Validation Report #028755

Validation Date: 09/16/13

Summary

Antigen	Tumor Protein P53 (TP53) antibody
Catalog number	ABIN967416
Supplier	BD Bioscience
Supplier catalog number	554157
Lot number	2335876
Method validated	Western Blotceexchange.com/validations/28755)
Laboratory	Alamo Laboratories Inc
Validation number	28755
Positive Control	U251-MG cells
Negative Control	PC3 cells
Notes	Strong bands of the expected size were observed in the positive control sample, and not in the negative control sample.

Full Methods

Primary Antibody
- Antibody: Tumor Protein P53 (TP53) antibody
- Catalog number: ABIN967416
- Supplier: BD Bioscience
- Supplier catalog number: 554157
- Lot number: 2335876

Loading Control Antibody
- Antibody: Actin antibody
- Supplier: antibodies-online
- Catalog number: ABIN968903
- Lot number: 2251669

Secondary Antibody
- Antibody: Goat anti-Mouse IgG-HRP
- Supplier: Santa Cruz Biotechnology
- Catalog number: SC-2005
- Lot number: 0312

Controls
- U251-MG (positive) and PC3 (negative) cell line extracts were prepared using RIPA buffer (R0278, Sigma Aldrich).
- Loading control: blots were stripped and re-probed for beta-actin to ensure equal loading of lysates.

Protocol

1. Total protein extracts were boiled in 1X SDS Sample Buffer containing 1% SDS and 1.25% Beta-mercaptoethanol at 95°C for 5 min prior to loading.
2. 32 µg of boiled extracts were loaded and resolved on a 8-16% SDS-polyacrylamide gel.
3. The Spectra Multicolor Broad Range molecular mass marker (26634 Thermo Scientific) was used as a standard.
4. Proteins were then transferred onto PVDF membrane by tank transfer and protein transfer was confirmed with Ponceau S staining.
5. The immunoblot membrane was blocked in PBS containing 3% (W/V) non-fat dry milk at room temperature for 1 h.
6. The membrane was rinsed with PBS containing 0.05% Tween-20 once.
7. The membrane was immersed with the protein side up in the antibody solution in PBS containing 1% (W/V) non-fat dry milk and incubated for 2 h at room temperature (~26°C).
8. The membrane was rinsed in PBS containing 0.05% Tween-20 thrice for 10 min each.
9. The membrane was incubated in the HRP-conjugated secondary antibody solution in PBS containing 1% (W/V) non-fat dry milk and incubated for 1 h at room temperature (~26°C) with gentle agitation.
10. The membrane was rinsed thrice PBS containing 0.05% Tween-20 thrice for 10 min each.
11. The membrane was washed in PBS twice for 30 sec each.
12. Signals were detected with Pierce ECL Western Blotting Substrate (32109, Thermo Scientific). The blot was scanned for 300 sec.
13. The membrane was rinsed three times with PBS containing 0.05% Tween-20.4. Incubated in Acidic Glycine Stripping Buffer at room temperature with gentle agitation for 3 times, 10 min each.
14. The membrane was washed in PBS containing 0.05% Tween-20 times for 10 min each.
15. Repeated Steps 5-12 with the loading control antibody (beta-actin) and its matching secondary antibody.

Experimental Notes

None

Figures

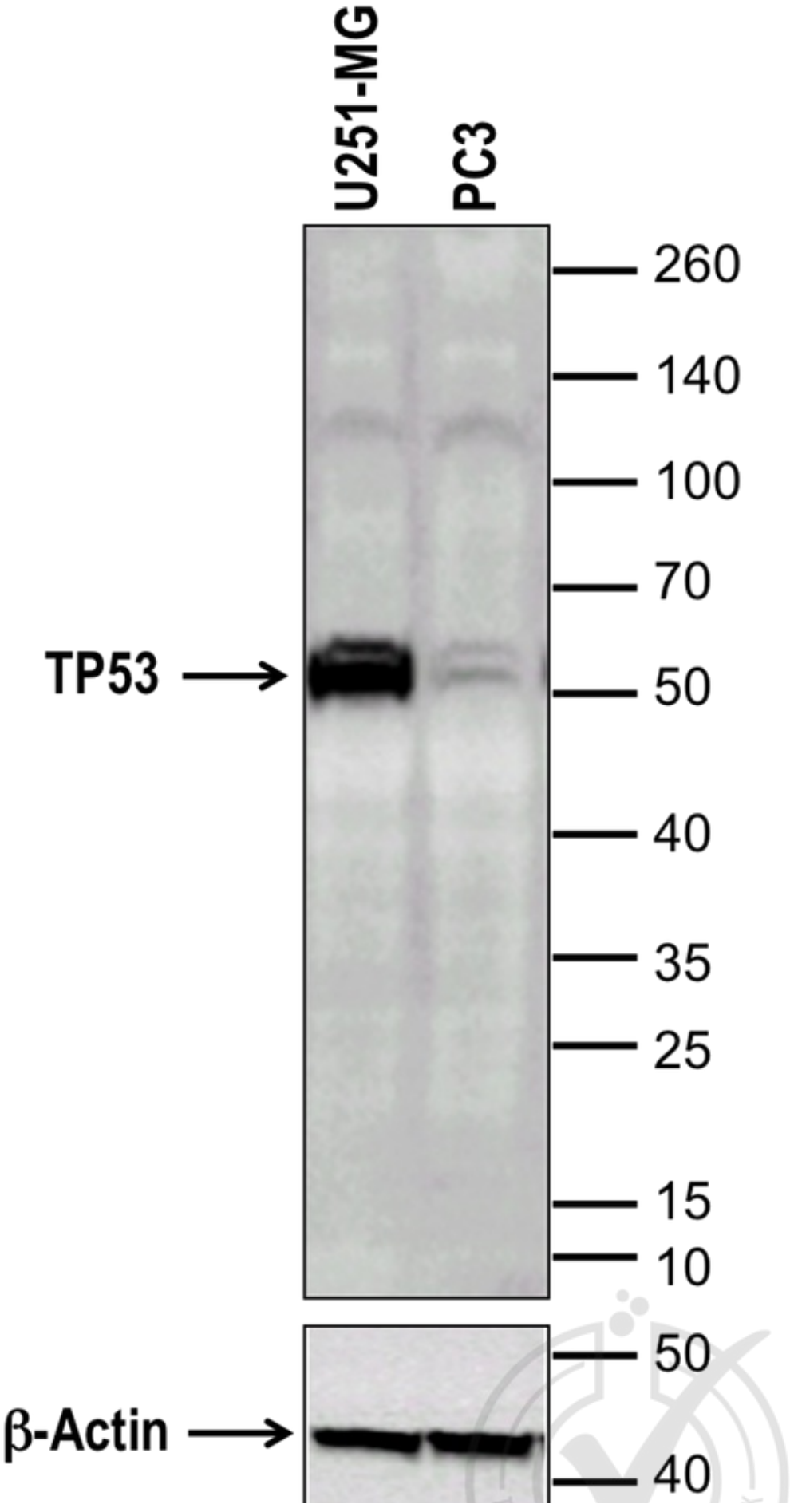

Figure 1: Western blot analysis of U251-MG and PC3 cell line extracts using Tumor Protein P53 (TP53) antibody (Catalog number ABIN967416, Lot number 2335876). TP53 is present in the positive control sample (U251-MG) and absent from the negative control sample (PC3). The arrowhead indicates the expected position of TP53 (predicted MW ~53kDa). 32 micrograms of total protein lysates from each sample were loaded into each lane. Upper panel: scanned image of the TP53 antibody probed with the U251-MG and PC3 extracts in lanes 1 and 2 respectively. Lower panel: scanned image of the loading control (beta-actin).