Anti-Cyclin B1 Antibody [SU33-03]

ET1608-27

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	SU33-03

Description: G2/mitotic-specific cyclin-B1 is a protein that in humans is encoded by the CCNB1 gene. Cyclin B1 is a regulatory protein involved in mitosis. The gene product complexes with p34 (Cdk1) to form the maturation-promoting factor (MPF). Cyclin B1 contributes to the switch-like all or none behavior of the cell in deciding to commit to mitosis. Its activation is well-regulated, and positive feedback loops ensure that once the cyclin B1-Cdk1 complex is activated, it is not deactivated. Cyclin B1-Cdk1 is involved in the early events of mitosis, such as chromosome condensation, nuclear envelope breakdown, and spindle pole assembly. Once activated, cyclin B1-Cdk1 promotes several of the events of early mitosis. The active complex phosphorylates and activates 13S condensin, which helps to condense chromosomes. Another important function of the cyclin B1-Cdk1 complex is to break down the nuclear envelope. Phosphorylation of the lamins by cyclin B1-Cdk1 causes them to dissociate, compromising the structural integrity of the nuclear envelope so that it breaks down. The destruction of the nuclear envelope is important because it allows the mitotic spindle to access the chromosomes.

Immunogen: Synthetic peptide within Human Cyclin B1 aa 384-433 / 433.

Positive control: HeLa cell lysate, Jurkat cell lysate, HepG2 cell lysate, human tonsil tissue, human colon carcinoma tissue, human lymph nodes tissue, human breast carcinoma tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P14635 Human

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:400
IP	Use at an assay dependent concentration.

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

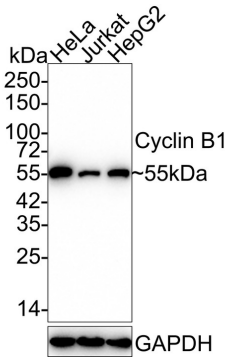

Fig1: Western blot analysis of Cyclin B1 on different lysates with Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: HepG2 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 55 kDa

Exposure time: 3 minutes 10 seconds;

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 (ET1608-27) at 1/5,000 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.

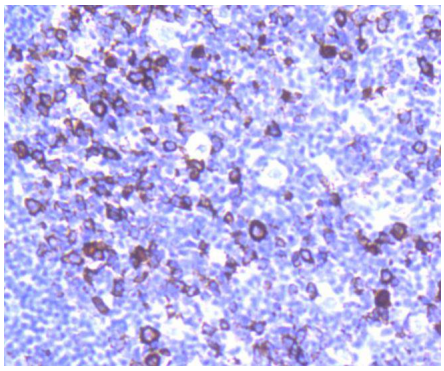

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Cyclin B1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-27, 1/50) for 30 minutes 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.

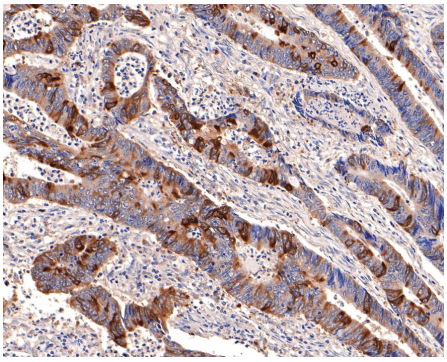

Fig3: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/400 dilution.

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 (ET1608-27) at 1/400 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.

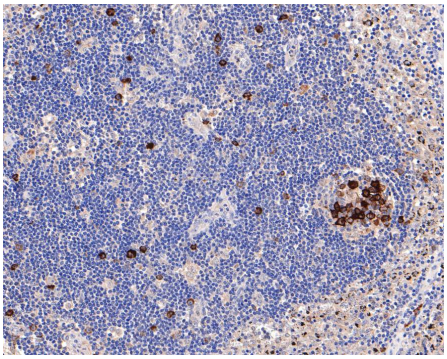

Fig4: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/400 dilution.

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 (ET1608-27) at 1/400 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.

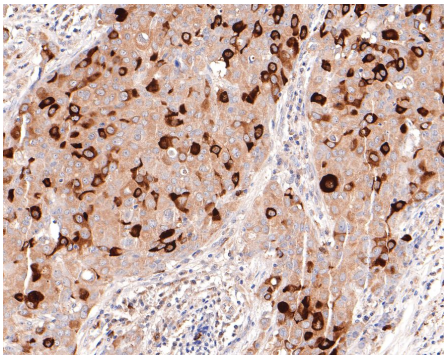

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/400 dilution.

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 (ET1608-27) at 1/400 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.

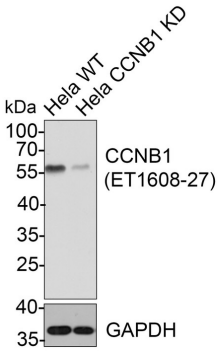

Fig6: All lanes: Western blot analysis of CCNB1 with anti-CCNB1 antibody[SU33-03] (ET1608-27) at 1:500 dilution.
Lane 1: Wild-type Hela whole cell lysate (10 µg).
Lane 2: CCNB1 knockdown Hela whole cell lysate (10 µg).

ET1608-27 was shown to specifically react with CCNB1 in wild-type Hela cells. Weakened bands were observed when CCNB1 knockdown samples were tested. Wild-type and CCNB1 knockdown 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 (ET1608-27, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

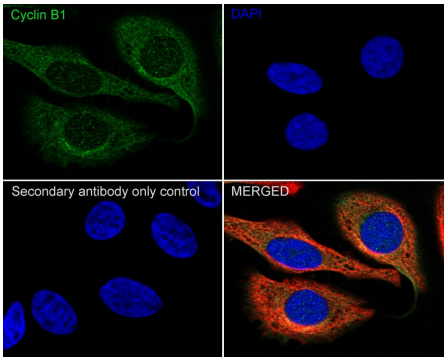

Fig7: Immunocytochemistry analysis of HeLa cells labeling Cyclin B1 with Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/100 dilution.

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 Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

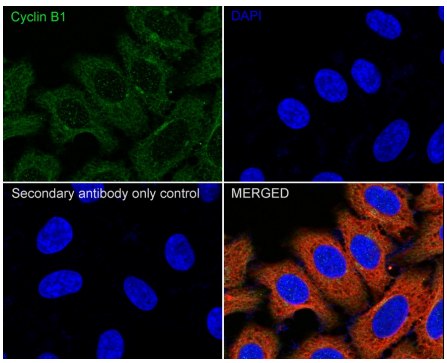

Fig8: Immunocytochemistry analysis of HePG2 cells labeling Cyclin B1 with Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/50 dilution.

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 Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Nabti I et al. Dual-mode regulation of the APC/C by CDK1 and MAPK controls meiosis I progression and fidelity. J Cell Biol 204:891-900 (2014).
2. Penas C et al. Casein kinase 1d-dependent Wee1 protein degradation. J Biol Chem 289:18893-903 (2014).