Validation Report #029809

Validation Date: 09/03/14

Summary

Antigen	Interleukin-1 Receptor-Associated Kinase 1 (IRAK1)
Catalog number	ABIN1387749
Supplier	Bioss
Supplier catalog number	bs-6464R
Lot number	140120
Method validated	Western Blot
Laboratory	Alamo Laboratories Inc
Validation number	29809
Positive Control	PC3 cells
Negative Control	C6/36 cells (non-reactive species)
Notes	A band was observed in the positive control sample at the correct molecular weight, which was absent from the negative control sample. Additional bands were also observed in the positive control sample, which were absent from the negative control. These bands may represent alternative IRAK1 isoforms.

Full Methods

Primary Antibody
- Antigen: Interleukin-1 Receptor-Associated Kinase 1 (IRAK1)
- Catalog number: ABIN1387749
- Supplier: Bioss
- Supplier catalog number: bs-6464R
- Lot number: 140120
- Antibody Dilution: 1:100

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: PC3 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 culture-media 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 24 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 45 minutes.
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

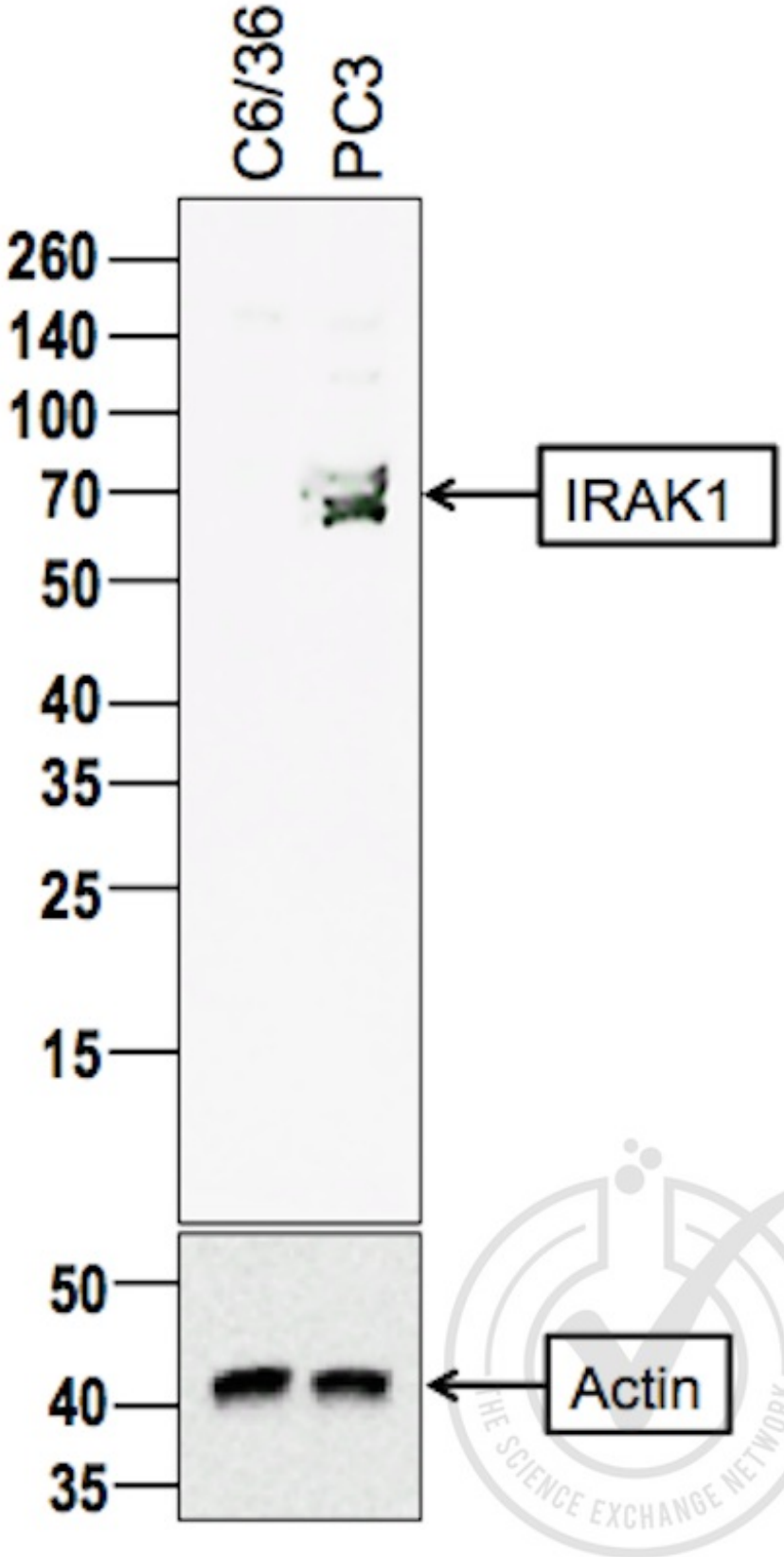

Figure 1: Western Blot for IRAK1. Arrowhead indicates the expected molecular weight of ~78 kDa.