td></td>
<td></td>
<td>×</td>
<td></td>
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<tr>
<td>Immunoprecipitation</td>
<td></td>
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§ Thiol-reactive reagents (e.g. β-mercaptoethanol, DTT) must be avoided as they destroy the epitope recognized by the antibody.

* This antibody work on cryosection and acetone fixation, and also in paraffin sections using proteinase K (20 µg/mL) as antigen retrieval technic but give a lower signal than on frozen section.

Cross-reactivity

In Western blot, mN1-2c(I4,I5) cross-reacts with rat NTPDase1 but not with human NTPDase1 (see below).

Western blot

A: Peritoneal macrophage lysate (12.5 µg) from wild-type (+/+ and knockout CD39 (-/-) mice.

B: Protein samples (6 µg) from a lysate from COS-7 cells (ctrl) or from COS-7 transiently transfected with a plasmid encoding for rat (r), human (h) or mouse (m) NTPDase1.

In both panels, proteins were loaded on a NuPAGE® Novex® Bis-Tris 4-12% gel under non-reducing conditions, transferred to an Immobilon-P membrane and incubated with mN1-2c.I8. A 78-kDa band is detected only in samples from cells expressing rat or mouse NTPDase1.
Immuno(cyto/histo)chemistry

A: Immunocytochemistry of untransfected COS-7 cells or transfected with a plasmid encoding mouse NTPDase1 both incubated with mN1-2cI5 or preimmune serum at the same dilution (1:2000). A strong signal is observed only with the antiserum in cells expressing mouse NTPDase1. No signal is detected in any of the control cells.

B: A mouse pancreas section incubated with mN1-2cI5 displays a positive reaction in blood vessels, on the apical surface of ascini, and in zymogen granules.

In both panels, nuclei are stained with hematoxylin (blue).

Flow cytometry

βTC3 cells transfected with mouse NTPDase1 cDNA vector using lipofectamine LTX (Invitrogen). Cells were incubated with mN1-2cI5 (1:400) or preimmune serum (Pi), followed by detection with a FITC-labeled secondary antibody. 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:

Few other references where these antibodies were used

1: Protocols and experimental conditions are available at www.ectonucleotidases-ab.com/Protocols.php.
2: This is the pre-peer reviewed version of the following article: Lévesque et al. 2010, NTPDase1 governs P2X7-dependent functions in murine macrophages. *Eur J Immunol*, 40(5):1473-1485, which has been published in final form at http://onlinelibrary.wiley.com.