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

Validation Report

Report #029849 | Validated On: **11/06/15**

Summary

Antigen	Glutathione S Transferase (GST)
Catalog number	ABIN1998462
Supplier	Sino Biological
Supplier catalog number	11213-MM01
Lot number	HD04FE1006
Method validated	Western Blot
Laboratory	Alamo Laboratories Inc
Validation number	29849
Positive Control	GST- AcNFkBp(C-terminal) 293-Lysate
Negative Control	Empty vector- 293 Lysate
Notes	A strong specific band was observed in the positive control at the expected size (~82 kDa) that is not observed in the negative control.

Full Methods

Primary Antibody

- Antigen: Glutathione S Transferase (GST) protein
- Catalog number: ABIN1998462
- Supplier: Sino Biological
- Supplier catalog number: 11213-MM01
- Lot number: HD04FE1006
- Antibody Dilution: 1:7000

Loading Control Antibody

- Antigen: Mouse Anti-Actin
- Supplier: BD Transduction Laboratories
- Catalog number: 612657
- Antibody Dilution: 1:6,000

Secondary Antibody

- Antigen: Goat Anti-Mouse IgG (H + L)-HRP Conjugate
- Supplier: Bio-Rad
- Catalog number: #170-5047
- Lot number: N/A
- Antibody Dilution: 1:15,000

Controls

- Positive control: GST- AcNFkBp(C-terminal) 293-Lysate
- Negative control: Empty vector- 293 Lysate

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 were loaded and resolved on 8-16% SDS-polyacrylamide gel.
3. The Bio-Rad Precision Plus (Cat 161-0374) were used as molecular mass markers.
4. Proteins were then transferred onto PVDF membrane by wet transfer.
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 1 minute.
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

None reported

Figures

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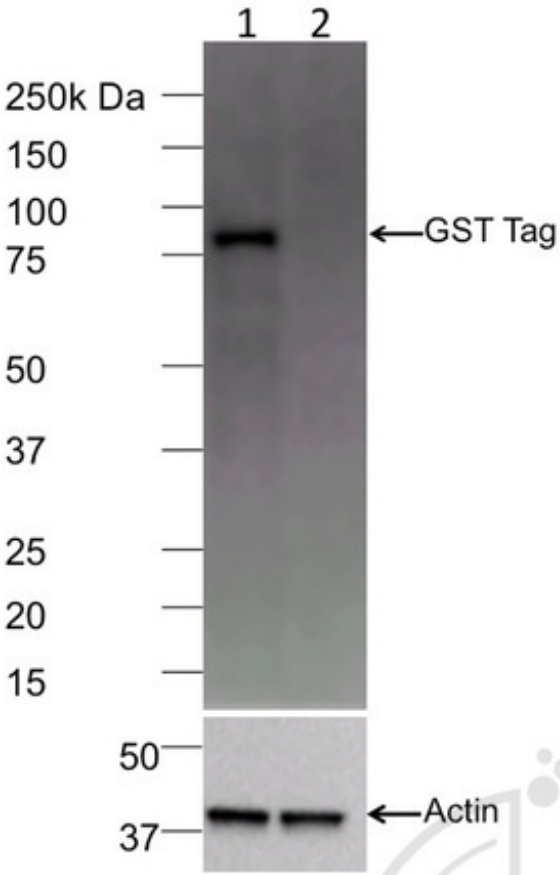

Figure 1. Probing of cell extracts with GST Tag (upper panel) or loading control Actin (lower panel) antibodies. Lane 1: GST-AcNFkBp(C-terminal)293-Lysate; Lane 2: Empty vector - 293 Lysate