ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: Podoplanin (PDPN) (Uniprot# Q86YL7)

Method tested: Western Blotting

Laboratory ID: LAB07

Project ID: AR119

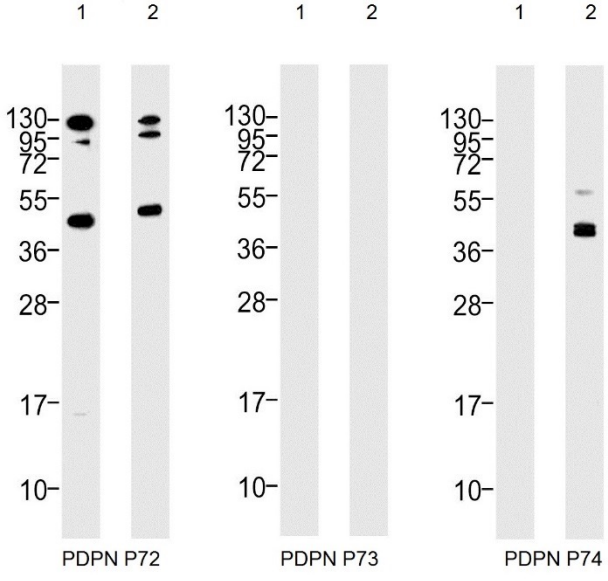
BACKGROUND

With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical protocol. In doing so, we hope to enable scientists to form an unrivalled opinion of which is the most suitable antibody for their research and in particular, which is going to require the least amount of optimisation, a process which can often take weeks or months.

For the purposes of the antibody comparison initiative, we select the best antibodies from each manufacturer and then compare them side-by-side using the same experimental conditions to provide a direct comparison. The antibodies are collected centrally, repackaged and given an internal reference ID prior to delivery to independent laboratories to ensure objective testing and to minimise bias.

Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS

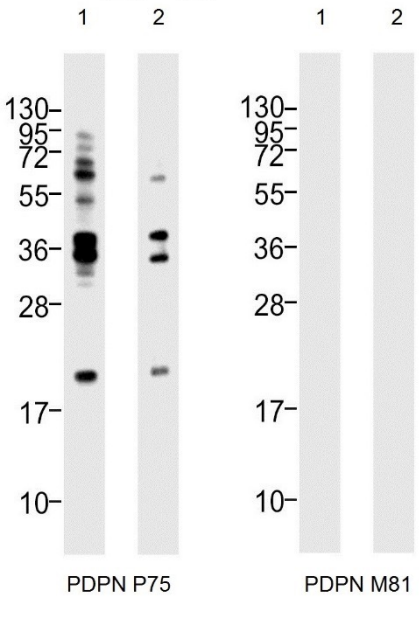


Western blot analysis of:-

(1) 293T/17 whole cell lysate

(2) HeLa whole cell lysate

using various anti-PDPN antibodies (see Method for primary and secondary antibody details).



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METHOD

Antibodies

Primary antibody	Secondary antibody
PDPN P72 at 1/100 (Atlas)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
PDPN P73 at 1/1000 (Supplier 14)	Peroxidase AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 805-035-180) at 1/10,000
PDPN P74 at 1/1000 (Everest)	Peroxidase AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 805-035-180) at 1/10,000
PDPN P75 at 1/1000 (Boster)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
PDPN M81 at 1/100 (Supplier 06)	Anti-Mouse IgG (Fc specific)–Peroxidase antibody produced in goat (Sigma, Cat no. A0168) at 1/10,000

Samples

Sample	Description
MW markers (Thermo Fisher Scientific, Cat no. 26619)	MW markers at 10, 17, 28, 36, 55, 72, 95, 130 and 250kDa.
293T/17 (Human epithelial cells from embryonic kidney) whole cell lysate at 20 µg/lane	Lane 1 - Test
HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 20 µg/lane	Lane 2 - Test

Detection Kit

Clarity™ Western ECL Blotting Substrate (Bio-rad, Cat no: 170-5061, Lot number: 102030607).

PROTOCOL

Western Blotting was performed using Invitrogen's Novex® XCell SureLock® Mini-Cell electrophoresis system followed by semi dry transfer onto PVDF membranes using Bio-Rad's Trans-Blot® SD Semi-Dry Transfer Cell and visualized using X-ray film as follows:-

1. Samples (see table above) were incubated with 1X SDS Sample Buffer containing 2% SDS and 100mM DTT at 95^{o}C for 5 minutes prior to loading.
2. The samples were then loaded and resolved on a 15% SDS-polyacrylamide gel (see table above for amount protein per lane).
3. Proteins were transferred onto PVDF membrane by semi dry transfer and confirmed by amido black staining.
4. The immunoblot membrane was blocked in Tris buffered saline (TBS) containing Tween-20 (TBST) and 5% non-fat dry milk powder (blocking buffer) for 2 hours at room temperature with gentle agitation and then washed for 5 minutes in TBST.
5. The membrane was then immersed with the protein side up in the primary antibody solution (for details see table above) diluted in blocking buffer overnight at 4^{o}C with gentle agitation.
6. Following two washes for 5 minutes each and one wash for 10 minutes at room temperature with TBST, the membrane was incubated in the secondary antibody (for details see table above) diluted in blocking buffer for 1 hour at room temperature with gentle agitation.
7. The membrane was then washed three times for 5 minutes and then 8 minutes with TBST at room temperature.
8. After draining away excess TBST, signals were detected with the detection kit detailed above, the blots exposed on X-ray film and the final images obtained using PS software.

EXPERIMENTAL NOTES

Under these experimental conditions, PDPN P72 exhibited immunoreactivity in both Human cell lysates with bands at around 45kDa, 90KDa and 120kDa. PDPN P74 also demonstrated immunoreactivity in the HeLa whole cell lysate at around 45kDa but this was not seen using the 293T/17 cell lysate. PDPN P75 shows immunoreactive bands at around 20kDa, in the range 30-50kDa and, to a lesser extent, within the range 55-90kDa in both lysates. PDPN M81 and PDPN P73 did not exhibit immunoreactivity in any of the tested lysates.

SUPERSTARTER ANTIBODY PANELS

A panel of Superstar antibodies in trial sizes, to enable you to economically test the best antibodies, to determine which is going to be the best for your research project for only $400, €358, £265.

The Podoplanin Superstarter Antibody Panel consists of:
-1x Abcam ab11936 (star performer)
-1x AbD Serotec (Bio-Rad) MCA2543 (high reviews)
-1x R&D Systems AF3244 (high reviews)
http://www.antibodyresource.com/superstars

Images of Superstar Podoplanin antibodies: