

# Anti-alpha smooth muscle Actin Antibody [SY02-64]

## ET1607-53



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IF-Cell, IF-Tissue, IHC-P, FC, mIHC
<b>Molecular Wt:</b>	Predicted band size: 42 kDa
<b>Clone number:</b>	SY02-64

**Description:** All eukaryotic cells express Actin, which often constitutes as much as 50% of total cellular protein. Actin filaments can form both stable and labile structures and are crucial components of microvilli and the contractile apparatus of muscle cells. While lower eukaryotes, such as yeast, have only one Actin gene, higher eukaryotes have several isoforms encoded by a family of genes. At least six types of Actin are present in mammalian tissues and fall into three classes.  $\alpha$ -Actin expression is limited to various types of muscle, whereas  $\beta$ -Actin and  $\gamma$ -Actin are the principle constituents of filaments in other tissues. Members of the small GTPase family regulate the organization of the Actin cytoskeleton. Rho controls the assembly of Actin stress fibers and focal adhesion. Rac regulates Actin filament accumulation at the plasma membrane. Cdc42 stimulates formation of filopodia.

**Immunogen:** Synthetic peptide within N-terminal human alpha smooth muscle Actin.

**Positive control:** Saos-2 cell lysate, A431 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, Neuro-2a cell lysate, mouse skin tissue lysate, rat skin tissue lysate, human breast carcinoma tissue, HeLa cell lysate, A431 cell lysate, A549 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, L6 cell lysate, mouse heart tissue lysate, mouse skin tissue lysate, rat heart tissue lysate, rat skin tissue lysate, rat smooth muscle tissue lysate, human kidney tissue, human stomach cancer tissue, human pancreatic carcinoma, HepG2, human tonsil tissue, human liver tissue, human gastric cancer, Human non-small cell lung cancer, mouse pancreas, mouse liver, mouse kidney, human stomach cancer tissue, human small intestine tissue, mouse small intestine tissue, rat small intestine tissue, NIH/3T3, PC-12, HepG2.

**Subcellular location:** Cytoplasm.

**Database links:** SwissProt: P62736 Human | P62737 Mouse | P62738 Rat

### Recommended Dilutions:

<b>WB</b>	1:5,000-1:50,000
<b>IF-Cell</b>	1:250-1:2,500
<b>IF-Tissue</b>	1:100-1:500
<b>IHC-P</b>	1:50,000
<b>FC</b>	1:1,000-1:2,000
<b>mIHC</b>	1:2,000-1:10,000

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

**Storage Instruction:** Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

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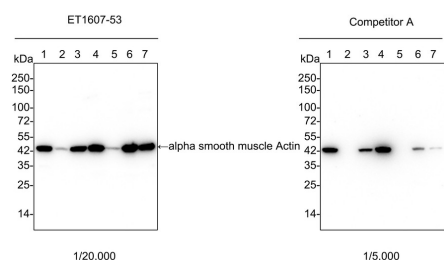
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## Images

**Fig1:** Western blot analysis of alpha smooth muscle Actin on different lysates with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/20,000 dilution and competitor's antibody at 1/2,000 dilution.



Lane 1: Saos-2 cell lysate (15 µg/Lane)  
 Lane 2: A431 cell lysate (15 µg/Lane)  
 Lane 3: NIH/3T3 cell lysate (15 µg/Lane)  
 Lane 4: C2C12 cell lysate (15 µg/Lane)  
 Lane 5: Neuro-2a cell lysate (15 µg/Lane)  
 Lane 6: Mouse skin tissue lysate (30 µg/Lane)  
 Lane 7: Rat skin tissue lysate (30 µg/Lane)

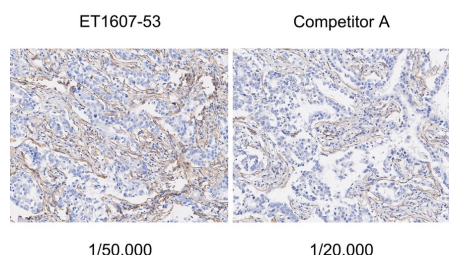
Predicted band size: 42 kDa  
 Observed band size: 42 kDa

Exposure time: 46 seconds

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (ET1607-53) at 1/20,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% NFDN/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:** Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/50,000 dilution and competitor's antibody at 1/20,000 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1607-53) at 1/50,000 dilution and competitor's antibody at 1/20,000 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.

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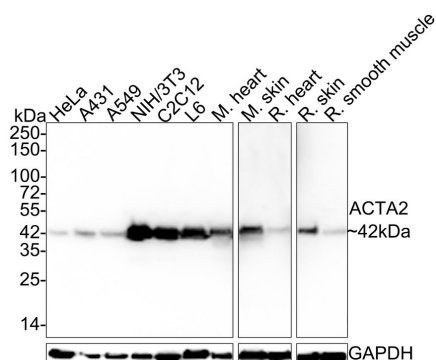
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**Fig3:** Western blot analysis of alpha smooth muscle Actin on different lysates with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/5,000 dilution.



Lane 1: HeLa cell lysate  
 Lane 2: A431 cell lysate  
 Lane 3: A549 cell lysate  
 Lane 4: NIH/3T3 cell lysate  
 Lane 5: C2C12 cell lysate  
 Lane 6: L6 cell lysate  
 Lane 7: Mouse heart tissue lysate  
 Lane 8: Mouse skin tissue lysate  
 Lane 9: Rat heart tissue lysate  
 Lane 10: Rat skin tissue lysate  
 Lane 11: Rat smooth muscle tissue lysate

Lysates/proteins at 20  $\mu$ g/Lane.

Predicted band size: 42 kDa

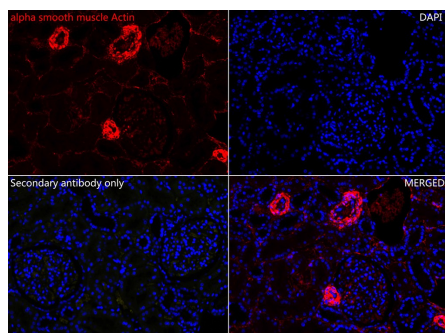
Observed band size: 42 kDa

Exposure time: 5 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 (ET1607-53) at 1/5,000 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.

**Fig4:** Immunofluorescence analysis of paraffin-embedded human kidney tissue labeling alpha smooth muscle Actin (ET1607-53).



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 alpha smooth muscle Actin (ET1607-53, red) at 1/1,000 dilution overnight at 4  $^{\circ}$ C, washed with PBS.

iFluor™ 647 conjugate-Goat anti-Rabbit IgG (HA1123) was used as the secondary antibody at 1/1,000 dilution. DAPI was used as nuclear counterstain.

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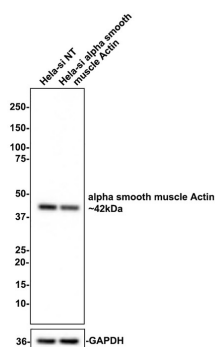
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**Fig5:** Western blot analysis of alpha smooth muscle Actin on different lysates with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/1,000 dilution.

Lane 1: HeLa-si NT cell lysate (10 µg/Lane)

Lane 2: HeLa-si alpha smooth muscle Actin cell lysate (10 µg/Lane)



Predicted band size: 42 kDa

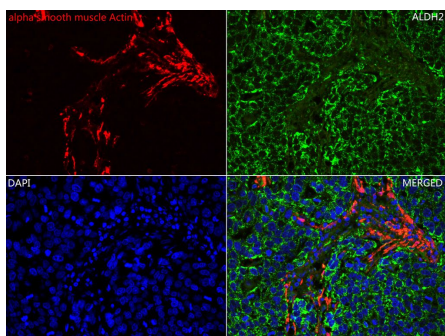
Observed band size: 42 kDa

Exposure time: 5 seconds;

4-20% SDS-PAGE gel.

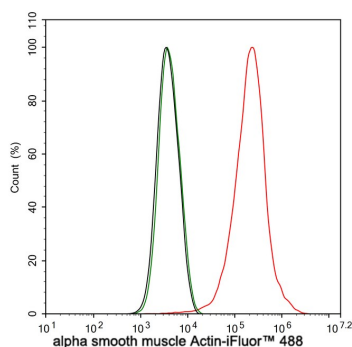
ET1607-53 was shown to specifically react with alpha smooth muscle Actin in HeLa-si NT cells. Weakened band was observed when HeLa-si alpha smooth muscle Actin sample was tested. HeLa-si NT and HeLa-si alpha smooth muscle Actin samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1607-53, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA 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.

**Fig6:** Immunofluorescence analysis of paraffin-embedded human stomach cancer tissue labeling alpha smooth muscle Actin (ET1607-53) and ALDH2 (M1509-1).



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 alpha smooth muscle Actin (ET1607-53, red) at 1/1,000 dilution and ALDH2 (M1509-1, green) at 1/100 dilution overnight at 4 °C, washed with PBS. iFluor™ 488 conjugate-Goat anti-Mouse IgG (HA1125) and iFluor™ 647 conjugate-Goat anti-Rabbit IgG (HA1123) was used as the secondary antibody at 1/1,000 dilution. DAPI was used as nuclear counterstain.

**Fig7:** Flow cytometric analysis of HepG2 cells labeling alpha smooth muscle Actin.



Cells were fixed and permeabilized. Then stained with the primary antibody (ET1607-53, 1/2,000) (red) 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)

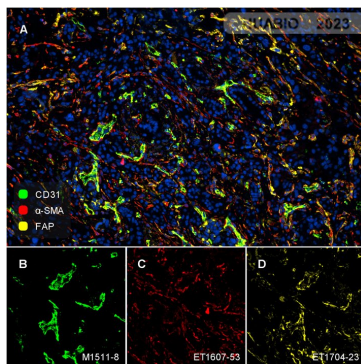
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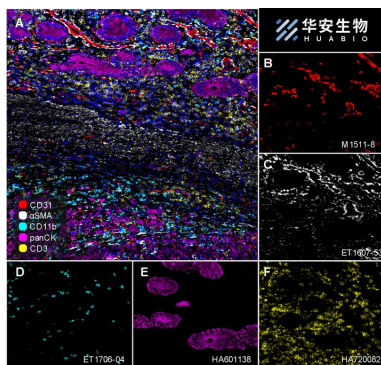
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**Fig8:** Fluorescence multiplex immunohistochemical analysis of the human pancreatic carcinoma (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD31 (M1511-8, green), anti- $\alpha$ -SMA (ET1607-53, red) and anti-FAP (ET1704-23, yellow) on human pancreatic carcinoma. Panel B: anti-CD31 stained on the endothelial cells. Panel C: anti- $\alpha$ -SMA stained on cancer-associated fibroblasts and smooth muscle cells. Panel D: anti-FAP stained on the cancer-associated fibroblasts. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of M1511-8 (1/5000 dilution), ET1704-23 (1/1000 dilution), and ET1607-53 (1/3000 dilution) for 20 mins at room temperature. 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 Nikon ECLIPSE Ni-E microscope.



**Fig9:** 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- $\alpha$ -SMA (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- $\alpha$ -SMA 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 Immunostaining 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.

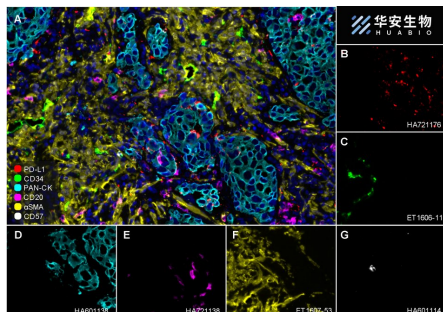
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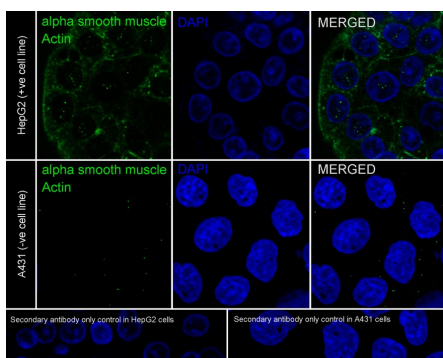
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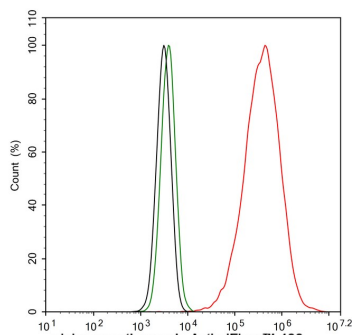


**Fig10:** Fluorescence multiplex immunohistochemical analysis of Human non-small cell lung cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-PD-L1 (HA721176, red), anti-CD34 (ET1606-11, green), anti-Pan-CK (HA601138, cyan), anti-CD20 (HA721138, magenta), anti-αSMA (ET1607-53, yellow) and anti-CD57 (HA601114, white) on NSCLC. Panel B: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel C: anti-CD34 stained on endothelial cells. Panel D: anti-Pan-CK stained on cancer cells. Panel E: CD20 stained on B cells. Panel F: anti-αSMA stained on cancer-associated fibroblasts and smooth muscle cells. Panel G: anti-CD57 stained on NK cells and 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 six rounds of staining: in the order of HA721176 (1/1,000 dilution), ET1606-11 (1/1,000 dilution), HA601138 (1/3,000 dilution), HA721138 (1/2,000 dilution), ET1607-53 (1/3,000 dilution) and HA601114 (1/1,000 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 Olympus VS200 Slide Scanner.



**Fig11:** Immunocytochemistry analysis of HepG2 (positive) and A431 (negative) labeling alpha smooth muscle Actin with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/2,000 dilution.

Cells were fixed in 100% precooled methanol 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-alpha smooth muscle Actin antibody (ET1607-53) 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.



**Fig12:** Flow cytometric analysis of NIH/3T3 cells labeling alpha smooth muscle Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1607-53, 1μg/mL) (red) 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).

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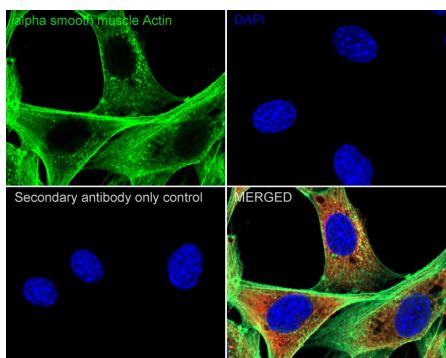
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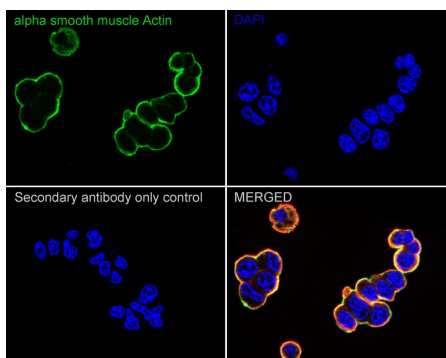
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**Fig13:** Immunocytochemistry analysis of NIH/3T3 cells labeling alpha smooth muscle Actin with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/2,500 dilution.



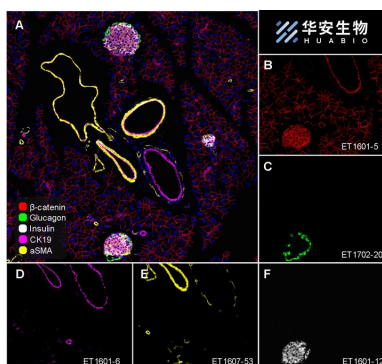
Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/2,500 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 PC-12 cells labeling alpha smooth muscle Actin with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/2,500 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-alpha smooth muscle Actin antibody (ET1607-53) at 1/2,500 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:** 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-αSMA (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



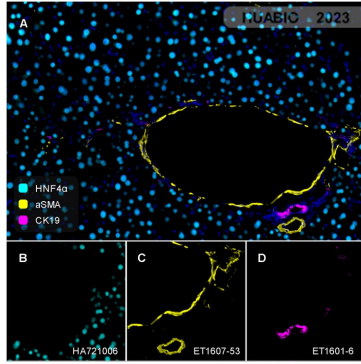
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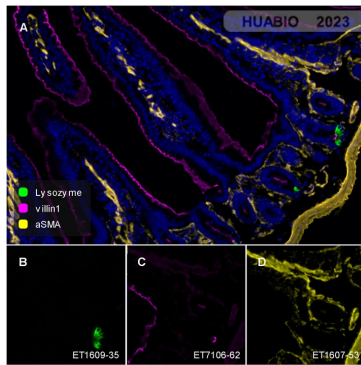
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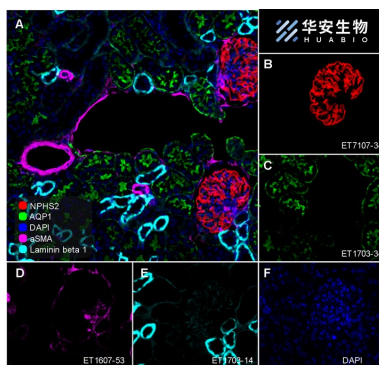
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**Fig16:** Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-HNF4 $\alpha$  (HA721006, Cyan), anti-CK19 (ET1601-6, Magenta) and anti- $\alpha$ SMA (ET1607-53, Yellow) 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 three rounds of staining: in the order of HA721006 (1/5,000 dilution), ET1601-6 (1/10,000 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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.



**Fig17:** Fluorescence multiplex immunohistochemical analysis of mouse small intestine (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Lysozyme (ET1609-35, Green), anti-villin1 (ET7106-62, Magenta) and anti- $\alpha$ SMA (ET1607-53, Yellow) on mouse small intestine. 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 ET1609-35 (1/2,000 dilution), ET7106-62 (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°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 kidney (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NPHS2 (ET7107-34, Red), anti-AQP1 (ET1703-34, Green), anti-Laminin beta 1 (ET1703-14, Cyan) and anti- $\alpha$ SMA (ET1607-53, Magenta) on kidney. 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 four rounds of staining: in the order of ET7107-34 (1/1,000 dilution), ET1703-34 (1/5,000 dilution), ET1703-14 (1/1,000 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°C. DAPI (blue) was used as a nuclear counter stain. Image

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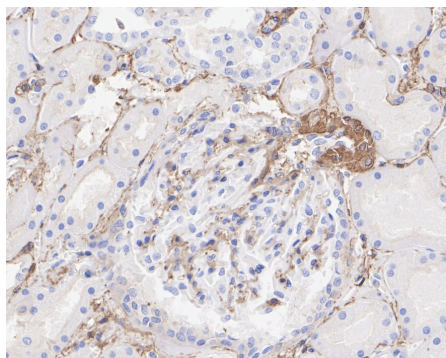
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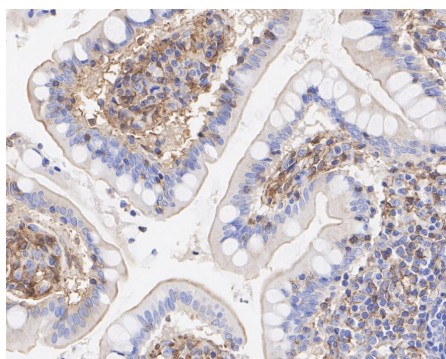
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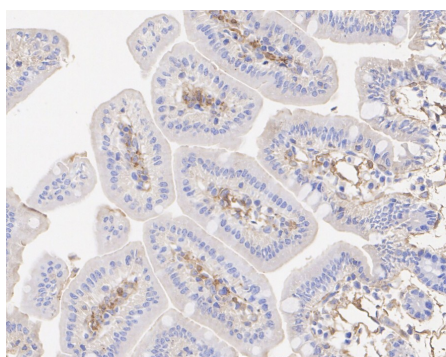
**Fig19:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/50,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1607-53) at 1/50,000 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.



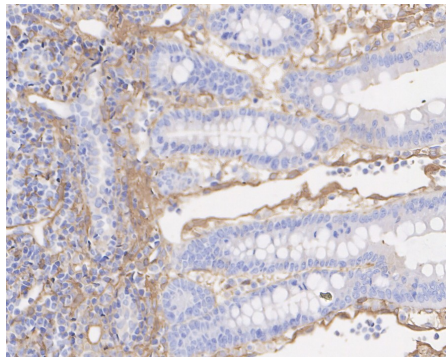
**Fig20:** Immunohistochemical analysis of paraffin-embedded human small intestine tissue with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/50,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1607-53) at 1/50,000 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.



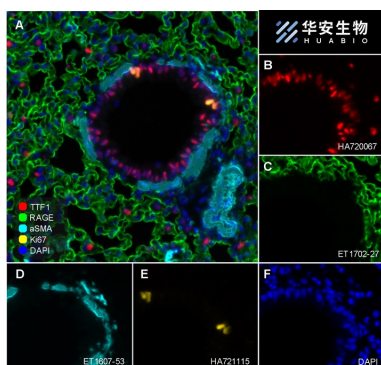
**Fig21:** Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/50,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1607-53) at 1/50,000 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.



**Fig22:** Immunohistochemical analysis of paraffin-embedded rat small intestine tissue with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-53) at 1/50,000 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1607-53) at 1/50,000 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.



**Fig23:** Fluorescence multiplex immunohistochemical analysis of mouse lung (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-TTF1 (HA720067, Red), anti-RAGE (ET1702-27, Green), anti-aSMA (ET1607-53, Cyan) and anti-Ki67 (HA721115, Yellow) on mouse lung. 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 four rounds of staining: in the order of HA720067 (1/4,000 dilution), ET1702-27 (1/3,000 dilution), ET1607-53 (1/10,000 dilution) and HA721115 (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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

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

## Background References

1. Izumi D et al. CXCL12/CXCR4 activation by cancer-associated fibroblasts promotes integrin 1 clustering and invasiveness in gastric cancer. *Int J Cancer* 138:1207-19 (2016).
2. Chung SI et al. Development of a transgenic mouse model of hepatocellular carcinoma with a liver fibrosis background. *BMC Gastroenterol* 16:13 (2016).

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