Validation Report #029836

Validation Date: 06/21/15

Summary

Antigen	Actin, beta (ACTB) (AA 2-16)
Catalog number	ABIN1742508
Supplier	Synaptic Systems
Supplier catalog number	251003
Lot number	251003/3
Method validated	Western Blot
Laboratory	Kinexus Bioinformatics Corporation
Validation number	29836
Positive Control	mouse brain, mouse kidney, mouse muscle, mouse spleen, mouse testes
Negative Control	none [ubiquitously expressed]
Notes	A band was observed in the positive controls at the expected size (~42 kDa)

Full Methods

Primary Antibody
- Antigen: Beta-Actin, (ACTB) (AA 2-16)
- Catalog number: ABIN1742553
- Supplier: Synaptic Systems
- Supplier catalog number: 251 003
- Lot number: 251003/3
- Dilution: 1:1000

Loading Control
- Ponceau stain

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

Controls
- All lysates used were prepared by Kinexus Bioinformatics Corporation following standard protocols and quality controlled for protein integrity on a regular basis.
- Loading control: blot was stripped and re-probed with β3-Tubulin.

Protocol
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.
15 µg of boiled lysate were loaded and resolved on a 12% SDS-polyacrylamide gel.
The Precision Plus Protein™ All Blue Prestained Standards from BioRad (161-0373) were used as molecular mass markers.
Proteins were transferred onto nitrocellulose membrane by tank transfer and protein transfer was confirmed with Ponceau S staining.
The immunoblot membrane was blocked in 2.5% skim milk and 1.5% BSA solution in TTBS at room temperature for 60 minutes.
The membrane was washed in TTBS twice for 5 minutes each.
The membrane was immersed with the protein side up in the antibody solution in TBS and incubated overnight at 4°C with gentle agitation.
The membrane was rinsed twice with TTBS.
The membrane was washed in TTBS twice for 5 minutes each.
The membrane was washed in TTBS once for 15 minutes.
The membrane was incubated in the HRP-conjugated secondary antibody solution in TBS for 60 minutes at room temperature with gentle agitation.
The membrane was rinsed twice with TTBS.
The membrane was washed in TTBS twice for 5 minutes each.
The membrane was washed in TTBS once for 15 minutes.
Signals were detected by chemiluminescence (ECL). The blot was scanned for 160 seconds.
The membrane was rinsed three times with TTBS.
Incubated in Acidic Glycine Stripping Buffer at room temperature with gentle agitation for 3 times, 10 minutes each.
The membrane was washed in TTBS 3 times for 5 minutes each.
Repeated Steps 4-14 with the loading control antibody (for β3–Tubulin).
Repeated Steps 4-14 with the loading control antibody and its matching secondary antibody. The blot was scanned for 160 seconds.

Experimental Notes
A protein band was observed at the expected target size of β–Actin of 42 kDa in all mouse extracts tested. Lower molecular weight bands of approximately 28-30 kDa were also observed in the mouse kidney and spleen tissue samples. Three main groups of actin have been identified. The β-actins and γ-actins co-exist in most cell types as

components of the cytoskeleton, whereas the α-actin are found in muscle tissues. In our experience, we find that the level of actin does vary widely between different cell and tissue types.

Figures

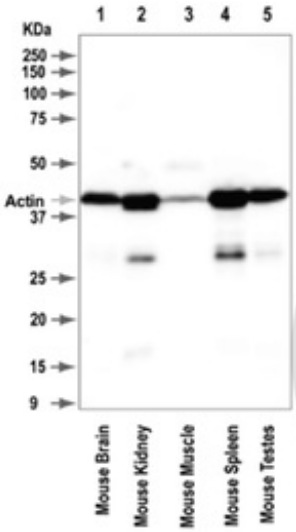

Figure 1. Western blot for beta actin (ACTB). Grey arrowhead indicates the expected molecular weight of ~42 kDa.

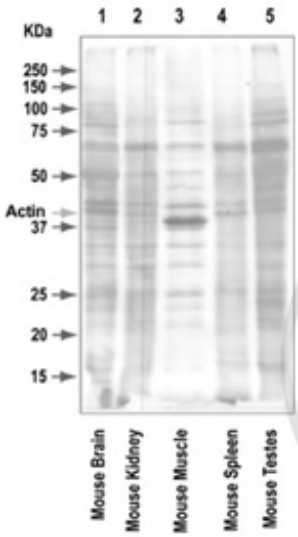

Figure 2. Ponceau stain loading control.

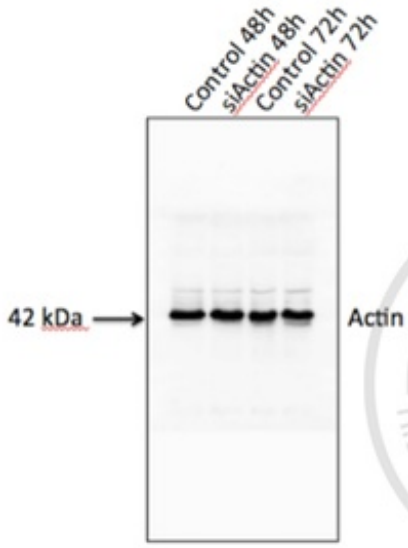

Figure 3. Western blot for beta actin (ACTB) using attempted siRNA silencing. Arrowhead indicates the expected molecular weight of ~42 kDa, however siRNA silencing was not detected.