

Anti-Cyclin D1 Antibody [SA38-08]

ET1601-31



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 34 kDa
Clone number:	SA38-08

Description: The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance throughout the cell cycle. Cyclins function as regulators of CDKs (Cyclin-dependent kinase). Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition. This protein has been shown to interact with tumor suppressor protein Rb and the expression of this gene is regulated positively by Rb. Mutations, amplification and overexpression of this gene, which alters cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis. Micrograph of cyclin D1 staining in a mantle cell lymphoma. Immunohistochemical staining of cyclin D1 antibodies is used to diagnose mantle cell lymphoma. Cyclin D1 has been found to be overexpressed in breast carcinoma. Its potential use as a biomarker was suggested.

Immunogen: Synthetic peptide within C-terminal human Cyclin D1.

Positive control: MCF7 cell lysate, K-562 cell lysate, A431 cell lysate, Neuro-2a cell lysate, NIH/3T3 cell lysate, C6 cell lysate, SH-SY5Y cell lysate, Neuro-2a, MCF7, human tonsil tissue, human colon carcinoma tissue, human liver carcinoma tissue, human small intestine tissue.

Subcellular location: Cytoplasm, Nucleus, Membrane, Mitochondrion

Database links: SwissProt: P24385 Human | P25322 Mouse | P39948 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:2,000
IF-Tissue	1:200-1:500
IHC-P	1:200-1:1,000
IP	Use at an assay dependent concentration.
FC	1:5,000

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

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

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

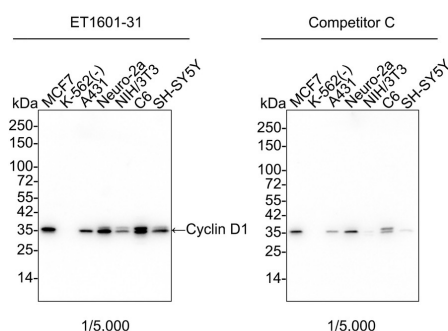
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Western blot analysis of Cyclin D1 on different lysates with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/5,000 dilution and competitor's antibody at 1/5,000 dilution.



Lane 1: MCF7 cell lysate
 Lane 2: K-562 cell lysate (negative)
 Lane 3: A431 cell lysate
 Lane 4: Neuro-2a cell lysate
 Lane 5: NIH/3T3 cell lysate
 Lane 6: C6 cell lysate
 Lane 7: SH-SY5Y cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 34 kDa
 Observed band size: 35 kDa

Exposure time: 20 seconds; ECL: K1802;
 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 (ET1601-31) at 1/5,000 dilution and competitor's antibody at 1/5,000 dilution were used in 5% NFDm/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

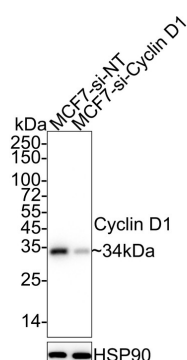
Fig2: Western blot analysis of Cyclin D1 on different lysates with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/5,000 dilution.

Lane 1: MCF7-si NT cell lysate
 Lane 2: MCF7-si Cyclin D1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 34 kDa
 Observed band size: 34 kDa

Exposure time: 17 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 (ET1601-31) at 1/5,000 dilution was used in 5% NFDm/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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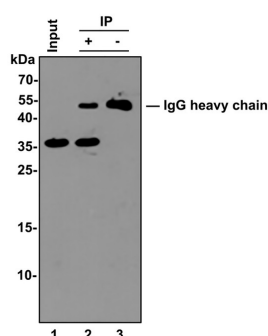


Fig3: Cyclin D1 was immunoprecipitated from 0.5 mg HeLa whole cell lysates with ET1601-31 at 2 µg/mL. Western blot was performed from the immunoprecipitate using ET1601-31 at 1/500 dilution for 45 minutes at room temperature. Goat anti-Rabbit IgG-HRP Secondary Antibody (HA1001) was used at 1:300,000 dilution for 30 minutes at room temperature.

Lane 1: HeLa whole cell lysates at 10 µg;
Lane 2: Cyclin D1 (ET1601-31) IP in HeLa whole cell lysates;
Lane 3: Rabbit IgG instead of Cyclin D1 (ET1601-31) in HeLa whole cell lysates.

Predicted band size: 34 kDa

Observed band size: 34 kDa

Exposure time: 5 minutes;

12% SDS-PAGE gel.

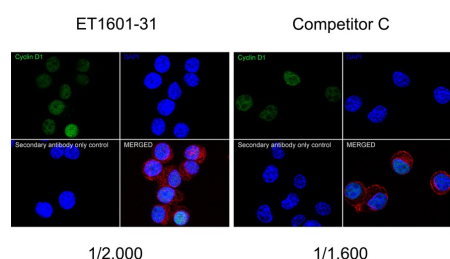


Fig4: Immunocytochemistry analysis of Neuro-2a cells labeling Cyclin D1 with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/2,000 dilution and competitor's antibody at 1/1,600 dilution.

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 D1 antibody (ET1601-31) at 1/2,000 dilution and competitor's antibody at 1/1,600 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

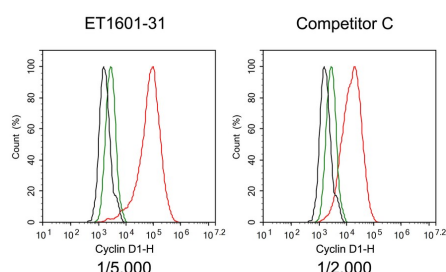


Fig5: Flow cytometric analysis of MCF7 cells labeling Cyclin D1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-31, red) at 1/5,000 dilution and competitor's antibody (red) at 1/2,000 dilution, compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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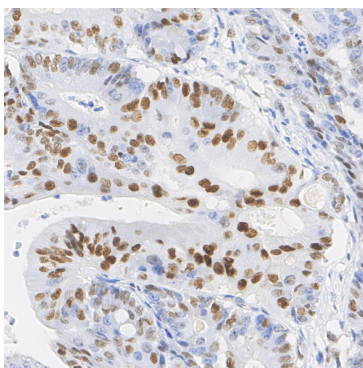


Fig6: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/1,000 dilution.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins. The section was incubated with ET1601-31 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

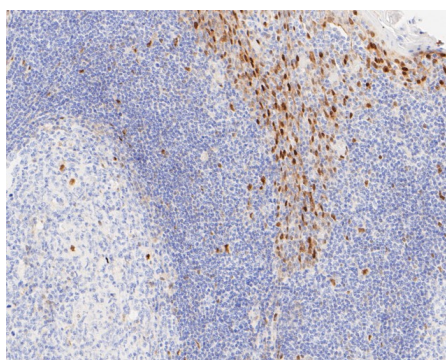


Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Cyclin D1 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1601-31, 1/200) 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.

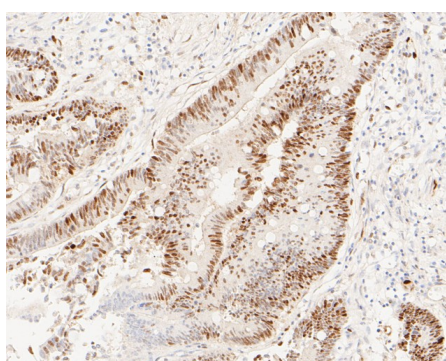


Fig8: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-Cyclin D1 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1601-31, 1/200) 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.

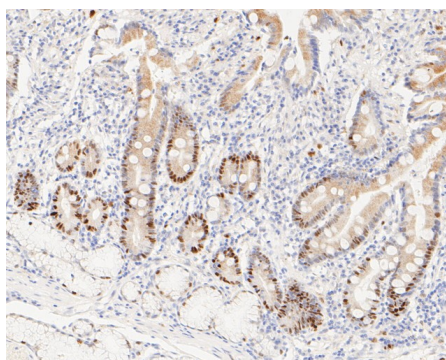


Fig9: Immunohistochemical analysis of paraffin-embedded human small intestine tissue using anti-Cyclin D1 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1601-31, 1/200) 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.

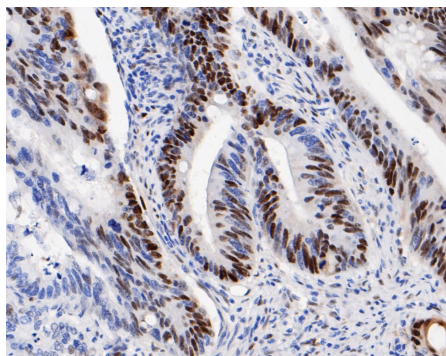


Fig10: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-Cyclin D1 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1601-31, 1/200) 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.

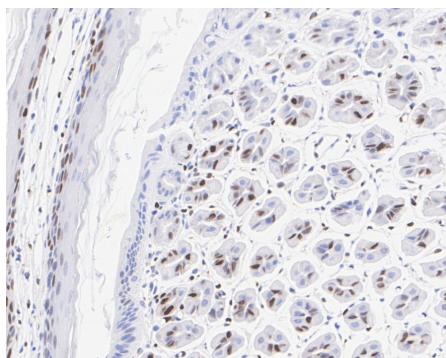


Fig11: Immunohistochemical analysis of paraffin-embedded mouse stomach tissue with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1601-31) 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.

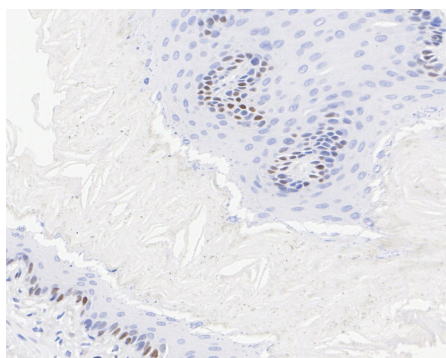
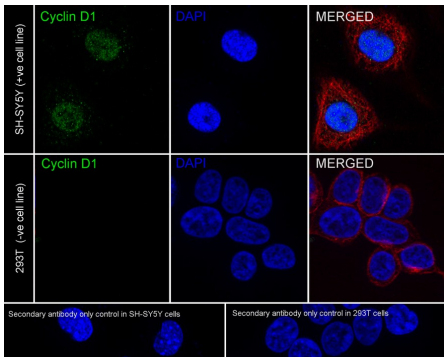


Fig12: Immunohistochemical analysis of paraffin-embedded rat esophagus tissue with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1601-31) 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.

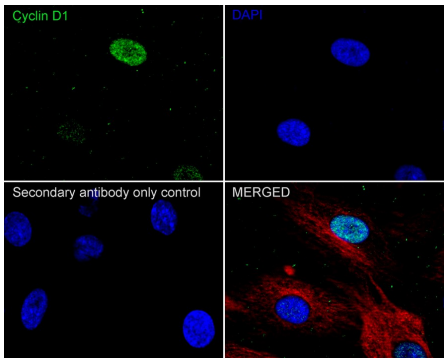
Fig13: Immunocytochemistry analysis of SH-SY5Y (positive) and 293T (negative) labeling Cyclin D1 with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/2,000 dilution.



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 D1 antibody (ET1601-31) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig14: Immunocytochemistry analysis of C6 cells labeling Cyclin D1 with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/2,000 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 D1 antibody (ET1601-31) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

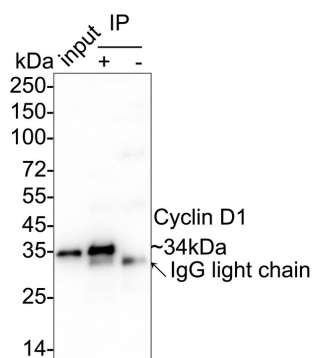


Fig15: Cyclin D1 was immunoprecipitated from 0.2 mg MCF7 cell lysate with ET1601-31 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1601-31 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: MCF7 cell lysate (input)

Lane 2: ET1601-31 IP in MCF7 cell lysate

Lane 3: Rabbit IgG instead of ET1601-31 in MCF7 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 5 seconds; ECL: K1802

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

Background References

1. Totta, P. et al. 2015. Clathrin heavy chain interacts with estrogen receptor α and modulates 17 β -estradiol signaling. *Molecular endocrinology* (Baltimore, Md.). : me20141385.
2. Luo, Y. et al. 2015. Lycorine induces programmed necrosis in the multiple myeloma cell line ARH-77. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine*. 36: 2937-45.

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