ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: BAX (Uniprot# Q07812)

Method tested: Western Blotting

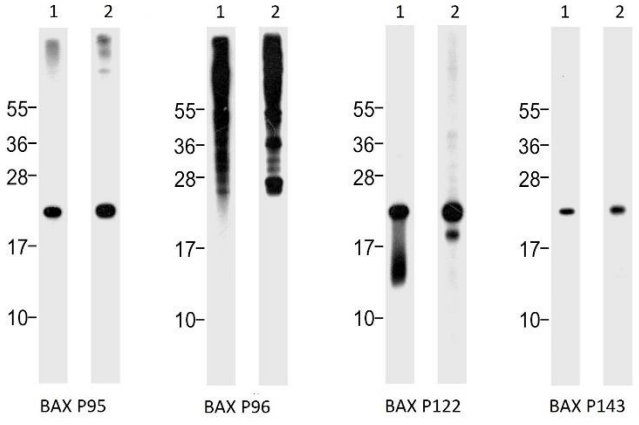
Laboratory ID: LAB07

Project ID: AR139

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

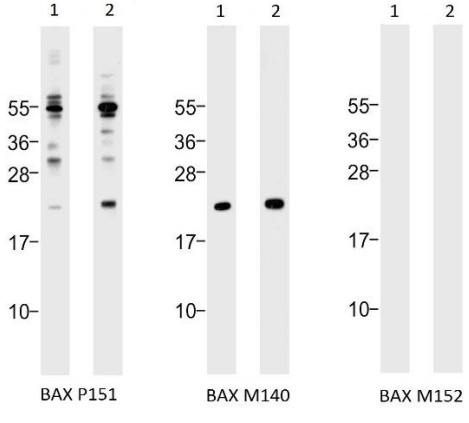


Western blot analysis of:-

(1) A549 whole cell lysate

(2) Daudi whole cell lysate

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



Western blot analysis of:-

(1) A549 whole cell lysate

(2) Daudi whole cell lysate

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

METHOD

Antibodies

Primary antibody	Secondary antibody
BAX P95 at 1/1000 (Cell Signalling)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
BAX P96 at 1/1000 (Supplier 22)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
BAX P122 at 1/1000 (Sicgen)	Peroxidase AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 805-035-180) at 1/10,000
BAX P143 at 1/2000 (USBio)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
BAX P151 at 1/100 (Supplier 32)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
BAX M140 at 1/1000 (Cell Signalling)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
BAX M152 at 1/100 (Supplier 22)	Goat anti-Mouse IgG, IgM (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31446) at 1/10,000

Samples

Sample	Description
MW markers (Thermo Fisher Scientific, Cat no. 26619)	MW markers at 10, 17, 28, 36, 55, 72, 95, 130 and 250kDa.
A549 (Human epithelial cells from lung carcinoma) whole cell lysate at 20 µg/lane	Lane 1 - Test
Daudi (Human Burkitt's lymphoma 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).

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, BAX P95, BAX P122, BAX P143, BAX 151 and BAX M140 exhibited immunoreactivity in both Human cell lysates with bands around expected MW of 21kDa. Although the 'smear' of the BAX P122 blots suggests that the antibody could be used at a lower concentration. In addition to these bands, higher MW non-specific bands were observed on the blot of BAX P151. BAX P96 and BAX M152 did not appear to demonstrate bands at the expected MW at the concentrations tested. Both may require the primary and/or secondary antibody dilutions to be adjusted for immunoreactivity to be observed, BAX P96 less concentrated and BAX M152 more concentrated.