

# Detection of Alpaca Podoplanin by Immunohistochemistry Using the Antibovine Podoplanin Monoclonal Antibody PMab-44

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Podoplanin (PDPN) is expressed in type I alveolar cells of the lungs, lymphatic endothelial cells, and podocytes of the kidneys, and induces platelet aggregation through the C-type lectin-like receptor-2. PDPNs of various animal species have been characterized using specific anti-PDPN monoclonal antibodies (mAbs). However, alpaca PDPN has not previously been characterized because antialpaca PDPN mAbs have not yet been developed. In this study, we investigated the potential cross-reaction between established antibovine PDPN mAbs and alpaca PDPN. Using immunohistochemical analysis, type I alveolar cells of the alpaca lungs were detected by the antibovine PDPN mAb, PMab-44. These results indicate that PMab-44 may be useful for the detection of alpaca PDPN.

**Keywords:** alpaca podoplanin, alpaca PDPN, PMab-44

## Introduction

**P**ODOPLANIN (PDPN) STUDIED in a variety of animal species is a type I transmembrane sialoglycoprotein.<sup>(1)</sup> Although PDPN lacks any recognizable catalytic activity, its N-terminus extracellular domain and C-terminus intracellular domain play important roles in cellular physiology through protein-protein interactions. The small cytoplasmic C-terminus (nine amino acids) interacts with the members of the ERM (ezrin, radixin, and moesin) protein family,<sup>(2-4)</sup> impacting the cytoskeleton dynamics and formation or stabilization of membrane structures, such as filopodia, invadopodia, or ruffles. The extracellular domain of PDPN is extensively *O*-glycosylated, enabling interactions with a variety of proteins, including the C-type lectin-like receptor-2 (CLEC-2),<sup>(5)</sup> CD44,<sup>(3)</sup> CD9,<sup>(6)</sup> galectin8,<sup>(7)</sup> CCL21,<sup>(8)</sup> or HspA9.<sup>(9)</sup> Importantly, three platelet aggregation-stimulating (PLAG) domains termed PLAG1, PLAG2, and PLAG3 (EDxxVTPG sequence) of the N-terminus induce platelet aggregation through CLEC-2 of the platelets.<sup>(10)</sup> We have previously shown that PLAG3 is the most important domain for platelet aggregation by human PDPN.<sup>(10-12)</sup>

Anti-PDPN monoclonal antibodies (mAbs) are useful in distinguishing type I from type II alveolar cells of the lungs, since PDPN serves as a specific marker for type I alveolar

cells.<sup>(11,13)</sup> We characterized the PDPNs from a variety of animal species using specific anti-PDPN mAbs and established antimouse (PMab-1),<sup>(14)</sup> antirat (PMab-2),<sup>(15)</sup> anti-rabbit (PMab-32),<sup>(16)</sup> antidog (PMab-38<sup>(17)</sup> and PMab-48<sup>(18)</sup>), antibovine (PMab-44),<sup>(19)</sup> and anticat (PMab-52) PDPN mAbs.<sup>(20)</sup>

Antialpaca PDPN mAbs have not previously been reported. In this study, we investigated the potential cross-reaction between the alpaca PDPN and our set of anti-PDPN mAbs established against diverse species using immunohistochemical analyses.

## Materials and Methods

### *Immunohistochemical analyses*

Normal alpaca lungs were collected after autopsy at Hokkaido University, fixed in 10% neutral-buffered formalin, and processed routinely to make paraffin-embedded tissue sections. Histological sections of thickness 4 μm were directly autoclaved in EnVision FLEX Target Retrieval Solution, high pH (Agilent Technologies, Inc., Santa Clara, CA), for 20 minutes. After blocking with SuperBlock T20 (PBS) Blocking Buffer (Thermo Fisher Scientific, Inc. Waltham, MA), sections were incubated with PMab-44 (10 μg/mL) for 1 hour at room temperature and treated using

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Envision+ Kit (Agilent Technologies, Inc.) for 30 minutes. Then color was developed using 3,3'-diaminobenzidine tetrahydrochloride (Agilent Technologies, Inc.) for 2 minutes, and counterstaining was performed with hematoxylin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

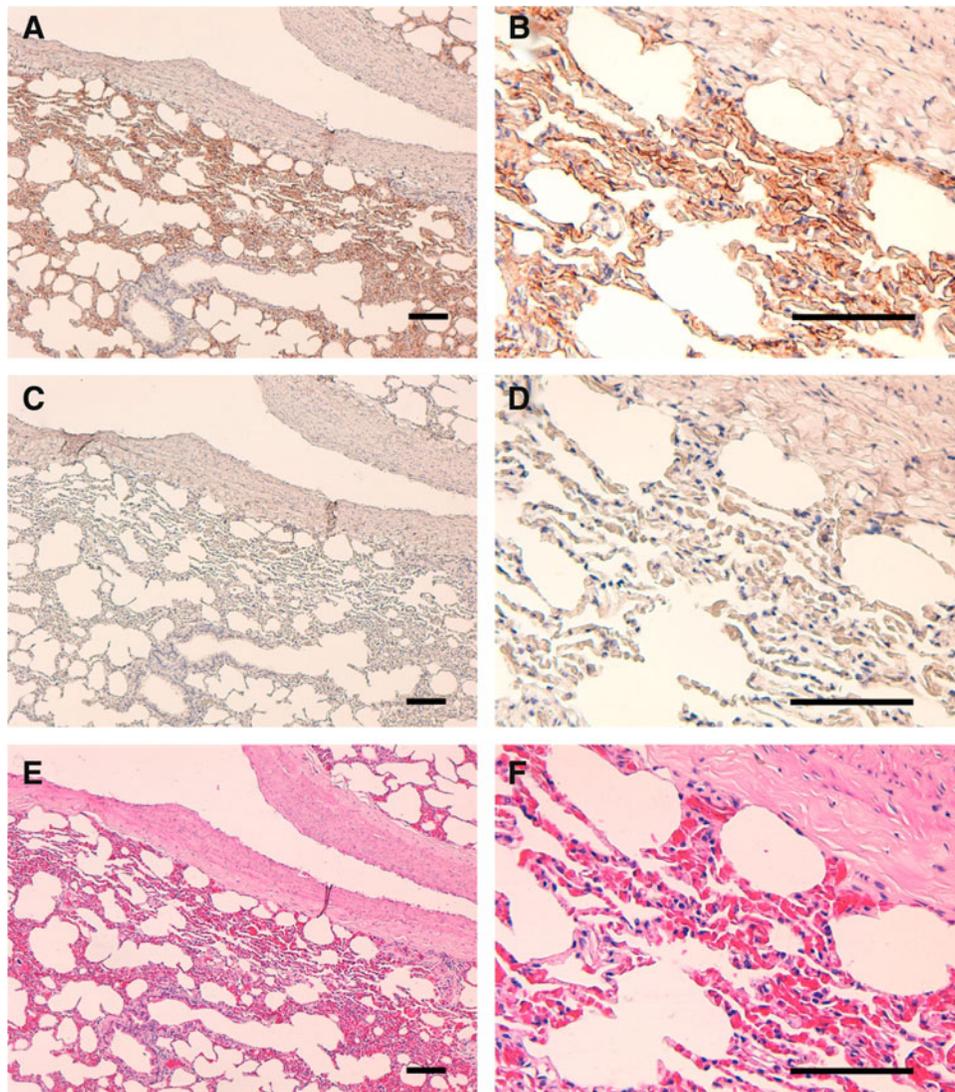
## Results and Discussion

In our previous work, we developed a mouse anti-bovine PDPN mAb, PMab-44 (IgG<sub>1</sub>, kappa), which specifically detects bovine PDPN in immunohistochemistry.<sup>(19)</sup> The PLAG3 of bovine PDPN was identified as a critical epitope of PMab-44<sup>(21)</sup> (Supplementary Fig. S1). The comparison of amino acid sequences revealed 85% homology between the bovine PDPN and alpaca PDPN. Furthermore, the alpaca PDPN possesses PMab-44 epitope (VEDYTT). Therefore, in this study, we investigated the cross-reaction between PMab-44 and alpaca PDPN.

In flow cytometry studies, PMab-44 reacted with alpaca PDPN-overexpressed CHO-K1 (CHO/aPDPN) cells (Supplementary Fig. S2). No reaction with CHO/aPDPN was observed for the other anti-PDPN mAbs, such as PMab-1,<sup>(14)</sup> PMab-2,<sup>(15)</sup> PMab-32,<sup>(16)</sup> PMab-38<sup>(17)</sup> and PMab-48,<sup>(18)</sup> and PMab-52<sup>(20)</sup> (data not shown). These results indicate that only PMab-44, among the anti-PDPN mAbs, is useful for experimental detection of alpaca PDPN.

We investigated the expression of alpaca PDPN in several alpaca tissues. Previously, it has been demonstrated that PMab-44 reacts with podocytes of bovine kidney<sup>(19)</sup> (Supplementary Fig. S3). As depicted in Figure 1, a membrane staining pattern was observed, with strong staining of type I alveolar cells of the alpaca lungs by PMab-44. In contrast, alpaca kidney and colon cells were not stained by PMab-44 in this study (data not shown). This may be attributed to the varying expression levels of alpaca PDPN in different tissues.

In conclusion, anti-bovine PDPN (PMab-44) is useful for the detection of alpaca PDPN using immunohistochemical



**FIG. 1.** Immunohistochemical analyses using alpaca tissues. Histological sections of the alpaca tissues were directly autoclaved in EnVision FLEX Target Retrieval Solution, high pH, for 20 minutes. After blocking, the sections were incubated with 10  $\mu$ g/mL of PMab-44 (A, B) or with blocking buffer (C, D), followed by detection using Envision+ Kit. (E, F) Hematoxylin and eosin staining. Scale bar = 100  $\mu$ m.

analyses. Further studies are necessary to explore whether PMAb-44 is able to detect alpaca PDPN in tissues other than type 1 alveolar cells of the lungs.

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### Author Disclosure Statement

No competing financial interests exist.

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