ANTIBODY TESTING REPORT

SUMMARY

Antigen: Histone H2A.X (Phospho, Ser139)

Method tested: Western Blotting

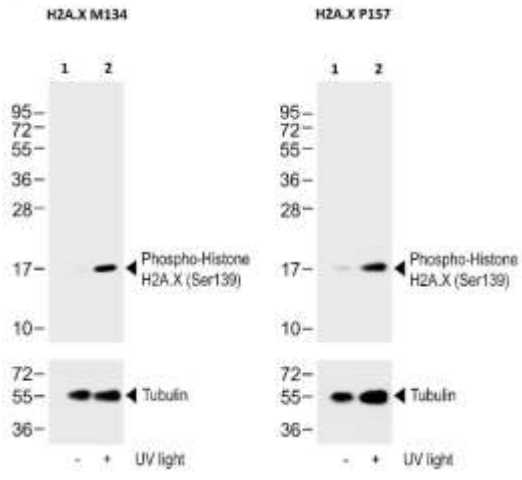
Laboratory ID: LAB07

Project ID: AR156

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

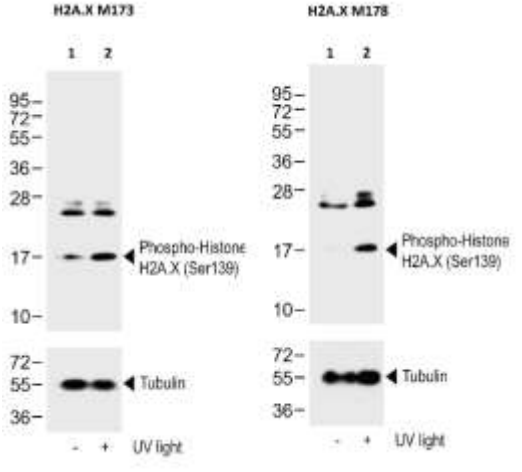


Western blot analysis of:-

(1) HeLa whole cell lysate

(2) UV light treated HeLa whole cell lysate

using various anti-H2A.X (phospho, Ser139) antibodies (see Method for primary and secondary antibody details).



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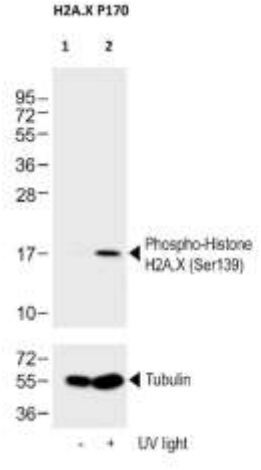

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METHOD

Antibodies

	Primary antibody	Secondary antibody	Loading antibody control
	H2A.X M134 at 1/1000 (Cell Signalling)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5000	Mouse anti-TBB5 antibody (Abgent, Cat no. AM1031a) at 1/10000
	H2A.X P157 at 1/5000 (Novus)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5000	Mouse anti-TBB5 antibody (Abgent, Cat no. AM1031a) at 1/10000
	H2A.X M173 at 1/1000 (Supplier 37)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5000	Mouse anti-TBB5 antibody (Abgent, Cat no. AM1031a) at 1/10000
	H2A.X M178 at 1/1000 (Supplier 22)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31446) at 1/5000	Mouse anti-TBB5 antibody (Abgent, Cat no. AM1031a) at 1/10000
	H2A.X P170 at 1/5000 (Invitrogen)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (ThermoFisher Scientific, Cat no. 31462) at 1/5000	Mouse anti-TBB5 antibody (Abgent, Cat no. AM1031a) at 1/10000

= Component of the Histone H2A.X 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.
HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 20 µg/lane	Lane 1 - Test
HeLa (Human epithelial cells from cervix adenocarcinoma), treated by UV light for 2 hours, 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^{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 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, H2A.X M134, H2A.X P157 and H2A.X P170 exhibited a band at approximately 15kDa in the UV treated cells only demonstrating specificity for phospho Histone H2A.X and consistent with the expected MW. H2A.X M173 and H2A.X M178 also demonstrate this band along with higher MW bands within both the non-treated and UV treated cells.

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 $361, €335, £248.

The Histone H2A.X Superstarter Antibody Panel consists of:

-1x Novus Biologicals NBP2-27427 (high ratings)
–1x Cell Signalling 9718 (high ratings)
-1x Millipore 05-636 (high ratings)
http://www.antibodyresource.com/superstars

Images of Superstar Histone H2A.X antibodies: