Anti-UBE4B Antibody [JE55-76]

ET7111-11

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P
Molecular Wt:	Predicted band size: 146 kDa.
Clone number:	JE55-76

Description: The modification of proteins with ubiquitin is an important cellular mechanism for targeting abnormal or short-lived proteins for degradation. Ubiquitination involves at least three classes of enzymes: ubiquitin-activating enzymes, or E1s, ubiquitin-conjugating enzymes, or E2s, and ubiquitin-protein ligases, or E3s. This gene encodes an additional conjugation factor, E4, which is involved in multiubiquitin chain assembly. This gene is also the strongest candidate in the neuroblastoma tumor suppressor genes. Alternatively spliced transcript variants encoding distinct isoforms have been found for this gene.

Immunogen: Synthetic peptide within N-terminal Human UBE4B.

Positive control: HeLa cell lysate, Raji cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, Mouse testis tissue lysate, Rat testis tissue lysate, human lung carcinoma tissue, human kidney tissue, mouse kidney tissue, rat kidney tissue, rat skeletal muscle tissue.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: O95155 Human | Q9ES00 Mouse
Entrez Gene: 298652 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:50-1:200

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

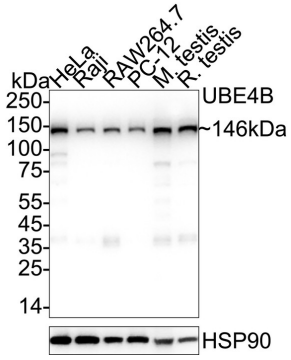

Fig1: Western blot analysis of UBE4B on different lysates with Rabbit anti-UBE4B antibody (ET7111-11) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: Raji cell lysate (10 µg/Lane)
Lane 3: RAW264.7 cell lysate (10 µg/Lane)
Lane 4: PC-12 cell lysate (10 µg/Lane)
Lane 5: Mouse testis tissue lysate (20 µg/Lane)
Lane 6: Rat testis tissue lysate (20 µg/Lane)

Predicted band size: 146 kDa
Observed band size: 146 kDa

Exposure time: 6 seconds; ECL: K1801;
4-20% 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 (ET7111-11) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

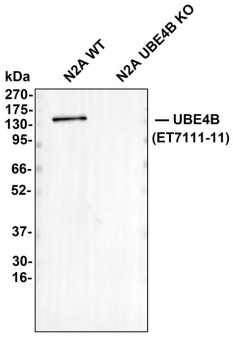

Fig2: All lanes: Western blot analysis of UBE4B with anti-UBE4B antibody [JE55-76] (ET7111-11) at 1:1,000 dilution.
Lane 1: Wild-type N2A whole cell lysate (30 µg).
Lane 2: UBE4B knockout N2A whole cell lysate (30 µg).

ET7111-11 was shown to specifically react with UBE4B in wild-type N2A cells. No band was observed when UBE4B knockdout sample was tested. Wild-type and UBE4B knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET7111-11, 1:1,000) was used in 5% BSA at room temperature for 2 hours.

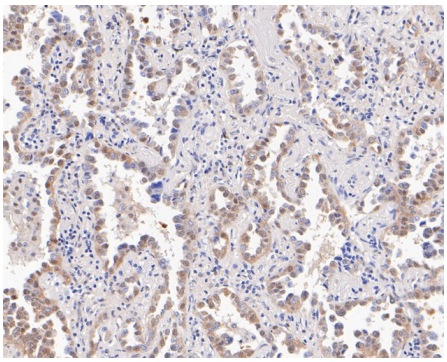

Fig3: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue with Rabbit anti-UBE4B antibody (ET7111-11) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (ET7111-11) at 1/200 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.

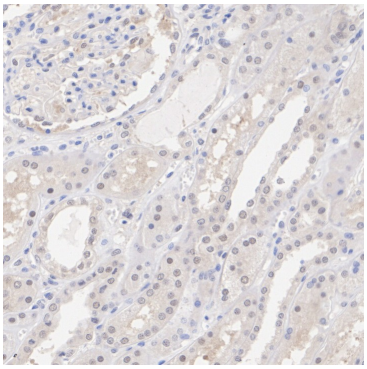

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-UBE4B antibody (ET7111-11) at 1/200 dilution.

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 (ET7111-11) at 1/200 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.

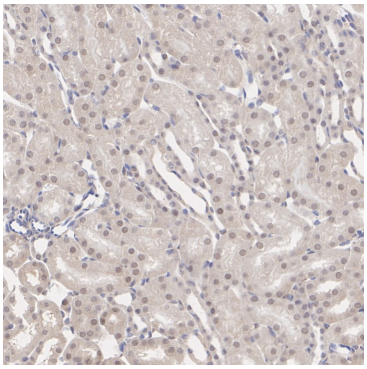

Fig5: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-UBE4B antibody (ET7111-11) at 1/200 dilution.

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 (ET7111-11) at 1/200 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.

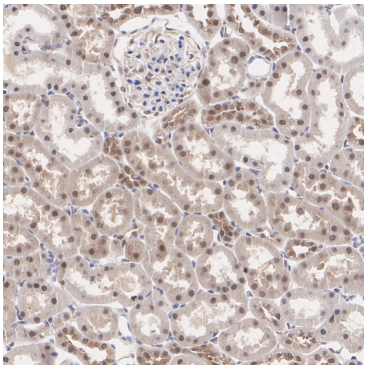

Fig6: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-UBE4B antibody (ET7111-11) at 1/200 dilution.

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 (ET7111-11) at 1/200 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.

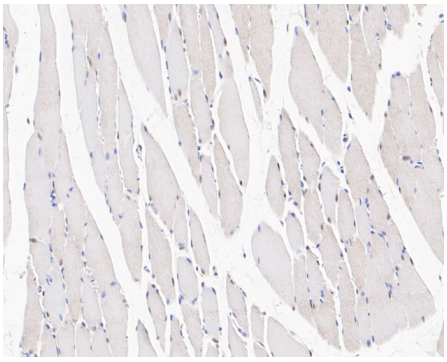

Fig7: Immunohistochemical analysis of paraffin-embedded rat skeletal muscle tissue with Rabbit anti-UBE4B antibody (ET7111-11) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (ET7111-11) at 1/50 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. Antoniou N. et. al. The Role of E3, E4 Ubiquitin Ligase (UBE4B) in Human Pathologies. Cancers (Basel). 2019 Dec
2. Du C. et. al. UBE4B targets phosphorylated p53 at serines 15 and 392 for degradation. Oncotarget. 2016 Jan