Anti-Hes1 Antibody [SC06-21]

ET1610-97

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IHC-Fr
Molecular Wt:	Predicted band size: 30 kDa
Clone number:	SC06-21

Description: Transcription factor HES1 (hairy and enhancer of split-1) is a protein that is encoded by the Hes1 gene, and is the mammalian homolog of the hairy gene in Drosophila. HES1 is one of the seven members of the Hes gene family (HES1-7). Hes genes code nuclear proteins that suppress transcription. This protein belongs to the basic helix-loop-helix (bHLH) family of transcription factors. It is a transcriptional repressor of genes that require a bHLH protein for their transcription. The protein has a particular type of basic domain that contains a helix interrupting protein that binds to the N-box promoter region rather than the canonical enhancer box (E-box). As a member of the bHLH family, it is a transcriptional repressor that influences cell proliferation and differentiation in embryogenesis. HES1 regulates its own expression via a negative feedback loop, and oscillates with approximately 2-hour periodicity.

Immunogen: Synthetic peptide within Human Hes1 aa 298-241 / 280.

Positive control: SH-SY5Y cell lysate, MDA-MB-231 cell lysate, MCF7 cell lysate, SK-MEL-28 cell lysate, SH-SY5Y, mouse brain tissue, rat brain tissue, human breast carcinoma tissue, mouse cerebellum tissue.

Subcellular location: Nucleus.

Database links: SwissProt: Q14469 Human | P35428 Mouse | Q04666 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:2,000
IF-Tissue	1:200
IHC-P	1:50-1:400
FC	1:1:1,000
IHC-Fr	1:500

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

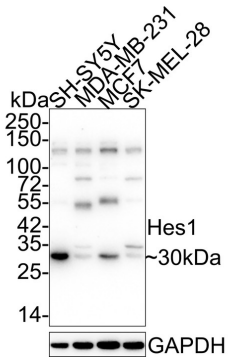

Fig1: Western blot analysis of Hes1 on different lysates with Rabbit anti-Hes1 antibody (ET1610-97) at 1/5,000 dilution.

Lane 1: SH-SY5Y cell lysate
Lane 2: MDA-MB-231 cell lysate
Lane 3: MCF7 cell lysate
Lane 4: SK-MEL-28 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 30 kDa
Observed band size: 30 kDa

Exposure time: 1 minute 20 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 (ET1610-97) 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.

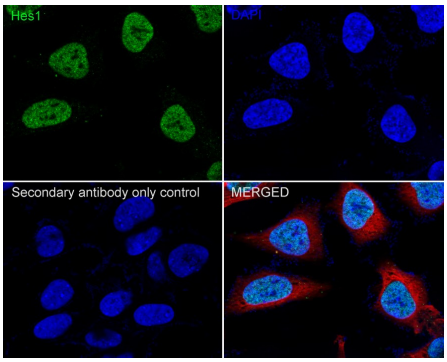

Fig2: Immunocytochemistry analysis of SH-SY5Y cells labeling Hes1 with Rabbit anti-Hes1 antibody (ET1610-97) at 1/2,000 dilution.

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-Hes1 antibody (ET1610-97) at 1/2,000 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.

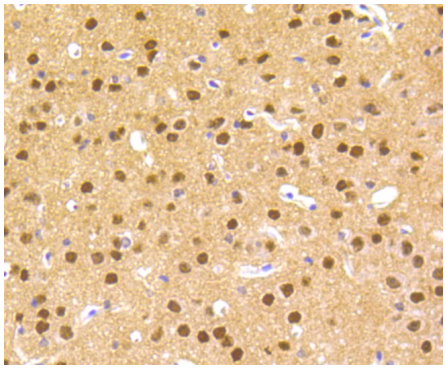

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-Hes1 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-97, 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.

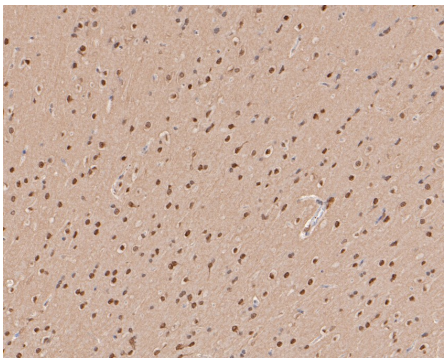

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue using anti-Hes1 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-97, 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.

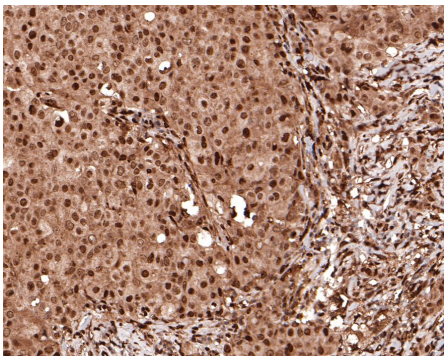

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Hes1 antibody (ET1610-97) at 1/400 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1610-97) 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.

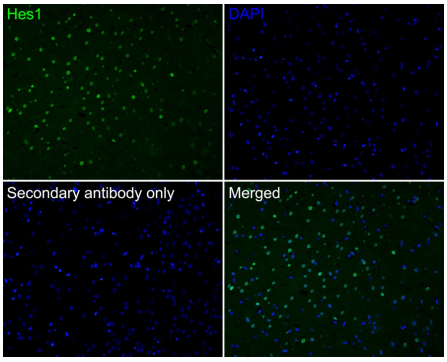

Fig6: Immunofluorescence analysis of paraffin-embedded rat brain tissue labeling Hes1 with Rabbit anti-Hes1 antibody (ET1610-97) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1610-97, green) at 1/200 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

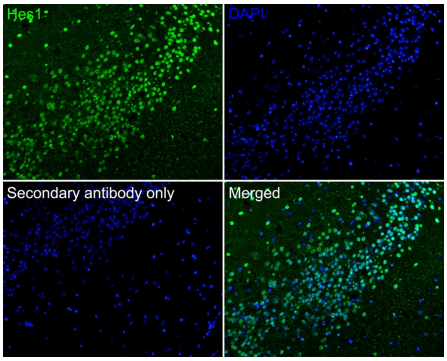

Fig7: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling Hes1 with Rabbit anti-Hes1 antibody (ET1610-97) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1610-97, green) at 1/200 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

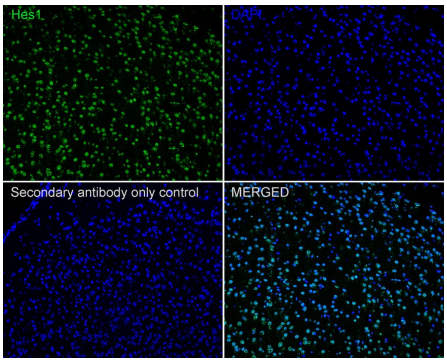

Fig8: Immunofluorescence analysis of frozen mouse cerebellum tissue with Rabbit anti-Hes1 antibody (ET1610-97) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1610-97, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

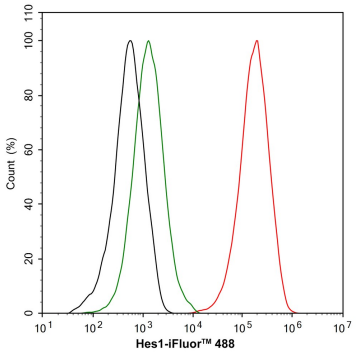

Fig9: Flow cytometric analysis of SH-SY5Y cells labeling Hes1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1610-97, 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).

Background References

1. Liu W et al. MicroRNA-206 overexpression promotes apoptosis, induces cell cycle arrest and inhibits the migration of human hepatocellular carcinoma HepG2 cells. Int J Mol Med 34:420-8 (2014).
2. Tang Y et al. Rnd3 regulates lung cancer cell proliferation through notch signaling. PLoS One 9:e111897 (2014).