555 Bryant Street, #939, Palo Alto, CA 94301-1704

Validation Report

Report #029761 | Validated On: **07/03/14**

Summary

Antigen	Mechanistic Target of Rapamycin (serine/threonine Kinase) (FRAP1)
Catalog number	ABIN676403
Supplier	Bioss
Supplier catalog number	bs-1992R
Lot number	131209
Method validated	Western Blot
Laboratory	Alamo Laboratories Inc
Validation number	29761
Positive Control	HeLa cells
Negative Control	c6/36 mosquito cells (non-reactive species)
Notes	A strong band was observed in the positive control sample at the correct molecular weight. Multiple faint bands were also observed in the positive sample, but these bands did not represent a significant background signal. A band at the correct molecular weight was also observed in the negative control; however, it may be impossible to find a true negative control for this antigen.

Full Methods

Primary Antibody
- Antigen: Mechanistic Target of Rapamycin (serine/threonine Kinase) (FRAP1) (1:200 dilution)
- Catalog number: ABIN676403
- Supplier: Bioss
- Supplier catalog number: bs-1992R
- Lot number: 131209

Loading Control Antibody
- Antigen: Mouse Anti-Actin
- Supplier: BD Transduction Laboratories
- Catalog number: 612657
- Lot number: N/A

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

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

Protocol
- 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.
- 20 µg of boiled extracts were loaded and resolved on 8-16% SDS-polyacrylamide gel.
- The Thermo Scientific - Spectra Multicolor Broad Range (Cat # 26634) were used as molecular mass markers.
- Proteins were then transferred onto PVDF membrane by wet transfer and protein transfer was confirmed with Ponceau-S staining.
- 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.
- The membrane was rinsed with TBST once.
- The membrane was immersed with the protein side up in the primary antibody solution (FRAP1; 1:200) in TBST containing 5% (W/V) BSA and incubated for 16 h at 4°C.
- The membrane was rinsed in TBST thrice for 5 min each.
- 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.
- The membrane was rinsed thrice TBST thrice for 5 min each.
- The membrane was rinsed in TBS twice for 30 s each.
- Signals were detected with ECL-2 Substrate. The blot was scanned for 300 s.
- The membrane was rinsed three times TBST.
- Incubated in Acidic Glycine Stripping Buffer at room temperature with gentle agitation for 3 times, 10 min each.
- The membrane was washed in TBST 2 times for 10 min each.
- 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 experimental challenges noted.

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Figures

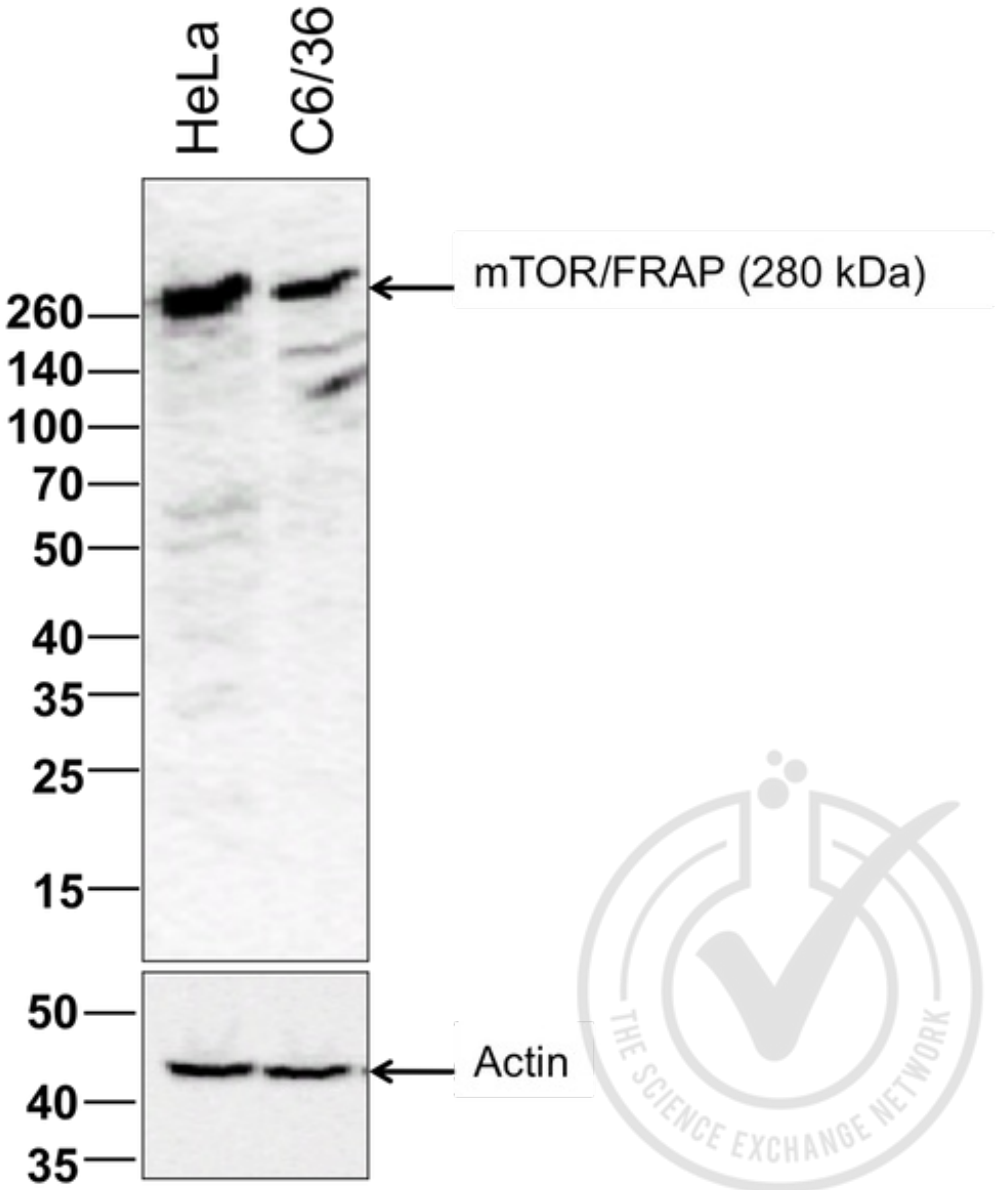

Figure 1. Western blot of lysates from HeLa cells (Lane 1) and c6/36 cells (Lane 2) probed with anti-mTOR (upper panel) or with anti-Actin for loading control (lower panel).