

ANTIBODY TESTING REPORT

SUMMARY

Antigen: HA Tag

Method tested: Western Blotting

Laboratory ID: LAB07

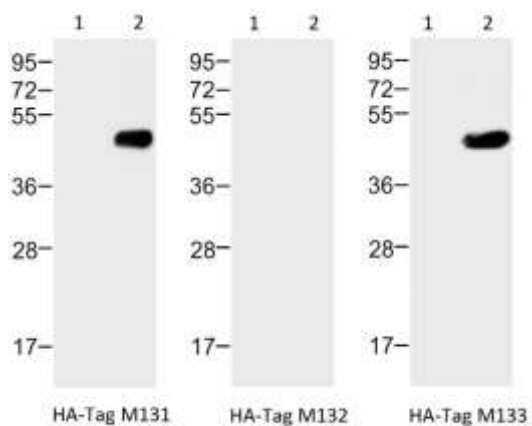
Project ID: AR153

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.

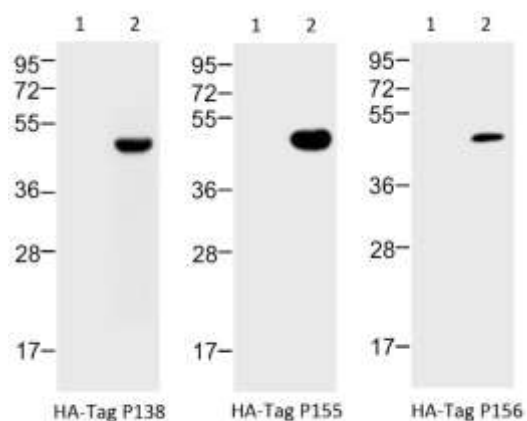
Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS



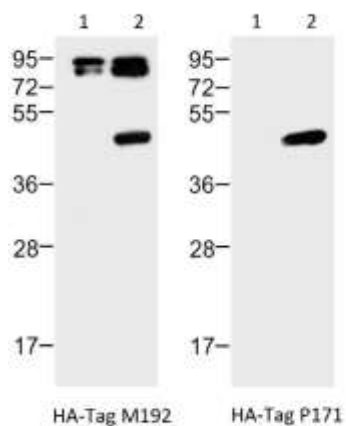
Western blot analysis of:-

- (1) 293T/17 whole cell lysate
 - (2) HA-Tag transfected 293T/17 whole cell lysate
- using various anti-HA-Tag antibodies (see Method for primary and secondary antibody details).



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METHOD

Antibodies

	Primary antibody	Secondary antibody
	HA-Tag M131 at 1/5000 (Cell Signalling)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5000
	HA-Tag M132 at 1/5000 (Supplier 27)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5000
	HA-Tag M133 at 1/5000 (Sigma)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5000
	HA-Tag P138 at 1/5000 (Sicgen)	Peroxidase AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch 805-035-180) at 1/20000
	HA-Tag P155 at 1/5000 (Novus)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5000
	HA-Tag P156 at 1/5000 (Rockland)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5000
	HA-Tag M192 at 1/5000 (Supplier 19)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5000
	HA-Tag P171 at 1/5000 (Invitrogen)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5000



= Component of the HA Tag 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.
293T/17 (Human epithelial cells from embryonic kidney) whole cell lysate at 20 µg/lane	Lane 1 - Test
293T/17-HA-Tag (Human epithelial cells from embryonic transfected with HA-Tag) 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 95°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°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, the majority of the antibodies tested (HA-Tag M131, HA-Tag M133, HA-Tag P138, HA-Tag P155, HA-Tag P156 and HA-Tag P171) exhibited bands at approximately 40kDa, consistent with the expected MW of the HA-Tag, in the HA-Tag transfected 293T/17 cells but not in the non-transfected cells. HA-Tag M192 also demonstrated this band along with higher MW bands in both the transfected and non-transfected cell lysates. No bands were seen for HA-Tag M132, this 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 \$269, €250, £185.

The HA Tag Superstarter Antibody Panel consists of:

- 1 x [H9658](#) (Sigma-Aldrich)
- 1 x [sc-7392](#) (Santa Cruz Biotechnology)
- 1 x [3724](#) (Cell Signaling Technology)

<http://www.antibodyresource.com/superstars>

Images of Superstar HA Tag antibodies:

