Cytoskeletal Marker Antibody Sampler Kit

HAK21084

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Desmin [ET1606-30]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,FC,mIHC	H,M,R	54 kDa
Cytokeratin 17 [ET1602-16]	20µl	WB,IF-Cell,IF-Tissue,IHC-P	H,M,R	48 kDa
pan Cytokeratin [HA601138]	20µl	IHC-P,mIHC,IF-Cell,IF-Tissue,WB,FC	H	
Vimentin [ET1610-39]	20µl	WB,IHC-P,IHC-Fr,IF-Tissue,IF-Cell,FC,IP	H,M,R	54 kDa
beta Actin [HA722023]	20µl	WB,IF-Cell,FC,IP	H,M,R	42 kDa
beta Tubulin [ET1602-4]	20µl	WB,IF-Cell,IHC-P,FC	H,M,R	50 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100µl	WB,ELISA,IHC-P	Rab	
HRP-Goat Anti-Mouse IgG (H+L) [HA1006]	100µl	WB,ELISA,IHC-P	M	

Description: The Cytoskeletal Marker Antibody Sampler Kit provides an economical means to evaluate the presence and status of select cytoskeleton associated proteins.

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Background The cytoskeleton consists of three different types of cystosolic fibers: microtubules, microfilaments (actin) and intermediate filaments. Actin, a ubiquitous eukaryotic protein, is the major component of the cytoskeleton. At least six isoforms are known in mammals. Nonmuscle β- and γ-actin, also known as cytoplasmic actin, are predominantly expressed in nonmuscle cells, controlling cell structure and motility. Major types of intermediate filaments are distinguished in part by the tissue in which they are expressed, for example; cytokeratins (epithelial cells), vimentin (mesenchyme origin), and desmin (skeletal, visceral and certain vascular smooth muscle cells). Keratin heterodimers composed of an acidic keratin (or type I keratin, keratins 9 to 23) and a basic keratin (or type II keratin, keratins 1 to 8) assemble to form intermediate filaments. Research studies have demonstrated that vimentin is present in sarcomas, but not carcinomas, and its expression is examined relative to other markers in order to distinguish between the two forms of neoplasm. Desmin is a myogenic marker expressed in early development that forms a network of filaments that extends across the myofibril and surrounds Z discs. α/β-tubulin heterodimers form the tubulin subunit that comprises the microtubule building block.

Database links: UniProt ID: P17661, P31001, P48675, Q04695, Q9QWL7, Q6IFU8, Q01546, Q7Z794, P48668, P13645, P08670, P20152, P31000, P60709, P60710, P60711, P07437, P99024, P69897

Images

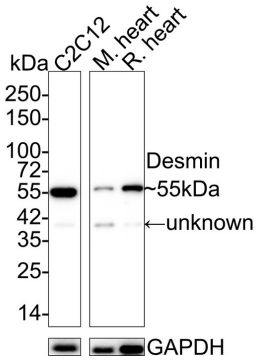

Fig1: Western blot analysis of Desmin on different lysates with Rabbit anti-Desmin antibody (ET1606-30) at 1/20,000 dilution.

Lane 1: C2C12 cell lysate
Lane 2: Mouse heart tissue lysate
Lane 3: Rat heart tissue lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 55 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 (ET1606-30) at 1/20,000 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.

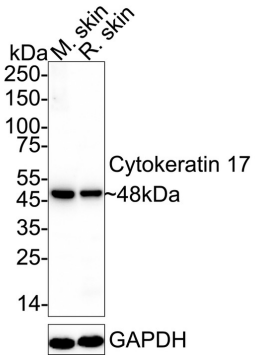

Fig2: Western blot analysis of Cytokeratin 17 on different lysates with Rabbit anti-Cytokeratin 17 antibody (ET1602-16) at 1/1,000 dilution.

Lane 1: Mouse skin tissue lysate
Lane 2: Rat skin tissue lysate

Lysates/proteins at 40 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 48 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 (ET1602-16) 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.

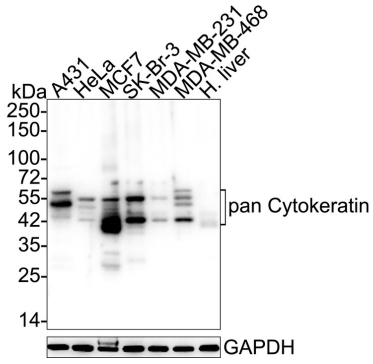

Fig3: Western blot analysis of pan Cytokeratin on different lysates with Mouse anti-pan Cytokeratin antibody (HA601138) at 1/2,000 dilution.

Lane 1: A431 cell lysate (10 µg/Lane)
Lane 2: HeLa cell lysate (10 µg/Lane)
Lane 3: MCF7 cell lysate (10 µg/Lane)
Lane 4: SK-Br-3 cell lysate (10 µg/Lane)
Lane 5: MDA-MB-231 cell lysate (10 µg/Lane)
Lane 6: MDA-MB-468 cell lysate (10 µg/Lane)
Lane 7: Human liver tissue lysate (20 µg/Lane)

Exposure time: 4 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 (HA601138) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

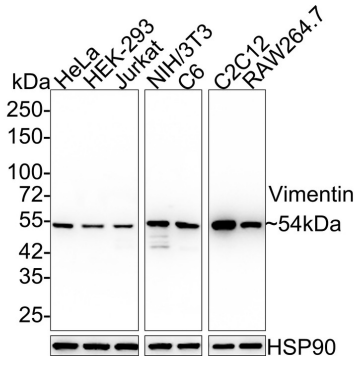

Fig4: Western blot analysis of Vimentin on different lysates with Rabbit anti-Vimentin antibody (ET1610-39) at 1/20,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: HEK-293 cell lysate (10 µg/Lane)
Lane 3: Jurkat cell lysate (10 µg/Lane)
Lane 4: NIH/3T3 cell lysate (10 µg/Lane)
Lane 5: C6 cell lysate (10 µg/Lane)
Lane 6: C2C12 cell lysate (10 µg/Lane)
Lane 7: RAW264.7 cell lysate (10 µg/Lane)

Predicted band size: 54 kDa
Observed band size: 54 kDa

Exposure time: Lane 1-5: 3 seconds; Lane 6-7: 14 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 (ET1610-39) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

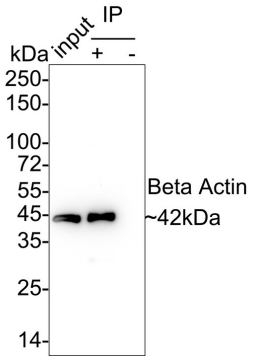

Fig5: beta Actin was immunoprecipitated from 0.2 mg NIH/3T3 cell lysate with HA722023 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA722023 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH/3T3 cell lysate (input)
Lane 2: HA722023 IP in NIH/3T3 cell lysate
Lane 3: Rabbit IgG instead of HA722023 in NIH/3T3 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute 2 seconds; ECL: K1801

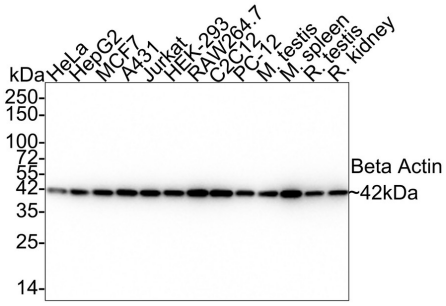

Fig6: Western blot analysis of beta Actin on different lysates with Rabbit anti-beta Actin antibody (HA722023) at 1/20,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: HepG2 cell lysate (10 µg/Lane)
Lane 3: MCF7 cell lysate (10 µg/Lane)
Lane 4: A431 cell lysate (10 µg/Lane)
Lane 5: Jurkat cell lysate (10 µg/Lane)
Lane 6: HEK-293 cell lysate (10 µg/Lane)
Lane 7: RAW264.7 cell lysate (10 µg/Lane)
Lane 8: C2C12 cell lysate (10 µg/Lane)
Lane 9: PC-12 cell lysate (10 µg/Lane)
Lane 10: Mouse testis tissue lysate (10 µg/Lane)
Lane 11: Mouse spleen tissue lysate (10 µg/Lane)
Lane 12: Rat testis tissue lysate (10 µg/Lane)
Lane 13: Rat kidney tissue lysate (10 µg/Lane)

Predicted band size: 42 kDa
Observed band size: 42 kDa

Exposure time: 3 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 (HA722023) at 1/20,000 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.

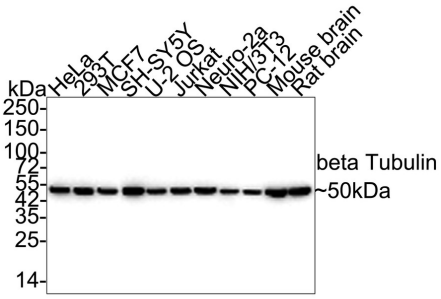

Fig7: Western blot analysis of beta Tubulin on different lysates with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/10,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: 293T cell lysate (15 µg/Lane)
Lane 3: MCF7 cell lysate (15 µg/Lane)
Lane 4: SH-SY5Y cell lysate (15 µg/Lane)
Lane 5: U-2 OS cell lysate (15 µg/Lane)
Lane 6: Jurkat cell lysate (15 µg/Lane)
Lane 7: Neuro-2a cell lysate (15 µg/Lane)
Lane 8: NIH/3T3 cell lysate (15 µg/Lane)
Lane 9: PC-12 cell lysate (15 µg/Lane)
Lane 10: Mouse brain tissue lysate (20 µg/Lane)
Lane 11: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 50 kDa
Observed band size: 50 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 (ET1602-4) at 1/10,000 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.

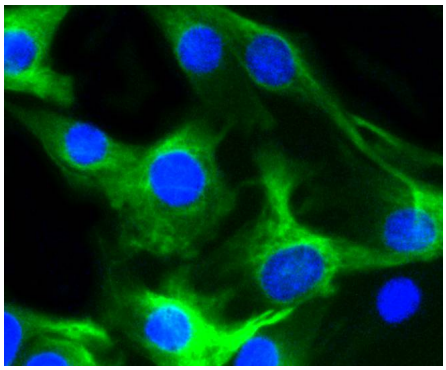

Fig8: ICC staining of Desmin in C2C12 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1606-30, 1/200) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

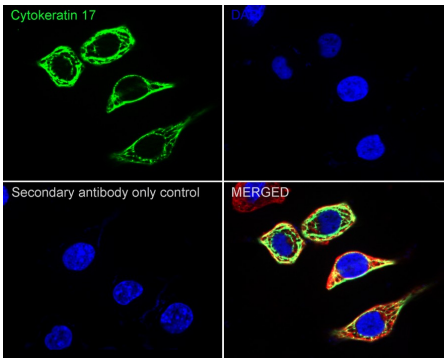

Fig9: Immunocytochemistry analysis of SiHa cells labeling Cytokeratin 17 with Rabbit anti-Cytokeratin 17 antibody (ET1602-16) at 1/1,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Cytokeratin 17 antibody (ET1602-16) at 1/1,000 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 (HA601187, 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.

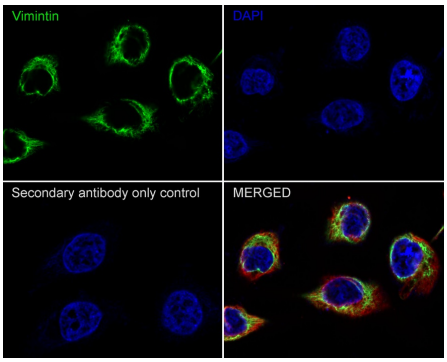

Fig10: Immunocytochemistry analysis of HeLa cells labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Vimentin antibody (ET1610-39) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. 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.

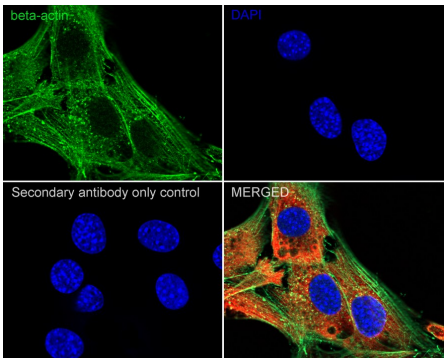

Fig11: Immunocytochemistry analysis of NIH/3T3 cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/100 dilution.

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 Actin antibody (HA722023) 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.

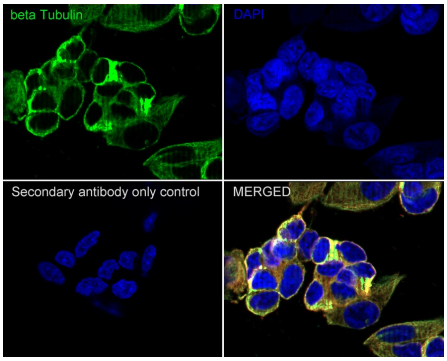

Fig12: Immunocytochemistry analysis of SH-SY5Y cells labeling beta Tubulin with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

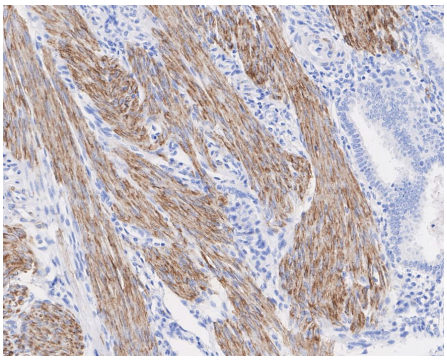

Fig13: Immunohistochemical analysis of paraffin-embedded human endometrium tissue with Rabbit anti-Desmin antibody (ET1606-30) at 1/1,000 dilution.

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 (ET1606-30) 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.

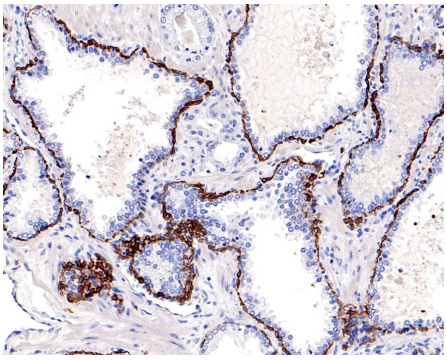

Fig14: Immunohistochemical analysis of paraffin-embedded human prostate tissue using anti-Cytokeratin 17 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-16, 1/400) 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.

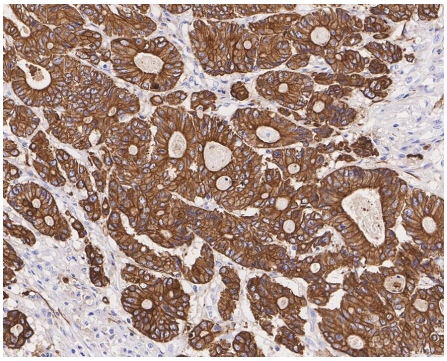

Fig15: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Mouse anti-pan Cytokeratin antibody (HA601138) at 1/2,000 dilution.

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 (HA601138) at 1/2,000 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.

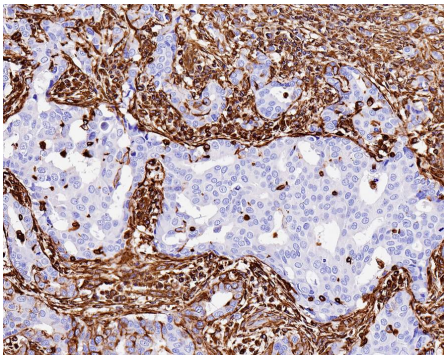

Fig16: Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue with Rabbit anti-Vimentin antibody (ET1610-39) at 1/1,000 dilution.

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 (ET1610-39) 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.

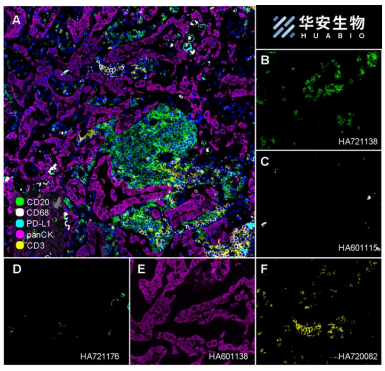

Fig17: Fluorescence multiplex immunohistochemical analysis of the human non-small cell lung cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-CD68 (HA601115, gray), anti-PD-L1 (HA721176, cyan), anti-panCK (HA601138, magenta) and anti-CD3 (HA720082, yellow) on human non-small cell lung cancer. Panel B: anti-CD20 stained on B cells. Panel C: anti-CD68 stained on macrophage M1 and macrophage M2. Panel D: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel E: anti-panCK stained on cancer cells. Panel F: anti-CD3 stained on T cells. 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 HA721138 (1/1,500 dilution), HA601115 (1/2,000 dilution), HA721176 (1/1,000 dilution), HA601138 (1/3,000 dilution), and HA720082 (1/500 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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

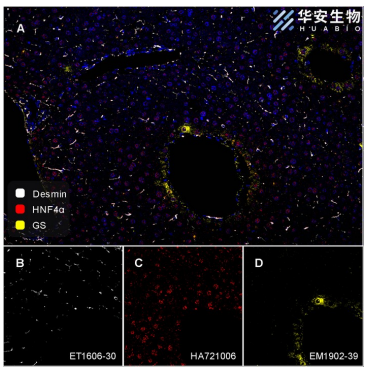

Fig18: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Desmin (ET1606-30, White), anti-HNF4α (HA721006, Red) and anti-GS (EM1902-39, Yellow) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody 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 ET1606-30 (1/800 dilution), HA721006 (1/5,000 dilution) and EM1902-39 (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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

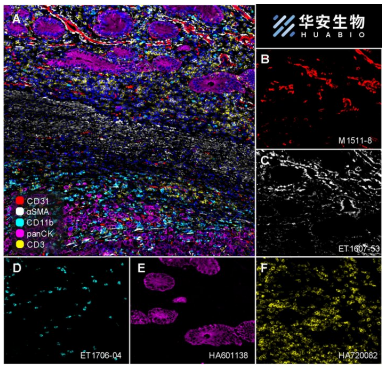

Fig19: Fluorescence multiplex immunohistochemical analysis of the human gastric cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD31 (M1511-8, red), anti-αSMA (ET1607-53, gray), anti-CD11b (ET1706-04, cyan), anti-panCK (HA601138, magenta) and anti-CD3 (HA720082, yellow) on human gastric cancer. Panel B: anti- CD31 stained on the endothelial cells. Panel C: anti-αSMA stained on cancer-associated fibroblasts and smooth muscle cells. Panel D: anti-CD11b stained on myeloid cells. Panel E: anti-panCK stained on cancer cells. Panel F: anti-CD3 stained on T cells. 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 M1511-8 (1/1,000 dilution), ET1607-53 (1/2,000 dilution), ET1706-04 (1/1,000 dilution), HA601138 (1/3,000 dilution), and HA720082 (1/500 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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

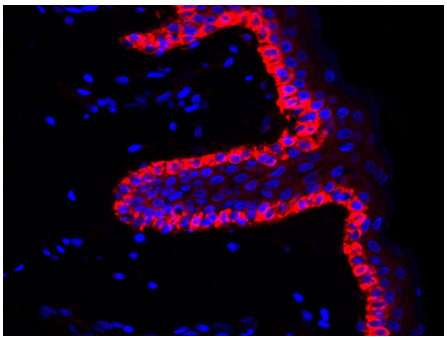

Fig20: Immunofluorescence analysis of paraffin-embedded human skin tissue labeling Cytokeratin 17 (ET1602-16).

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 antibodies Cytokeratin 17 (ET1602-16, red) at 1/200 dilution at +4℃ overnight, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) was used as the secondary antibodies at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

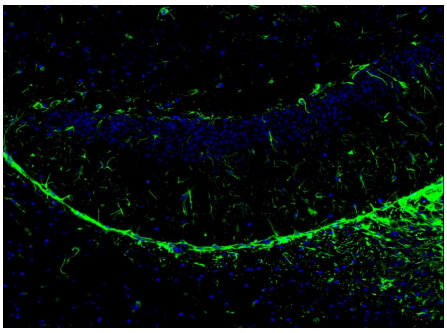

Fig21: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1610-39, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

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

Background References

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