Anti-USP14 Antibody [JU30-49]

ET1706-37

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Zebrafish
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	JU30-49

Description: The ubiquitin (Ub) pathway involves three sequential enzymatic steps that facilitate the conjugation of Ub and Ub-like molecules to specific protein substrates. Through the use of a wide range of enzymes that can add or remove ubiquitin, the Ub pathway controls many intracellular processes such as signal transduction, transcriptional activation and cell cycle progression. USP14 (ubiquitin specific peptidase 14), also known as TGT (tRNA-guanine transglycosylase), is a cytoplasmic protein that belongs to the ubiquitin-specific processing family of deubiquitinating enzymes. Existing as a homodimer within the cell, USP14 functions to cleave ubiquitin residues from both ubiquitinylated proteins and ubiquitin-fused precursors, thereby saving these proteins from proteasomal degradation. In mice, defects or mutations in the gene encoding USP14 cause retarded growth or fetal death, indicating that USP14 plays a key role in early developmental processes. Multiple isoforms of USP14 are expressed due to alternative splicing events.

Immunogen: Recombinant protein within Human USP14 aa 351-494 / 494.

Positive control: K562 cell lysate, Hela cell lysate, zebrafish lysates, A431, HUVEC, LOVO, human colon carcinoma tissue, human kidney tissue, mouse prostate tissue, rat epididymis tissue, Jurkat.

Subcellular location: Cytoplasm. Cell membrane.

Database links: SwissProt: P54578 Human

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

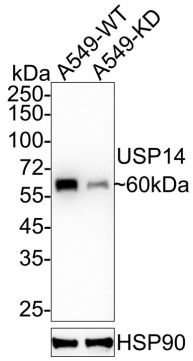

Fig1: Western blot analysis of USP14 on different lysates with Rabbit anti-USP14 antibody (ET1706-37) at 1/1,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si USP14 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 56 kDa
Observed band size: 60 kDa

Exposure time: 12 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 (ET1706-37) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

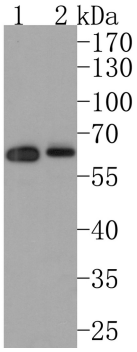

Fig2: Western blot analysis of USP14 on different lysates with Rabbit anti-USP14 antibody (ET1706-37) at 1/500 dilution.

Lane 1: K562 cell lysate
Lane 2: Hela cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 56 kDa
Observed band size: 60 kDa

Exposure time: 2 minutes;

10% 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 (ET1706-37) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 200,000 dilution was used for 1 hour at room temperature.

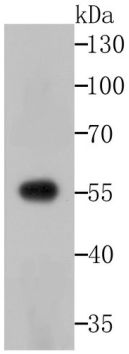

Fig3: Western blot analysis of USP14 on zebrafish lysates with Rabbit anti-USP14 antibody (ET1706-37) at 1/500 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 56 kDa
Observed band size: 60 kDa

Exposure time: 2 minutes;

10% 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 (ET1706-37) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

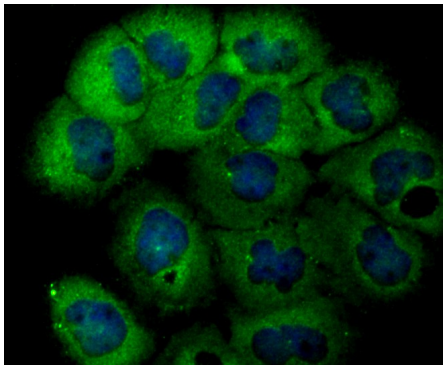

Fig4: Immunocytochemistry analysis of A431 cells labeling USP14 with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

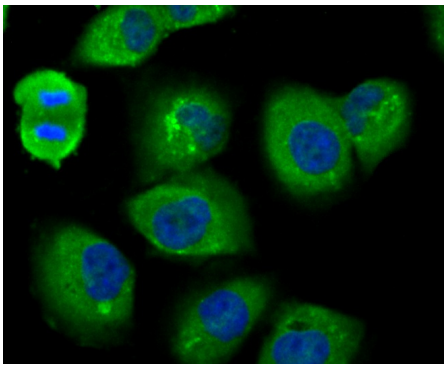

Fig5: Immunocytochemistry analysis of HUVEC cells labeling USP14 with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

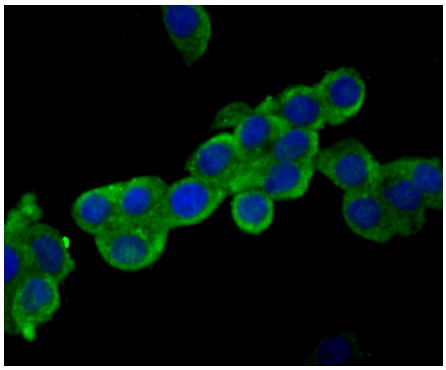

Fig6: Immunocytochemistry analysis of LOVO cells labeling USP14 with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

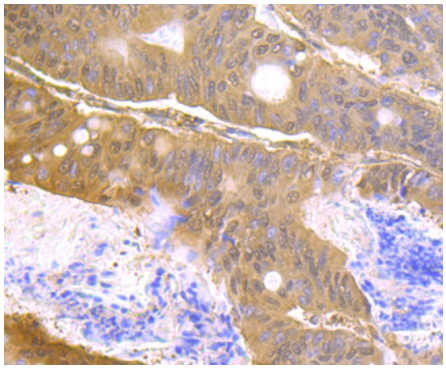

Fig7: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution.

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 (ET1706-37) at 1/50 dilution for 0.5 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.

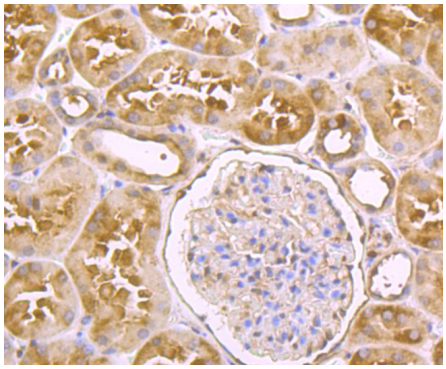

Fig8: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution.

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 (ET1706-37) at 1/50 dilution for 0.5 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.

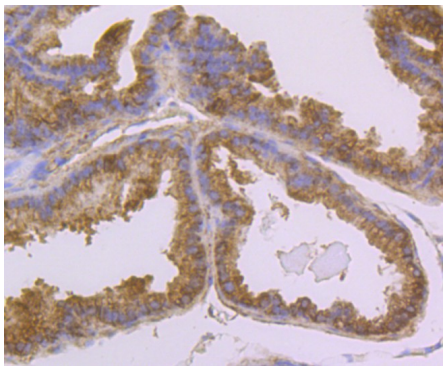

Fig9: Immunohistochemical analysis of paraffin-embedded mouse prostate tissue with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution.

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 (ET1706-37) at 1/50 dilution for 0.5 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.

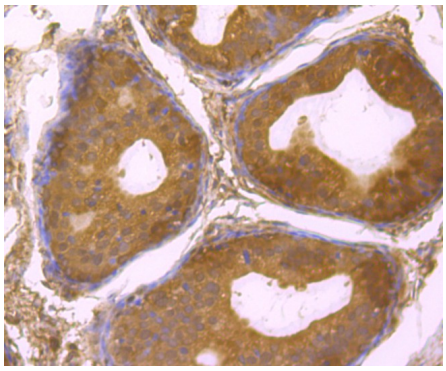

Fig10: Immunohistochemical analysis of paraffin-embedded rat epididymis tissue with Rabbit anti-USP14 antibody (ET1706-37) at 1/50 dilution.

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 (ET1706-37) at 1/50 dilution for 0.5 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.

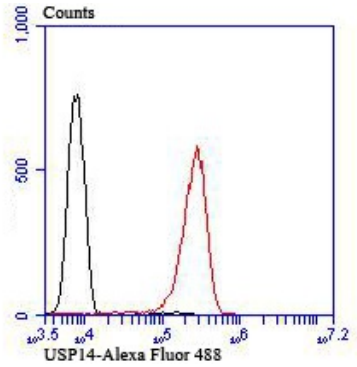

Fig11: Flow cytometric analysis of USP14 was done on Jurkat cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1706-37, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Perry JW et al. Antiviral activity of a small molecule deubiquitinase inhibitor occurs via induction of the unfolded protein response. PLoS Pathog 8:e1002783 (2012).
2. Xu D et al. Phosphorylation and activation of ubiquitin-specific protease-14 by Akt regulates the ubiquitin-proteasome system. Elife 4:e10510 (2015).