Validation Report #029802

Validation Date: 08/26/14

Summary

Antigen	BCL2-Associated Agonist of Cell Death (BAD)
Catalog number	ABIN674709
Supplier	Bioss
Supplier catalog number	bs-1304R
Lot number	090915
Method validated	Western Blot
Laboratory	Alamo Laboratories Inc
Validation number	29802
Positive Control	MCF7 cells
Negative Control	C6/36 cells (non-reactive species)
Notes	A strong band was observed in the positive control sample at the correct molecular weight. No bands were observed in the negative control sample.

Full Methods

Primary Antibody
- Antigen: BCL2-Associated Agonist of Cell Death (BAD)
- Catalog number: ABIN674709
- Supplier: Bioss
- Supplier catalog number: bs-1304R
- Lot number: 090915
- Antibody Dilution: 1:200

Loading Control Antibody
- Antigen: Mouse Anti-Actin
- Supplier: BD Transduction Laboratories
- Catalog number: 612657
- Antibody Dilution: 1:6,000

Secondary Antibody
- Antigen: Goat Anti-Rabbit IgG (H + L)-HRP Conjugate
- Supplier: Bio-Rad
- Catalog number: #170-6515
- Lot number: L170-6515
- Antibody Dilution: 1:10,000

Controls
- Positive control: MCF7 cells
- Negative control: C6/36 cells

Protocol

1. The cell extracts were heated at 95°C for 5 minutes in 1X SDS Sample Buffer containing 1% SDS and 1.25% β-mercaptoethanol.
2. 15 µl of heated extracts were loaded and resolved on 8-16% SDS-polyacrylamide gel.
3. The Thermo Scientific - Spectra Multicolor Broad Range (Cat # 26634) were used as molecular mass markers.
4. Proteins were then transferred onto PVDF membrane by wet transfer and protein transfer was confirmed with Ponceau-S staining.
5. The PVDF membrane was incubated with 25 ml of blocking buffer [Tris Buffered Saline, pH 7.4 plus 0.1% TW20 (TBST)] containing 5% (W/V) BSA at room temperature for 1 hour.
6. The membrane was rinsed with TBST once.
7. The membrane was immersed with the protein side up in the primary antibody solution in TBST containing 5% (W/V) BSA and incubated for 16 hours at 4°C.
8. The membrane was rinsed in TBST thrice for 5 minutes each.
9. The membrane was incubated in the HRP-conjugated secondary antibody solution in TBST containing 5% (W/V) BSA and incubated for 1 hour at room temperature (~26°C) with gentle agitation.
10. The membrane was rinsed thrice TBST thrice for 5 minutes each.
11. The membrane was rinsed in TBS twice for 30 seconds each.
12. Signals were detected with ECL-2 Substrate. The blot was scanned for 600 seconds.
13. The membrane was rinsed three times TBST.
14. Incubated in Acidic Glycine Stripping Buffer at room temperature with gentle agitation for 3 times, 10 minutes each.
15. The membrane was washed in TBST 2 times for 10 minutes each.
16. Repeated Steps 5-12 with the loading control antibody (for Anti-actin) and its matching secondary antibody.

Experimental Notes
- No experimental challenges noted.

Figures

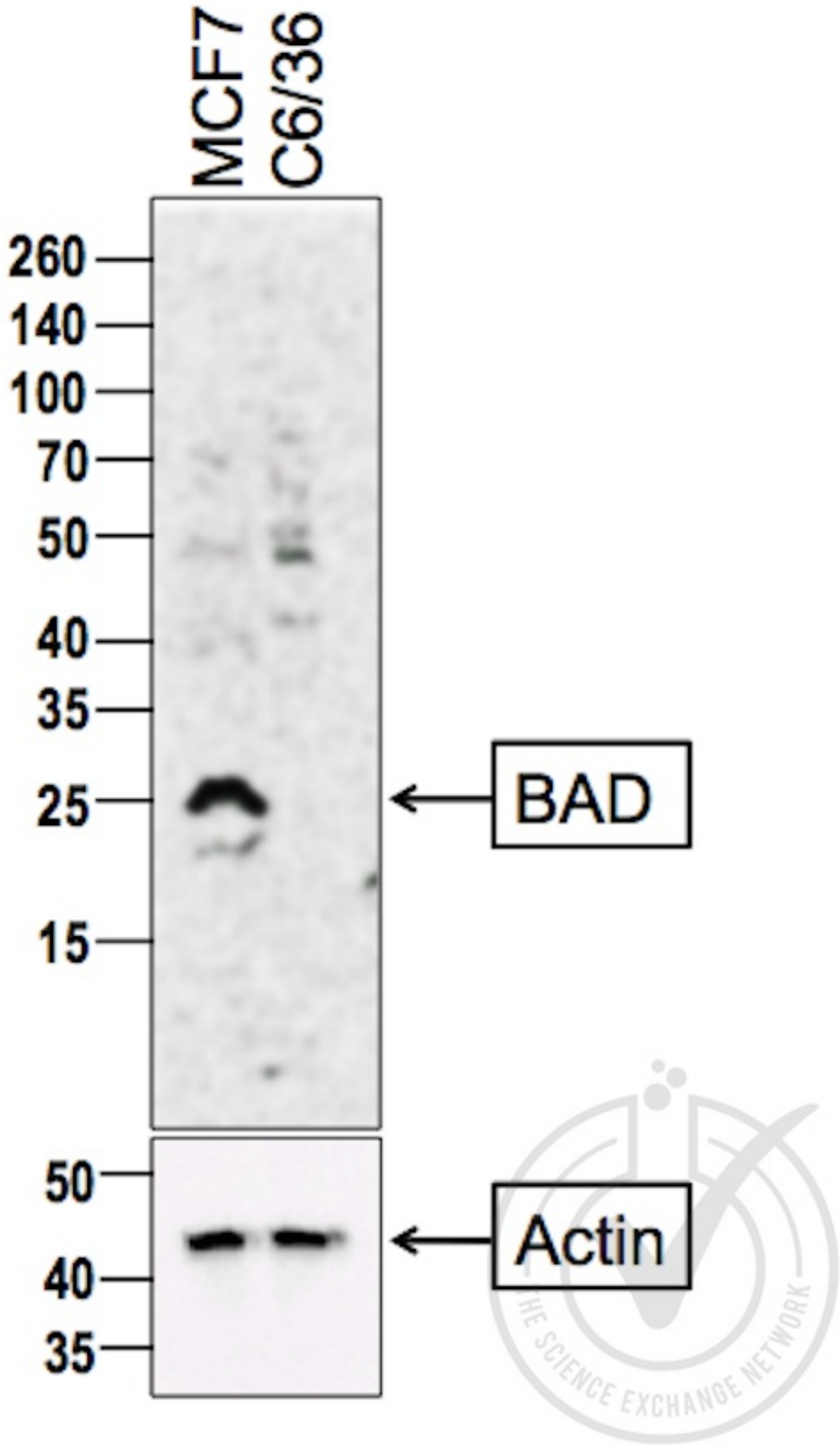

Figure 1: Western Blot for BAD. Arrowhead indicates the expected molecular weight of ~18 kDa.