ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: Cyclin D1 (Uniprot# P24385)

Method tested: Western Blotting

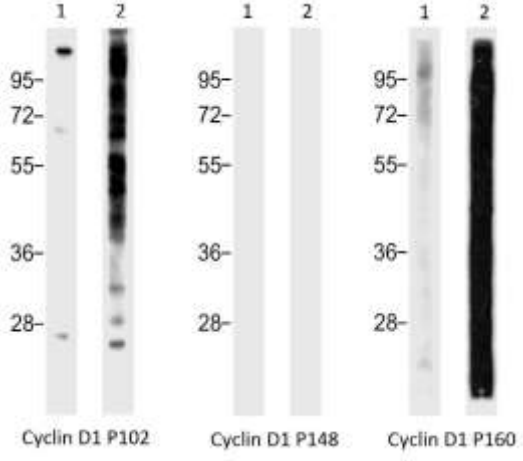
Laboratory ID: LAB07

Project ID: AR141

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.

RESULTS

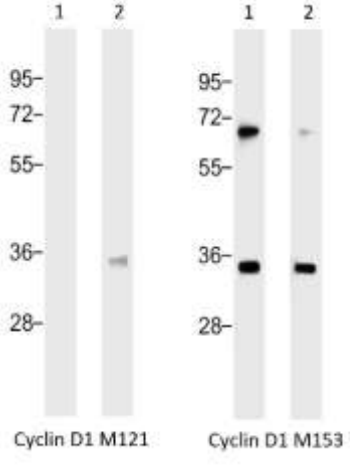


Western blot analysis of:-

(1) A431 whole cell lysate

(2) HepG2 whole cell lysate

using various anti-Cyclin D1 antibodies (see Method for primary and secondary antibody details).



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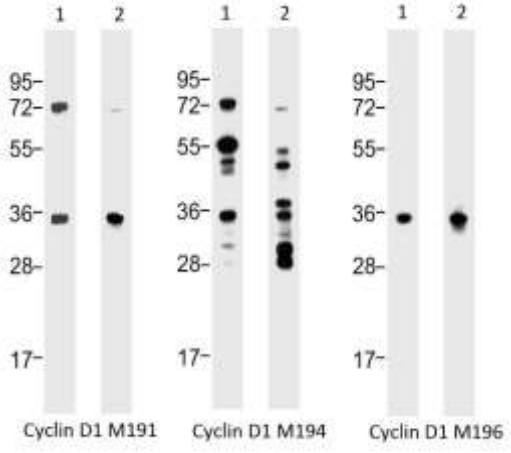

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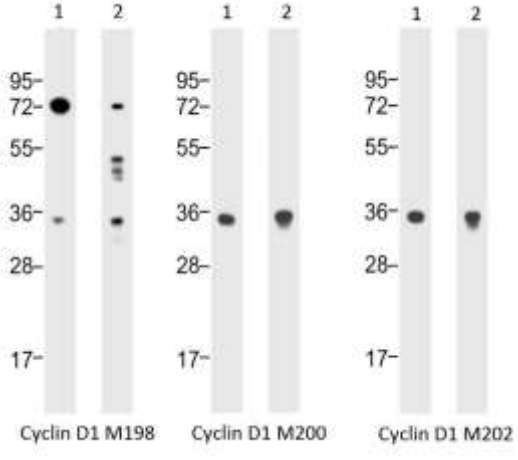

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METHOD

Antibodies

	Primary antibody	Secondary antibody
	Cyclin D1 P102 at 1/100 (Supplier 22)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/10,000
	Cyclin D1 P148 at 1/1000 (Supplier 31)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/10,000
	Cyclin D1 P160 at 1/1000 (Supplier 34)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/10,000
	Cyclin D1 M121 at 1/1000 (Cell Signalling)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/10,000
	Cyclin D1 M153 at 1/100 (Novus)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/10,000
	Cyclin D1 M191 at 1/1000 (Invitrogen)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000
	Cyclin D1 M194 at 1/100 (Supplier 19)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000
	Cyclin D1 M196 at 1/25 (Invitrogen)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5,000
	Cyclin D1 M198 at 1/100 (Supplier 19)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000
	Cyclin D1 M200 at 1/100 (Invitrogen)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5,000
	Cyclin D1 M202 at 1/25 (Invitrogen)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5,000

 = Component of the Cyclin D1 Superstarter Antibody Panel. See end of report for details.

Samples

Sample	Description
MW markers (Thermo Fisher Scientific, Cat no. 26619)	MW markers at 10, 17, 28, 36, 55, 72, 95, 130 and 250kDa.
A431 (Human epidermoid carcinoma cell line) whole cell lysate at 20 µg/lane	Lane 1 - Test
HepG2 (Human liver hepatocellular carcinoma cell line) 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: 102030671 and 102030896).

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 12% 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 diluted in TBST containing 3% non-fat dry milk powder (dilution buffer) overnight at 4^{o}C with gentle agitation. Each antibody was diluted according to the working range suggested by the supplier (for details see table above).
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 dilution buffer for 1 hour at room temperature with gentle agitation.

7. The membrane was then washed three times for 5 minutes and then one wash of 10 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, Cyclin D1 M196, Cyclin D1 M200 and Cyclin D1 M202 exhibited immunoreactivity in both Human cell lysates with a band around the expected MW of 34kDa. Cyclin D1 M153 and Cyclin D1 M191 also showed this band with a higher MW band at around 72kDa. Additional bands were seen in the blots of Cyclin D1 M194 and Cyclin D1 M198. Cyclin D1 M121 demonstrated a faint band at the expected MW in the HepG2 cell line lysate suggesting immunoreactivity. This could be confirmed using a higher concentration of the primary antibody. No immunoreactivity was observed using Cyclin D1 P148, again this could be re-investigated using a higher concentration of primary antibody. Using this method, neither Cyclin D1 P102 or Cyclin D1 P160 demonstrated specific bands at the expected MW.

NB Cyclin D1 P102 through to Cyclin D1 M153 were tested on a different occasion to Cyclin D1 M191 through to Cyclin D1 M202. The consistency of testing is shown by the results of Cyclin D1 M153 and Cyclin D1 M191 as these antibodies were obtained from different suppliers but were derived from the same clone. Cyclin D1 M194 was also derived from this clone but was in ascites form.

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 $279, €254, £188.

The Cyclin D1 Superstarter Antibody Panel consists of:

1 x	2978	(Cell Signaling Technology)
1 x	sc-8396	(Santa Cruz Biotechnology)
1 x	NBP2-24695	(Novus Biologicals)

http://www.antibodyresource.com/superstars

Images of Superstar Cyclin D1 antibodies: