Anti-beta Actin Antibody [A2-F6]

EM21002

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	A2-F6

Description: Beta-actin (human gene and protein abbreviation ACTB/ACTB) is one of six different actin isoforms which have been identified in humans. This is one of the two nonmuscle cytoskeletal actins. Actins are highly conserved proteins[5][6] that are involved in cell motility, structure and integrity. Alpha actins are a major constituent of the contractile apparatus. Beta-actin has been shown to interact with SPTBN2. In addition, RNA-binding protein Sam68 was found to interact with the mRNA encoding β-actin, which regulates the synaptic formation of the dendritic spines with its cytoskeletal components. Beta-actin has been shown to activate eNOS, thereby increasing NO production. An eight-amino acid residue (326-333) in actin has been shown to mediate the interaction between actin and eNOS. Recurrent mutations in this gene have been associated to cases of diffuse large B-cell lymphoma. Beta actin is often used in Western blotting as a loading control, to normalize total protein amounts and check for eventual protein degradation in the samples. Its transcript is also commonly used as a housekeeping gene standard in qPCR. Its molecular weight is approximately 42 kDa.

Immunogen: Synthetic peptide (KLH-coupled) within human Beta-actin aa 1-50.

Positive control: Hela cell lysate, THP-1 cell lysate, HepG2 cell lysate, MCF-7 cell lysate, PC-12 cell lysate, PMVEC cell lysate, RH-35 cell lysate, L6 cell lysate, NIH/3T3 cell lysate, L929 cell lysate, RAW264.7 cell lysate, B16F1 cell lysate, MEF cell lysate, Hela, A549, NIH/3T3, human colon carcinoma tissue, mouse prostate tissue, mouse kidney tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P60709 Human | P60710 Mouse | P60711 Rat

Recommended Dilutions:

WB	1:10,000-160,000
IF-Cell	1:100-1:200
IHC-P	1:1,000
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

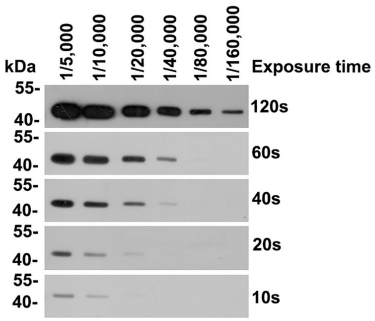


Fig1: Western blot analysis of beta Actin on Hela cell lysates with Mouse anti-beta Actin antibody (EM21002).

Hela cell lysates at 10 µg/Lane.

Predicted band size: 42 kDa
Observed band size: 42 kDa

12% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM21002) at serial dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

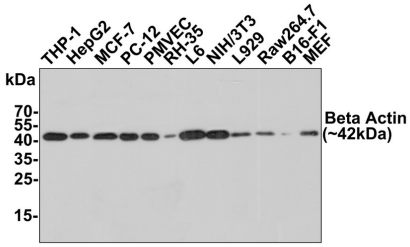


Fig2: Western blot analysis of beta Actin on different lysates with Mouse anti-beta Actin antibody (EM21002) at 1/40,000 dilution.

Cell lysates at 10 µg/Lane, tissue lysates at 20 µg/Lane.

Predicted band size: 42 kDa
Observed band size: 42 kDa

Exposure time: 1 minute;

12% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM21002) at 1/40,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

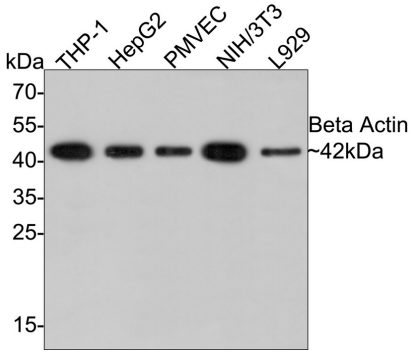


Fig3: Western blot analysis of beta Actin on different lysates with Mouse anti-beta Actin antibody (EM21002) at 1/20,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: HepG2 cell lysate
Lane 3: PMVEC cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: L929 cell lysate

Lysates/proteins at 10 µg/Lane.

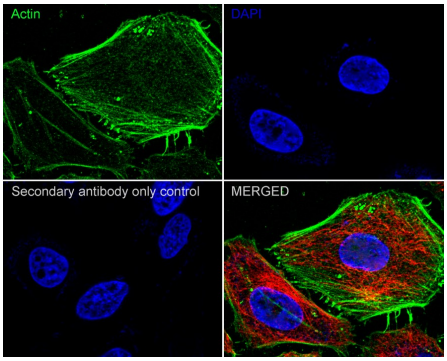
Predicted band size: 42 kDa
Observed band size: 42 kDa

Exposure time: 2 minutes;

12% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM21002) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:150,000 dilution was used for 1 hour at room temperature.

Fig4: Immunocytochemistry analysis of HeLa cells labeling beta Actin with Mouse anti-beta Actin antibody (EM21002) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-beta Actin antibody (EM21002) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) was used as the secondary antibody at 1/1,000 dilution.

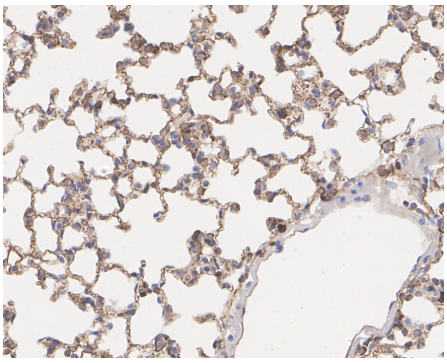


Fig5: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Mouse anti-beta Actin antibody (EM21002) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM21002) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

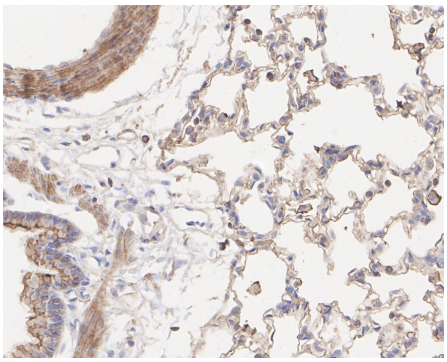


Fig6: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Mouse anti-beta Actin antibody (EM21002) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM21002) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Fujiki R et al. GlcNAcylation of a histone methyltransferase in retinoic-acid-induced granulopoiesis. Nature 459:455-459 (2009).
2. Fujiki R et al. Retraction: GlcNAcylation of a histone methyltransferase in retinoic-acid-induced granulopoiesis. Nature 505:574-574 (2014).