Validation Report #029759

Validation Date: 07/03/14

Summary

Antigen	Caspase 8, Apoptosis-Related Cysteine Peptidase (CASP8)
Catalog number	ABIN724205
Supplier	Bioss
Supplier catalog number	bs-0052R
Lot number	131127
Method validated	Western Blot
Laboratory	Alamo Laboratories Inc
Validation number	29759
Positive Control	HeLa cells
Negative Control	c6/36 mosquito cells (non-reactive species)
Notes	A strong band was observed at the correct molecular weight in the positive control sample. No major bands were observed in the negative sample.

Full Methods

Primary Antibody
- Antigen: Caspase 8, Apoptosis-Related Cysteine Peptidase (CASP8) (1:200 dilution)
- Catalog number: ABIN724205
- Supplier: Bioss
- Supplier catalog number: bs-0052R
- Lot number: 131127

Loading Control Antibody
- Antigen: Mouse Anti-Actin (1:6,000 dilution)
- Supplier: BD Transduction Laboratories
- Catalog number: 612657
- Lot number: N/A

Secondary Antibody
- Antigen: Goat Anti-Rabbit IgG (H + L)-HRP Conjugate (1:20,000 dilution)
- Supplier: Bio-Rad
- Catalog number: #170-6515
- Lot number: L170-6515

Controls
- Positive control: HeLa cell extract
- Negative control: c6/36 cell extract

Protocol
1. Total protein extracts were boiled in 1X SDS Sample Buffer containing 1% SDS and 1.25% β-mercaptoethanol at 95°C for 5 min prior to loading.
2. 20 µg of boiled 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 h.
6. The membrane was rinsed with TBST once.
7. The membrane was immersed with the protein side up in the primary antibody solution (CASP8; 1:200) in TBST containing 5% (W/V) BSA and incubated for 16 h at 4°C.
8. The membrane was rinsed in TBST thrice for 5 min each.
9. The membrane was incubated in the HRP-conjugated secondary antibody solution (Goat anti-rabbit IgG-HRP; 1:20,000) 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 min each.
11. The membrane was rinsed in TBS twice for 30 s each.
12. Signals were detected with ECL-2 Substrate. The blot was scanned for 300 s.
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 min each.
15. The membrane was washed in TBST 2 times for 10 min each.
16. Repeated Steps 5-12 with the loading control antibody (anti-Actin; 1:6,000) and its matching secondary antibody (Goat anti-rabbit IgG-HRP; 1:20,000).

Experimental Notes
- Nothing to note.

Figures

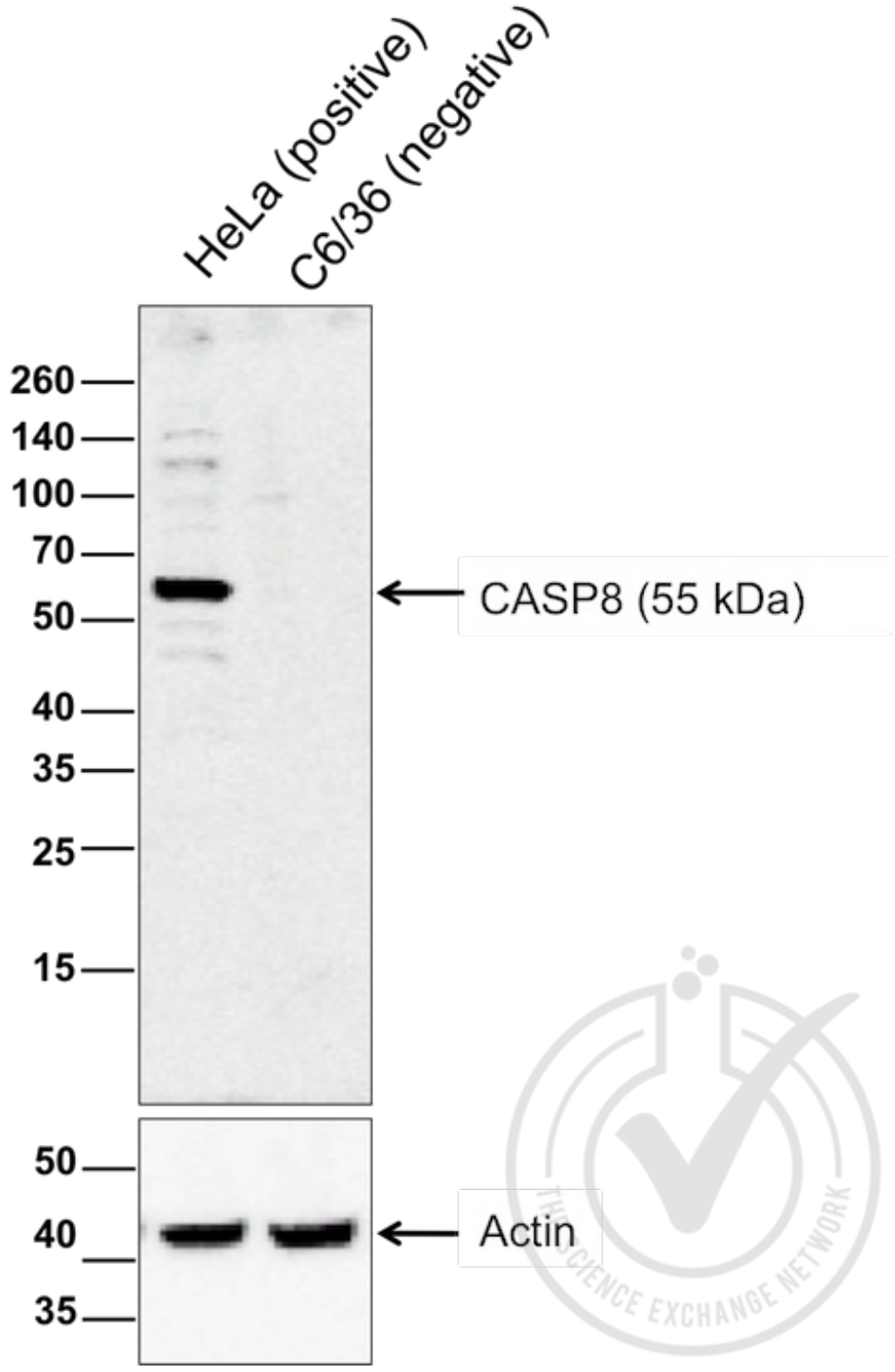

Figure 1: Western blot of lysates from HeLa cells (Lane 1), and c6/36 cells (Lane 2) probed with anti-CASP8 (upper panel) or with anti-Actin for loading control (lower panel).