ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: NALP3 (Uniprot# Q96P20)

Method tested: Western Blotting

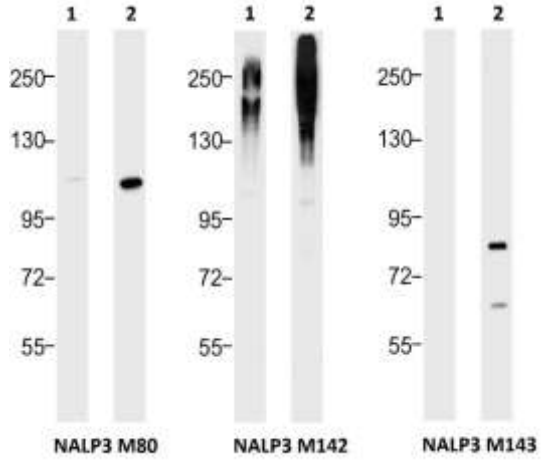
Laboratory ID: LAB07

Project ID: AR145

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

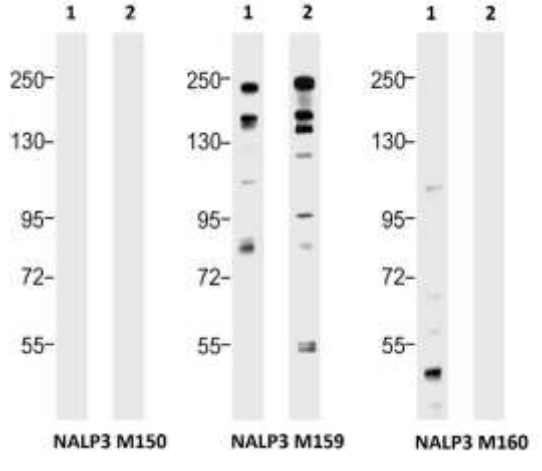


Western blot analysis of:-

(1) THP1 whole cell lysate

(2) Jurkat whole cell lysate

using various anti-NALP3 antibodies (see Method for primary and secondary antibody details).



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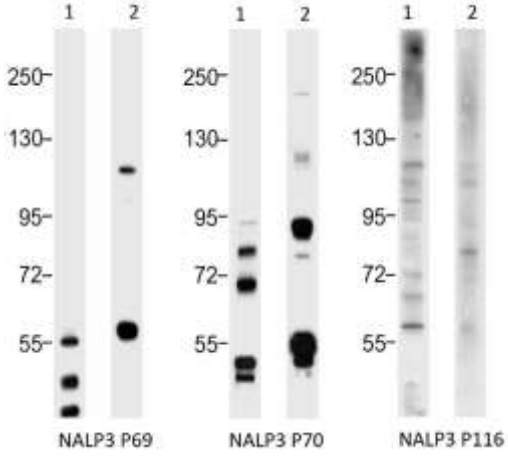

Western blot analysis of:-
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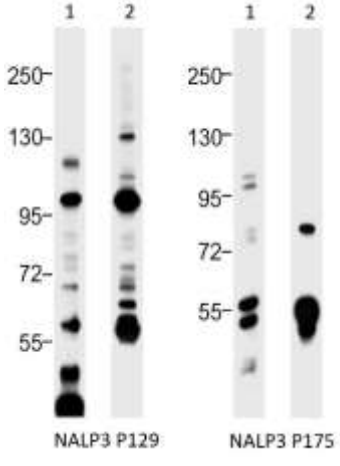

Western blot analysis of:-
(1) THP1 whole cell lysate
(2) Jurkat whole cell lysate
using various anti-NALP3 antibodies (see Method for primary and secondary antibody details).

METHOD

Antibodies

	Primary antibody	Secondary antibody
	NALP3 M80 at 1/1000 (Atlas)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/10,000
	NALP3 M142 at 1/100 (Supplier 22)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/10,000
	NALP3 M143 at 1/100 (Supplier 22)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/10,000
	NALP3 M150 at 1/1000 (Supplier 35)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/10,000
	NALP3 M159 at 1/1000 (Supplier 31)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/10,000
	NALP3 M160 at 1/1000 (Supplier 36)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/10,000
	NALP3 P69 at 1/1000 (ProSci)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/10,000
	NALP3 P70 at 1/1000 (Supplier 11)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/10,000
	NALP3 P116 at 1/1000 (Supplier 22)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/10,000

	NALP3 P129 at 1/1000 (Supplier 24)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/10,000
	NALP3 P175 at 1/1000 (Supplier 11)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at *1/5,000

Samples

Sample	Description
MW markers (Thermo Fisher Scientific, Cat no. 26619)	MW markers at 10, 17, 28, 36, 55, 72, 95, 130 and 250kDa.
THP1 (Human monocytic leukemia cell line) whole cell lysate at 20 µg/lane	Lane 1 - Test
Jurkat (Human T cell leukemia 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 wet transfer onto PVDF membranes using Bio-Rad's Trans-Blot® 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 an 8% SDS-polyacrylamide gel (see table above for amount protein per lane).
3. Proteins were transferred onto PVDF membrane by wet 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, NALP3 M80 exhibited immunoreactivity in both the Human cell lysates with bands around the expected MW of 118kDa. NALP3 P69 also showed a band at the expected MW in the Jurkat cell lysate along with some lower MW bands. NALP3 M150 did not appear to demonstrate immunoreactivity and NALP3 M160 only showed at very faint band on one of the blots at the expected MW. Both antibodies may require the primary and/or secondary antibody dilutions to be adjusted for immunoreactivity to be observed. Although, NALP3 M159, NALP3 P70 and NALP3 P129 show bands in the region of the expected MW, they also exhibit several bands at other MWs whilst NALP3 M142, NALP3 M143, NALP3 P116 and NALP3 P175 did not show the expected banding pattern using this method.

NB. Using the protocol described, NALP3 P175 was tested on a different occasion to the other antibodies with the changes in reagents lots or dilutions indicated by*