Validation Report #029822

Validation Date: 10/28/14

Summary

Antigen	Tubulin, Beta, 3 (TUBB3) (AA 443-450)
Catalog number	ABIN1742553
Supplier	Synaptic Systems
Supplier catalog number	302 302
Lot number	302302/3
Method validated	Western Blot
Laboratory	Kinexus Bioinformatics Corporation
Validation number	029822
Positive Control	Mouse brain
Negative Control	Mouse heart, HeLa cells, A431 cells
Notes	A band was observed in the positive controls at the expected size (~50 kDa), which is lower in the negative controls.

Full Methods

Primary Antibody
- Antigen: Tubulin, Beta, 3 (TUBB3) (AA 443-450)
- Catalog number: ABIN1742553
- Supplier: Synaptic Systems
- Supplier catalog number: 302 302
- Lot number: 302302/3
- Dilution: 1:1000

Loading Control Antibody
- Antigen: β-actin
- Supplier: Santa Cruz
- Supplier catalog number: sc-1616
- Lot number: A1910
- Dilution: 1:200

Secondary Antibody
- Antibody: Donkey anti-Rabbit IgG Antibody (HRP)
- Catalogue number: sc-2007
- Supplier: Santa Cruz
- Lot number: F0613
- Dilution: 1:10,000

Additional Information

Controls
- Lysates were prepared by Kinexus Bioinformatics Corporation following standard protocols and quality controlled for protein integrity on a regular basis.

Protocol
1. Cell/tissue total protein lysates were boiled in 1X SDS Sample Buffer containing 1% SDS and 1.25% β-mercaptoethanol at 95°C for 5 minutes prior to loading.
2. 15 µg of boiled lysate were loaded and resolved on a 12% SDS-polyacrylamide gel.
3. The Precision Plus Protein™ All Blue Prestained Standards from BioRad (161-0373) were used as molecular mass markers.
4. Proteins were transferred onto nitrocellulose membrane by tank transfer and protein transfer was confirmed with Ponceau S staining.
5. The immunoblot membrane was blocked in 2.5% skim milk and 1.5% BSA solution in TTBS at room temperature for 60 minutes.
6. The membrane was washed in TTBS twice for 5 minutes each.
7. The membrane was immersed with the protein side up in the antibody solution in TBS and incubated overnight at 4°C with gentle agitation.
8. The membrane was rinsed twice with TTBS.
9. The membrane was washed in TTBS twice for 5 minutes each.
10. The membrane was washed in TTBS once for 15 minutes.
11. The membrane was incubated in the HRP-conjugated secondary antibody solution in TBS for 60 minutes at room temperature with gentle agitation.
12. The membrane was rinsed twice with TTBS.
13. The membrane was washed in TTBS twice for 5 minutes each.
14. The membrane was washed in TTBS once for 15 minutes.
15. Signals were detected by chemiluminescence (ECL). The blot was scanned for 320 seconds.
16. The membrane was rinsed three times with TTBS.
17. Repeated Steps 4-15 with the loading control antibody and its matching secondary antibody. The blot was scanned for 160 seconds.

Experimental Notes
- No experimental challenges noted.

Figures

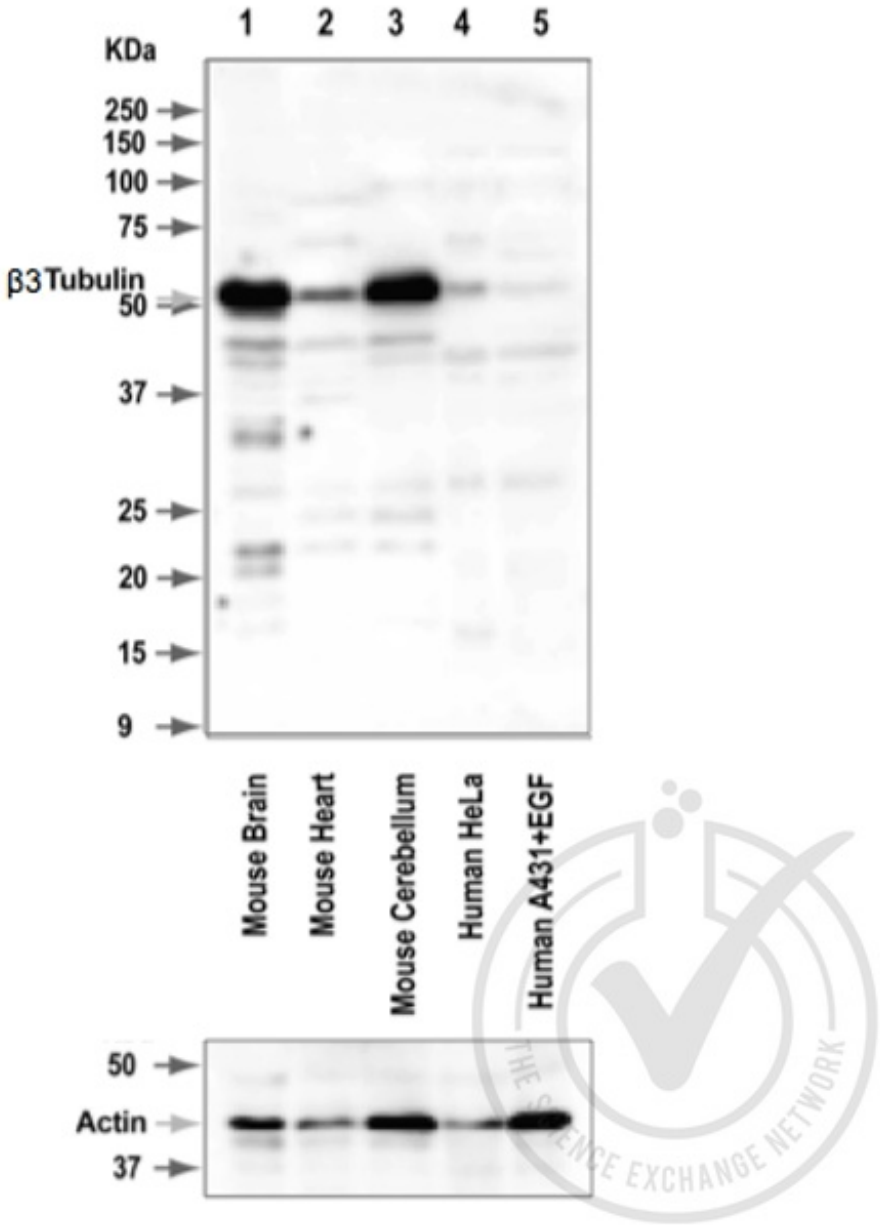

Figure 1: Western Blot for Tubulin, Beta, 3 (TUBB3). Grey arrowhead indicates the expected molecular weight of ~50 kDa.