ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: TLR4 (Uniprot# O00206)

Method tested: Western Blotting

Laboratory ID: LAB07

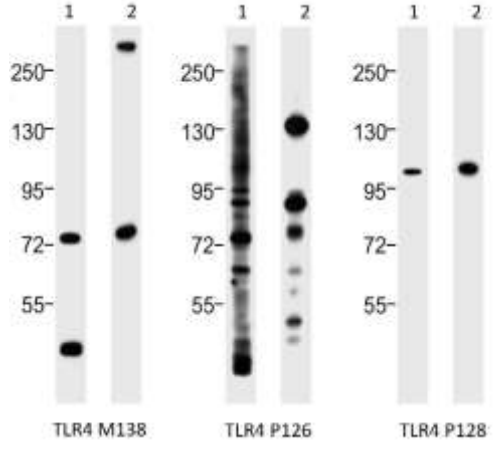
Project ID: AR158

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

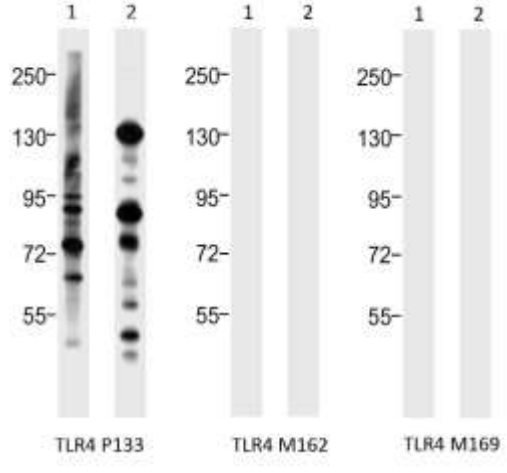


Western blot analysis of:-

(1) HL-60 whole cell lysate

(2) THP-1 whole cell lysate

using various anti-TLR4 antibodies (see Method for primary and secondary antibody details).



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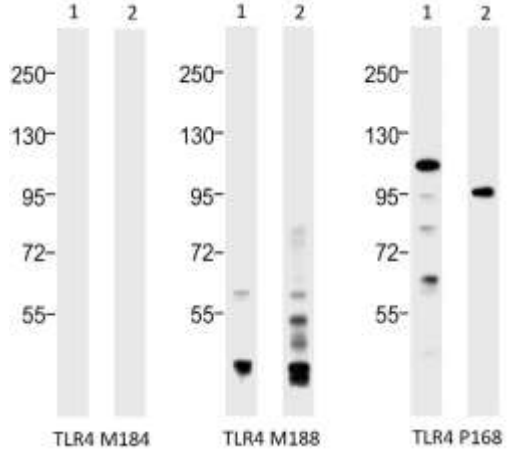

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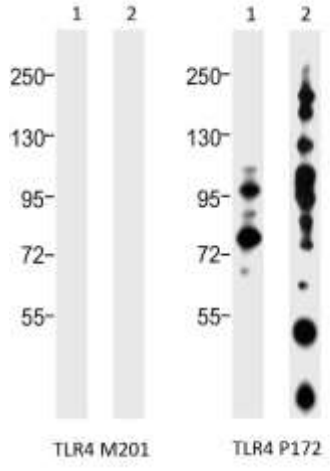

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METHOD

Antibodies

	Primary antibody	Secondary antibody
	TLR4 M138 at 1/500 (Supplier 22)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000
	TLR4 P126 at 1/500 (Supplier 01)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5,000
	TLR4 P128 at 1/500 (Santa Cruz)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5,000
	TLR4 P133 at 1/500 (Supplier 42)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5,000
	TLR4 M162 at 1/500 (Supplier 41)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000
	TLR4 M169 at 1/500 (Supplier 37)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000
	TLR4 M184 at 1/500 (Supplier 19)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000

	TLR4 M188 at 1/500 (Supplier 19)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000
	TLR4 P168 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
	TLR4 M201 at 1/500 (Supplier 19)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5,000
	TLR4 P172 at 1/100 (Supplier 19)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5,000

= Component of the TLR4 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.
HL-60 (Human promyelocytic leukaemia cells) whole cell lysate at 20 µg/lane	Lane 1 - Test
THP-1 (Human monocytic leukaemia cells) 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: 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 95ºC for 5 minutes prior to loading.
2. The samples were then loaded and resolved on a 8% 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 4ºC 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, TLR4 P128 exhibited immunoreactivity in both Human cell lysates with bands around expected MW. TLR4 P168 also demonstrated bands in this region but the banding pattern was different for each cell lysate. In addition to these bands, several non-specific bands were observed on the blots of TLR4 P126, TLR4 P133 and TLR4 P172. Although distinct bands were seen on the blot of TLR4 M138 and TLR4 M188, they did not appear to demonstrate bands at the expected MW at the concentrations tested whilst no bands were seen for TLR M162, TLR4 M169, TLR4 M184 or TLR4 M201. The latter four antibodies were derived from the same clone. These may require the primary and/or secondary antibody dilutions to be adjusted for immunoreactivity to be observed.

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 $307, €267, £198.

The TLR4 Superstarter Antibody Panel consists of:

1 x NB100-56566 (Novus Biologicals)

1 x sc-10741 (Santa Cruz Biotechnology)

http://www.antibodyresource.com/superstars

Images of Superstar TLR4 antibodies: