Validation Report #028752

Validation Date: 09/08/13

Summary

Antigen	NK2 Homeobox 1 (NKX2-1)
Catalog number	ABIN728713
Lot number	120319
Method validated	Western Blot
Laboratory	Alamo Laboratories Inc
Supplier	Bioss
Supplier catalog number	bs-0826r
Validation number	28752
Positive Control	Brain
Negative Control	Liver
Notes	A strong band was observed at the expected size in the positive control lysate but not in the negative control lysate.

Full Methods

Primary Antibody
- Antibody: NK2 Homeobox 1 (NKX2-1) antibody
- Catalog number: ABIN728713
- Lot number: 120319

Loading Control Antibody
- Antibody: Anti-Beta-Actin antibody
- Catalog number: bs-0061R
- Lot number: YYLS29W

Secondary Antibody
- Antibody: Goat anti-Rabbit IgG Antibody (HRP)
- Catalog number: ABIN1384779
- Lot number: YYDW62W

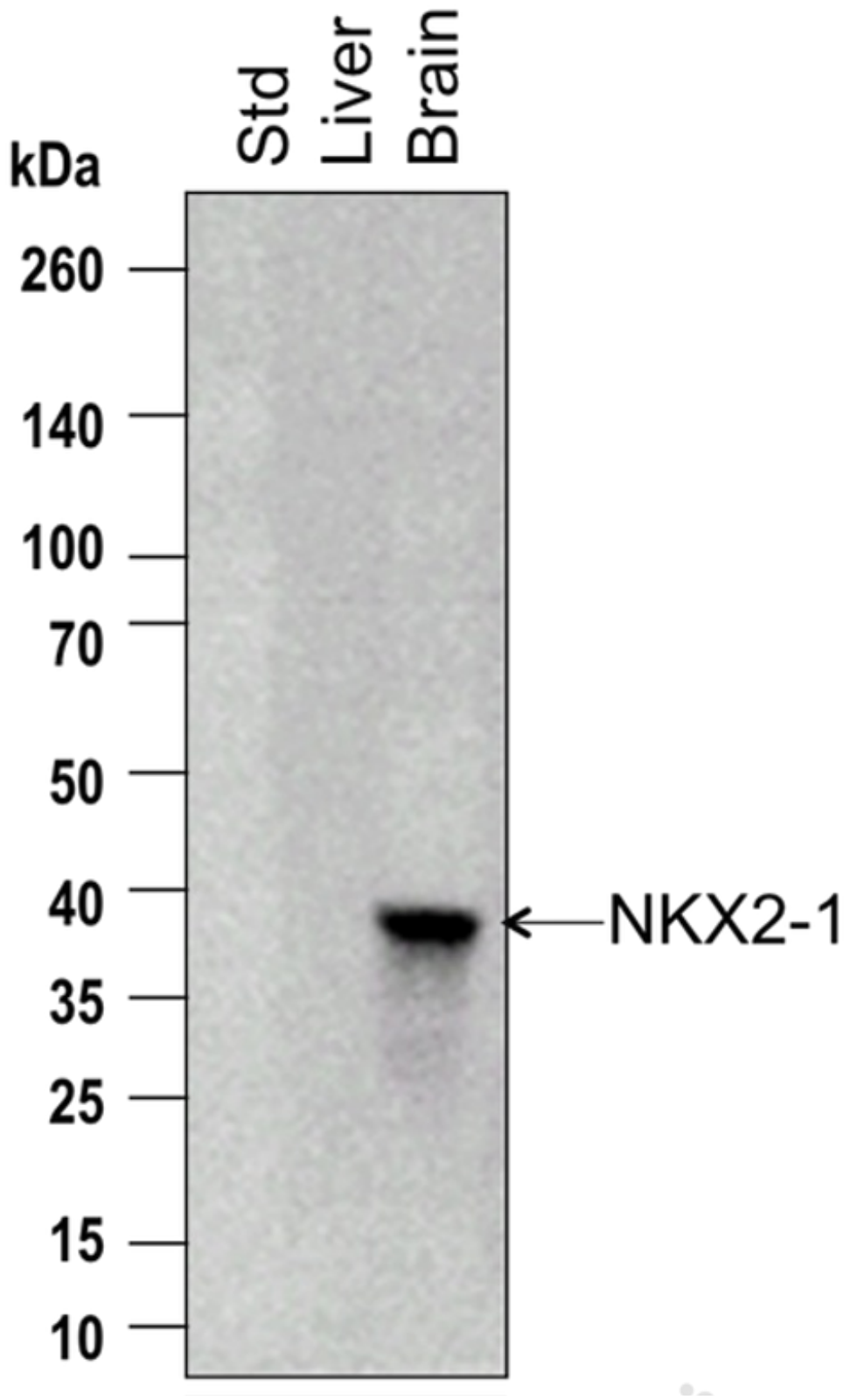
Controls
- Mouse brain and liver tissue extracts were prepared using N-PER (87792 Thermo Scientific) and T-PER (78510 Thermo Scientific) protein extraction reagents, respectively.
- Loading control: blots were stripped and re-probed for Beta-actin to ensure equal loading of lysates.

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 minutes prior to loading.
2. 24 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 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 hour.
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 hours 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 hour at room temperature (~26°C) with gentle agitation.
10. The membrane was rinsed in PBS containing 0.05% Tween-20 thrice for 10 min each.
11. The membrane was washed in PBS twice for 30 seconds each.
12. Signals were detected with Pierce ECL Western Blotting Substrate (32109, Thermo Scientific). The blot was scanned for 300 seconds.
13. The membrane was rinsed three times with PBS containing 0.05% Tween-20.
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 PBS containing 0.05% Tween-20 times for 10 min each.
16. Repeated Steps 5-12 with the loading control antibody (for Beta-actin) and its matching secondary antibody.

Experimental Notes

None

Figures



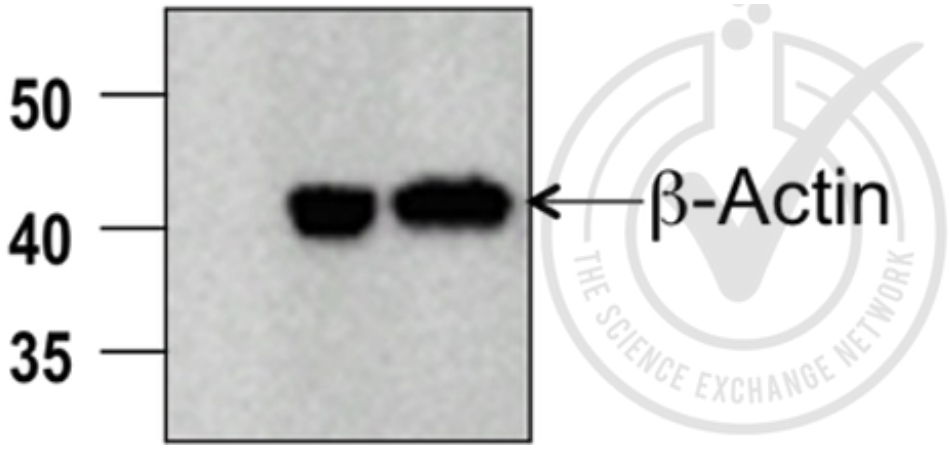


Figure 1: Western blot analysis of mouse brain and liver extracts using NK2 Homeobox 1 (NKX2-1) antibody (Catalog number ABIN728713, Lot number 120319). NKX2-1 is present in the positive control sample (brain) and absent from the negative control sample (liver). The arrowhead indicates the expected position of NKX2-1 (predicted MW ~38kDa). 24 micrograms of total protein extracts from each sample were loaded into each lane. Upper panel: scanned image of the NKX2-1 antibody probed with the liver and brain extracts in lanes 2 and 3, respectively. Lower panel: scanned image of the loading control (Beta-actin).