

# **Validation Report #029822**

## **Summary**

Antigen	Tubulin, Beta, 3 (TUBB3) (AA 443-450)
Catalog number	ABIN1742553
Supplier	Synaptic Systems
Supplier catalog number	<u>302 302</u>
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.



Validation Date: 10/28/14

## **Full Methods**

#### **Primary Antibody**

• Antigen: Tubulin, Beta, 3 (TUBB3) (AA 443-450)

Catalog number: ABIN1742553Supplier: Synaptic SystemsSupplier catalog number: 302 302

Lot number: 302302/3Dilution: 1:1000

#### **Loading Control Antibody**

Antigen: β-actinSupplier: Santa Cruz

Supplier catalog number: sc-1616

Lot number: A1910Dilution: 1:200

#### **Secondary Antibody**

• Antibody: Donkey anti-Rabbit IgG Antibody (HRP)

• Catalogue number: sc-2007

Supplier: Santa CruzLot number: F0613Dilution: 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%  $\beta$ -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**

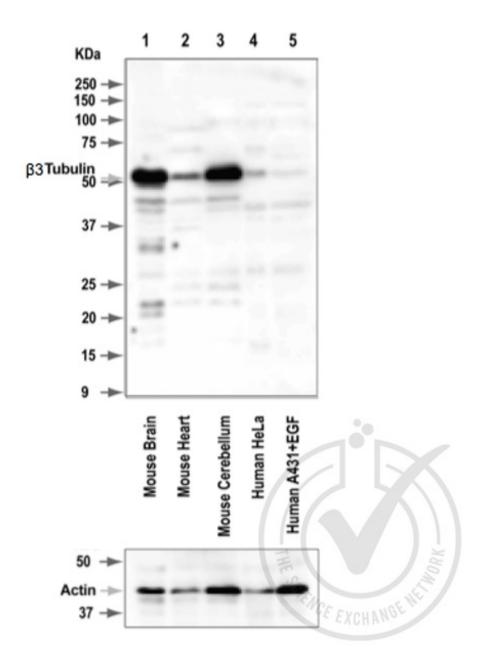


Figure 1: Western Blot for Tubulin, Beta, 3 (TUBB3). Grey arrowhead indicates the expected molecular weight of  $\sim$ 50 kDa.