Anti-E2F1 Antibody [JJ092-02]

ET1701-73

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: 47 kDa
Clone number:	JJ092-02

Description: The human retinoblastoma gene product appears to play an important role in the negative regulation of cell proliferation. Functional inactivation of Rb can be mediated either through mutation or as a consequence of interaction with DNA tumor virus-encoded proteins. Of all the Rb associations described to date, the identification of a complex between Rb and the transcription factor E2F most directly implicates Rb in regulation of cell proliferation. E2F was originally identified through its role in transcriptional activation of the adenovirus E2 promoter. Sequences homologous to the E2F binding site have been found upstream of a number of genes that encode proteins with putative functions in the G1 and S phases of the cell cycle. E2F-1 is a member of a broader family of transcription regulators including E2F-2, E2F-3, E2F-4, E2F-5, E2F-6 and E2F-7 each of which forms heterodimers with a second protein, DP-1, forming an "active" E2F transcriptional regulatory complex.

Immunogen: Synthetic peptide within Human E2F1 aa 371-404 / 437.

Positive control: HT-29 cell lysate, SW480 cell lysate, SW620 cell lysate, HepG2 cell lysate, NIH/3T3 cell lysate, rat heart tissue lysate, Hela, MCF-7, HepG2, human tonsil tissue, mouse pancreas tissue, rat heart tissue.

Subcellular location: Nucleus.

Database links: SwissProt: Q01094 Human | Q61501 Mouse | O09139 Rat

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:1,000-1:5,000
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

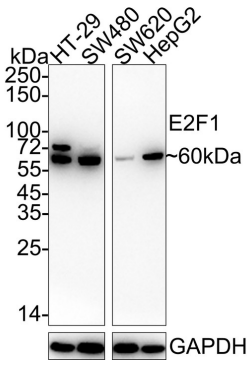


Fig1: Western blot analysis of E2F1 on different lysates with Rabbit anti-E2F1 antibody (ET1701-73) at 1/1,000 dilution.

Lane 1: HT-29 cell lysate
Lane 2: SW480 cell lysate
Lane 3: SW620 cell lysate
Lane 4: HepG2 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 47 kDa
Observed band size: 60 kDa

Exposure time: 3 minutes; 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 (ET1701-73) 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.

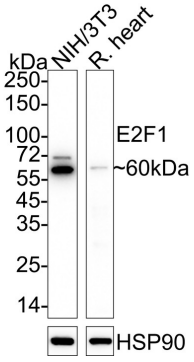


Fig2: Western blot analysis of E2F1 on different lysates with Rabbit anti-E2F1 antibody (ET1701-73) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate (10 µg/Lane)
Lane 2: Rat heart tissue lysate (20 µg/Lane)

Predicted band size: 47 kDa
Observed band size: 60 kDa

Exposure time: 1 minute 2 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 (ET1701-73) 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.

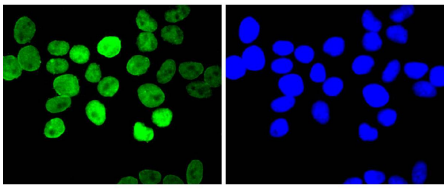


Fig3: ICC staining of E2F1 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-73, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

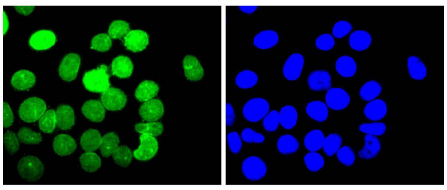


Fig4: ICC staining of E2F1 in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-73, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

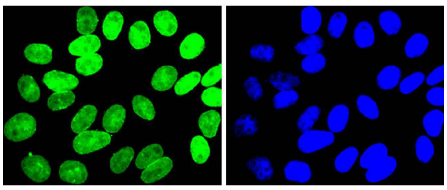


Fig5: ICC staining of E2F1 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-73, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

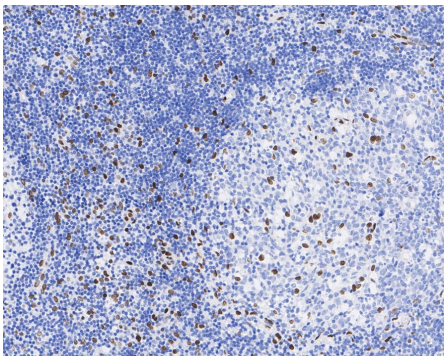


Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-E2F1 antibody (ET1701-73) at 1/1,000 dilution.

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 (ET1701-73) 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.

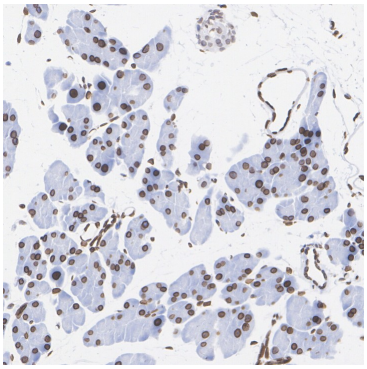


Fig7: Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue with Rabbit anti-E2F1 antibody (ET1701-73) at 1/5,000 dilution.

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 (ET1701-73) at 1/5,000 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.

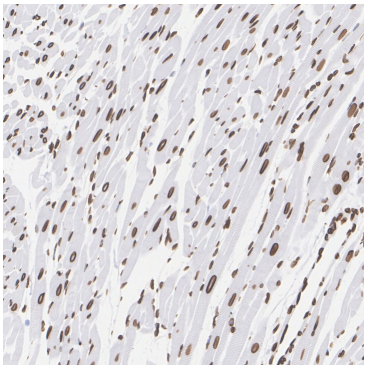


Fig8: Immunohistochemical analysis of paraffin-embedded rat heart tissue with Rabbit anti-E2F1 antibody (ET1701-73) at 1/5,000 dilution.

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 (ET1701-73) at 1/5,000 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.

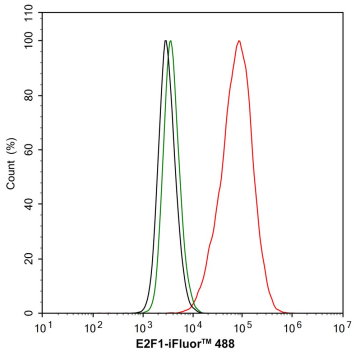


Fig9: Flow cytometric analysis of HepG2 cells labeling E2F1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1701-73, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

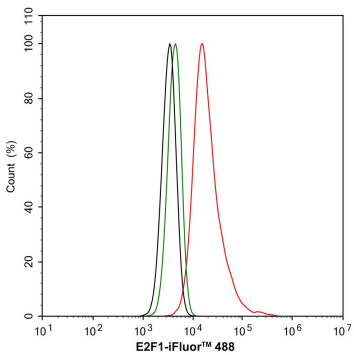


Fig10: Flow cytometric analysis of NIH/3T3 cells labeling E2F1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1701-73, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

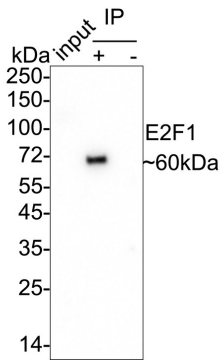


Fig11: E2F1 was immunoprecipitated from 0.2 mg HepG2 cell lysate with ET1701-73 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1701-73 at 1/1,000 dilution. Anti-Rabbit IgG for IP, AlpSdAbs® VHH(HRP) (025-100-005) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HepG2 cell lysate (input)
Lane 2: ET1701-73 IP in HepG2 cell lysate
Lane 3: Rabbit IgG instead of ET1701-73 in HepG2 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 26 seconds; ECL: K1801

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

Background References

1. Peng W & Feng J Long noncoding RNA LUNAR1 associates with cell proliferation and predicts a poor prognosis in diffuse large B-cell lymphoma. Biomed Pharmacother 77:65-71 (2016).
2. Millour J et al. ATM and p53 Regulate FOXM1 Expression via E2F in Breast Cancer Epirubicin Treatment and Resistance. Mol Cancer Ther 10:1046-58 (2011).