Anti-Beta Catenin Antibody [SA30-04]

ET1601-5

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, IP, mIHC, IF-Cell, IHC-Fr, FC
Molecular Wt:	Predicted band size: 85 kDa
Clone number:	SA30-04

Description: Catenin beta-1, also known as beta-catenin (β-catenin), is a protein that in humans is encoded by the CTNNB1 gene. Beta-catenin is a dual function protein, involved in regulation and coordination of cell–cell adhesion and gene transcription. In humans, the CTNNB1 protein is encoded by the CTNNB1 gene. In Drosophila, the homologous protein is called armadillo. β-catenin is a subunit of the cadherin protein complex and acts as an intracellular signal transducer in the Wnt signaling pathway. Mutations and overexpression of β-catenin are associated with many cancers, including hepatocellular carcinoma, colorectal carcinoma, lung cancer, malignant breast tumors, ovarian and endometrial cancer. Alterations in the localization and expression levels of beta-catenin have been associated with various forms of heart disease, including dilated cardiomyopathy. β-catenin is regulated and destroyed by the beta-catenin destruction complex, and in particular by the adenomatous polyposis coli (APC) protein, encoded by the tumour-suppressing APC gene. Therefore, genetic mutation of the APC gene is also strongly linked to cancers, and in particular colorectal cancer resulting from familial adenomatous polyposis (FAP).

Immunogen: Synthetic peptide within human Beta-Catenin aa 30-70.

Positive control: SW480 cell lysate, A431 cell lysate, HT-29 cell lysates, NIH/3T3 cell lysate, rat brain tissue lysate, mouse pancreas, mouse liver, human colon cancer tissue, mouse colon tissue, A431, C6.

Subcellular location: Cytoplasm, Nucleus, Cell membrane, Cell junction

Database links: SwissProt: P35222 Human | Q02248 Mouse | Q9WU82 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:200-1:1,000
IF-Tissue	1:100
IP	1-2μg/sample
mIHC	1:2,000
IF-Cell	1:100
IHC-Fr	1:200
FC	1:1,000

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

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

Purity: Protein A affinity purified.

Images

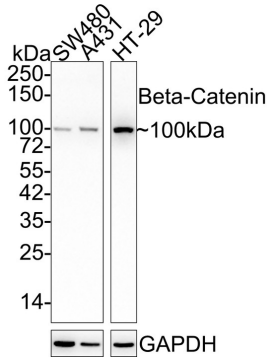

Fig1: Western blot analysis of Beta Catenin on different lysates with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/2,000 dilution.

Lane 1: SW480 cell lysate
Lane 2: A431 cell lysate
Lane 3: HT-29 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 85 kDa
Observed band size: 100 kDa

Exposure time: 3 minutes;

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 (ET1601-5) at 1/2,000 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.

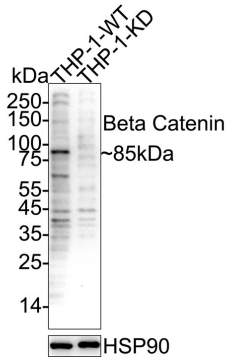

Fig2: Western blot analysis of Beta Catenin on different lysates with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/2,000 dilution.

Lane 1: THP-1-si NT cell lysate
Lane 2: THP-1-si Beta Catenin cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 85 kDa
Observed band size: 85 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 (ET1601-5) at 1/2,000 dilution was used in primary antibody dilution at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

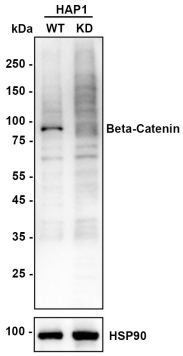

Fig3: Western blot analysis of Beta Catenin on different lysates with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Beta Catenin KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 85 kDa
Observed band size: 85 kDa

Exposure time: 40 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 (ET1601-5) at 1/2,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

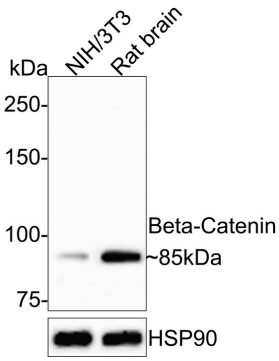

Fig4: Western blot analysis of Beta Catenin on different lysates with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/1,000 dilution.

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

Predicted band size: 85 kDa
Observed band size: 85 kDa

Exposure time: 30 seconds;

6% 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 (ET1601-5) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

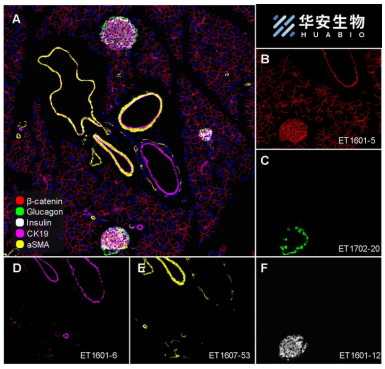

Fig5: Fluorescence multiplex immunohistochemical analysis of mouse pancreas (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-β-catenin (ET1601-5, Red), anti-Glucagon (ET1702-20, Green), anti-Insulin (ET1601-12, White), anti-CK19 (ET1601-6, Magenta) and anti-aSMA (ET1607-53, Yellow) on mouse pancreas. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of ET1601-5 (1/2,000 dilution), ET1702-20 (1/6,000 dilution), ET1601-12 (1/8,000 dilution), ET1601-6 (1/5,000 dilution) and ET1607-53 (1/10,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

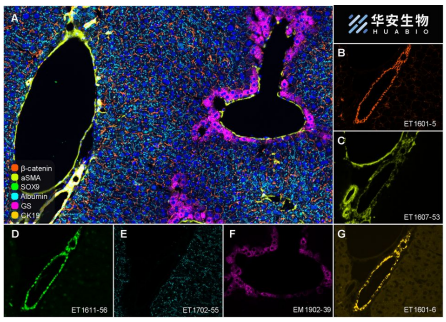

Fig6: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-β-catenin (ET1601-5, Tangerine), anti-αSMA (ET1607-53, Yellow), anti-SOX9 (ET1611-56, Green), anti-Albumin (ET1702-55, Cyan) anti-GS (EM1902-39, Magenta) and anti-CK19 (ET1601-6, Orange) on mouse liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in six rounds of staining: in the order of ET1601-5 (1/2,000 dilution), ET1607-53 (1/3,000 dilution), ET1611-56 (1/1,500 dilution), ET1702-55 (1/3,000 dilution), EM1902-39 (1/2,000 dilution) and ET1601-6 (1/3,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

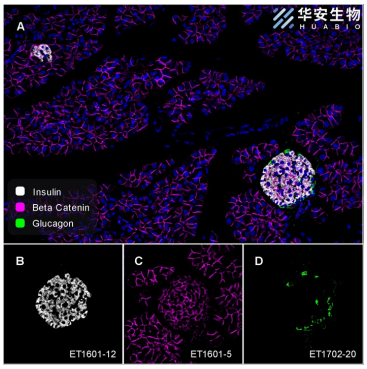

Fig7: Fluorescence multiplex immunohistochemical analysis of mouse pancreas (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Beta Catenin (ET1601-5, Violet), anti-Glucagon (ET1702-20, Green) and anti-Insulin (ET1601-12, White) on pancreas. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1601-5 (1/2,000 dilution), ET1702-20 (1/6,000 dilution) and ET1601-12 (1/8,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

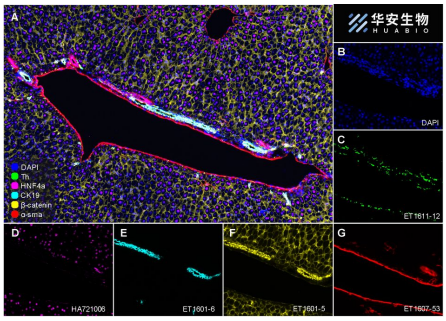

Fig8: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Th (ET1611-12, Green), anti-HNF4a (HA721006, Magenta), anti-CK19 (ET1601-6, Cyan), anti-α-sma (ET1607-53, Red) and anti-β-catenin (ET1601-5, Yellow) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1611-12 (1/1,000 dilution), HA721006 (1/2,000 dilution), ET1601-6 (1/3,000 dilution), ET1607-53 (1/10,000 dilution) and ET1601-5 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

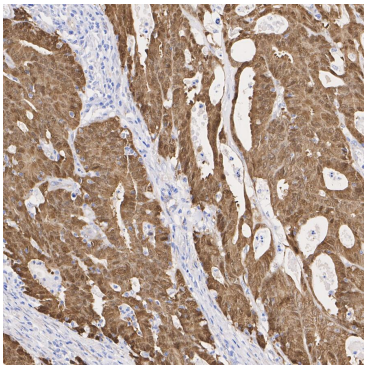

Fig9: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1601-5) 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.

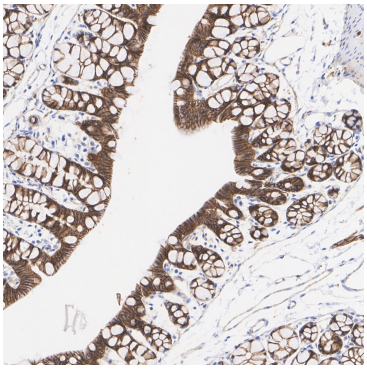

Fig10: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1601-5) 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.

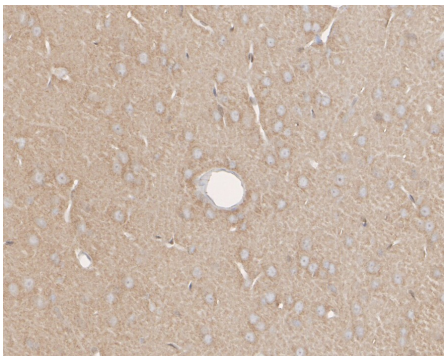

Fig11: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/200 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-5) at 1/200 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.

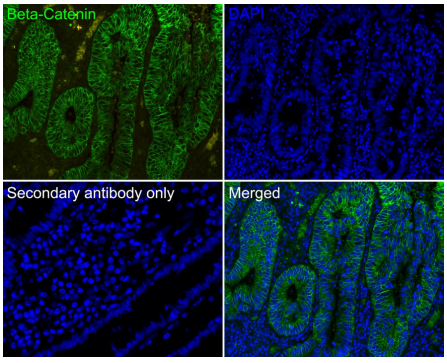

Fig12: Immunofluorescence analysis of paraffin-embedded human colon cancer tissue labeling Beta Catenin with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1601-5, green) at 1/100 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).

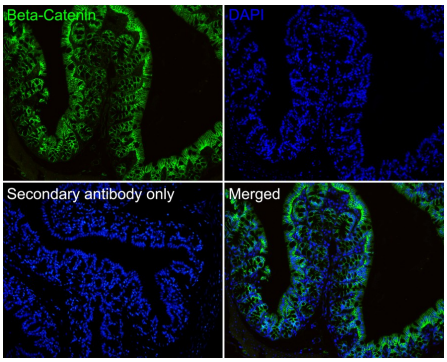

Fig13: Immunofluorescence analysis of paraffin-embedded mouse colon tissue labeling Beta Catenin with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1601-5, green) at 1/100 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).

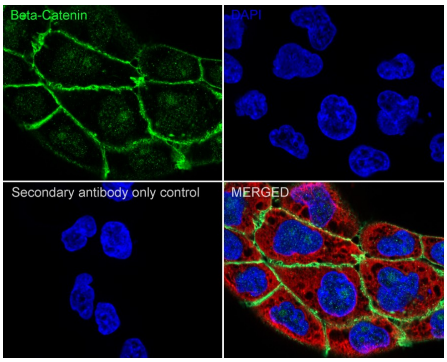

Fig14: Immunocytochemistry analysis of A431 cells labeling Beta Catenin with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/100 dilution.

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-Beta Catenin antibody (ET1601-5) 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.

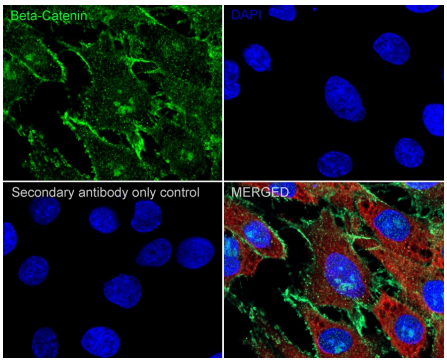

Fig15: Immunocytochemistry analysis of C6 cells labeling Beta Catenin with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/100 dilution.

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-Beta Catenin antibody (ET1601-5) 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.

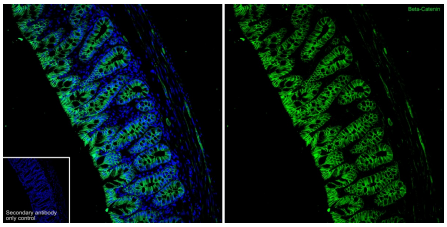

Fig16: Immunofluorescence analysis of frozen mouse colon tissue with Rabbit anti-Beta Catenin antibody (ET1601-5) at 1/200 dilution.

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 (ET1601-5, 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).

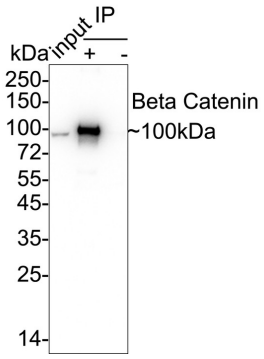

Fig17: Beta Catenin was immunoprecipitated from 0.2 mg rat brain tissue lysate with ET1601-5 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1601-5 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Rat brain tissue lysate (input)
Lane 2: ET1601-5 IP in rat brain tissue lysate
Lane 3: Rabbit IgG instead of ET1601-5 in rat brain tissue lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 2 seconds; ECL: K1801

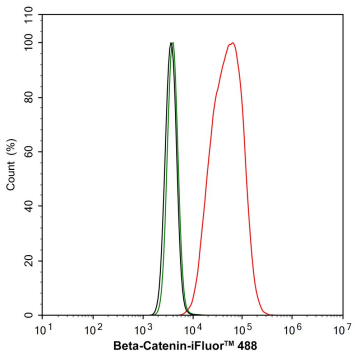

Fig18: Flow cytometric analysis of A431 cells labeling Beta Catenin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-5, 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).

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

Background References

1. "AlphaT-catenin: a novel tissue-specific beta-catenin-binding protein mediating strong cell-cell adhesion."Janssens B., Goossens S., Staes K., Gilbert B., van Hengel J., Colpaert C., Bruyneel E., Mareel M., van Roy F. J. Cell Sci. 114:3177-3188(2001).
2. "Characterisation of the phosphorylation of beta-catenin at the GSK-3 priming site Ser45." Hagen T., Vidal-Puig A. Biochem. Biophys. Res. Commun. 294:324-328(2002).

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