Validation Report #029749

Validation Date: 07/01/14

Summary

Antigen	Partner and Localizer of BRCA2 (PALB2)
Catalog number	ABIN670536
Supplier	Bioss
Supplier catalog number	bs-0588R
Lot number	120510
Method validated	Western Blot
Laboratory	Alamo Laboratories Inc
Validation number	29749
Positive Control	A549 cell extract
Negative Control	c6/36 mosquito cell extract (non-reactive species)
Notes	A single strong band was observed in the positive control at the correct molecular weight. Several faint bands appeared in the negative control, but they were much less intense than the positive control band.

Full Methods

Primary Antibody
- Antigen: Partner and Localizer of BRCA2 (PALB2) (1:500 dilution)
- Catalog number: ABIN670536
- Supplier: Bioss
- Supplier catalog number: bs-0588R
- Lot number: 120510

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

Secondary Antibody
- Antibody: 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: A549 cell extract
- Negative control: c6/36 Mosquito 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. 35 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 (anti-PALB2; 1:500) 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 h 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
- No challenges noted.

Figures

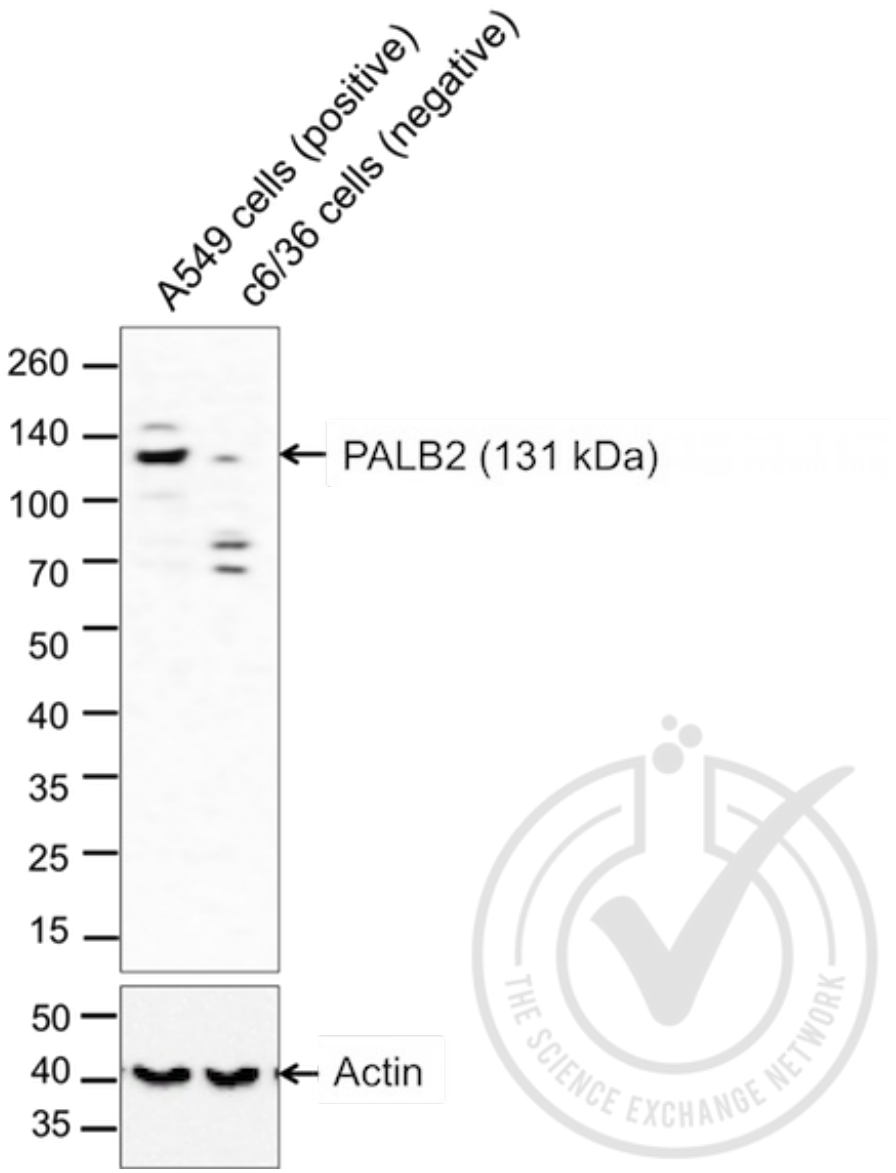

Figure 1. Western blot of lysates from A549 cells (Lane 1) and c6/36 cells (Lane 2) probed with anti-PALB2 (upper panel) or with anti-Actin for loading control (lower panel).