Anti-Insulin Antibody [SA0410] - BSA and Azide free

HA750004

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	IF-Cell, IF-Tissue, IHC-P, IHC-Fr, WB
Molecular Wt:	Predicted band size: 12 kDa
Clone number:	SA0410

Description: Insulin (from Latin insula, 'island') is a peptide hormone produced by beta cells of the pancreatic islets; it is considered to be the main anabolic hormone of the body. It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of glucose from the blood into liver, fat and skeletal muscle cells. In these tissues the absorbed glucose is converted into either glycogen via glycogenesis or fats (triglycerides) via lipogenesis, or, in the case of the liver, into both. Glucose production and secretion by the liver is strongly inhibited by high concentrations of insulin in the blood. Circulating insulin also affects the synthesis of proteins in a wide variety of tissues. It is therefore an anabolic hormone, promoting the conversion of small molecules in the blood into large molecules inside the cells. Low insulin levels in the blood have the opposite effect by promoting widespread catabolism, especially of reserve body fat.

Immunogen: Recombinant protein within human Insulin aa 15-110.

Positive control: Mouse pancreas tissue, human pancreas tissue, rat pancreas tissue, Mouse pancreas tissue lysate, Rat pancreas tissue lysate.

Subcellular location: Secreted.

Database links: SwissProt: P01308 Human | P01325 Mouse | P01322 Rat

Recommended Dilutions:

IF-Cell	1:200-1:500
IF-Tissue	1:200-1:500
IHC-P	1:20,000
mIHC	1:8,000
IHC-Fr	1:1,000
WB	1:1,000

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

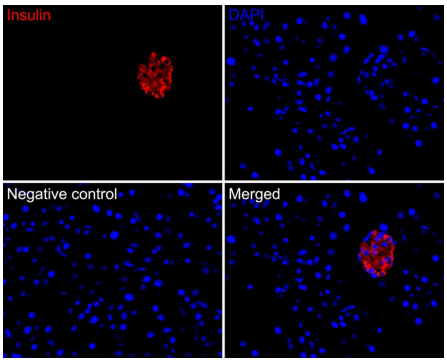

Fig1: Immunofluorescence analysis of paraffin-embedded mouse pancreas tissue labeling Insulin with Rabbit anti-Insulin antibody (HA750004) at 1/500 dilution.

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 (HA750004, red) at 1/500 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) was used as the secondary antibody at 1/500 dilution. Nuclei were counterstained with DAPI (blue).

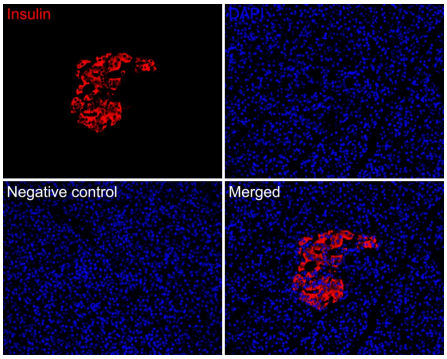

Fig2: Immunofluorescence analysis of paraffin-embedded human pancreas tissue labeling Insulin with Rabbit anti-Insulin antibody (HA750004) at 1/500 dilution.

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 (HA750004, red) at 1/500 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) was used as the secondary antibody at 1/500 dilution. Nuclei were counterstained with DAPI (blue).

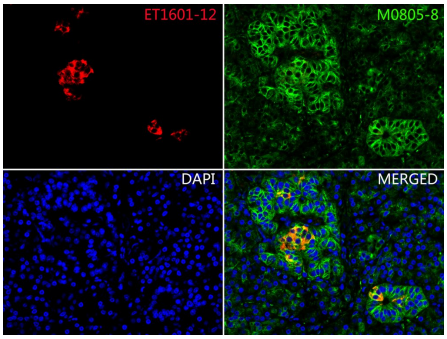

Fig3: Immunofluorescence analysis of paraffin-embedded human pancreas tissue labeling Insulin (HA750004) and beta III Tubulin (M0805-8).

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 Insulin (HA750004, red) at 1/200 dilution and beta III Tubulin (M0805-8, green) at 1/200 dilution at +4℃ overnight, washed with PBS.

Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) and Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

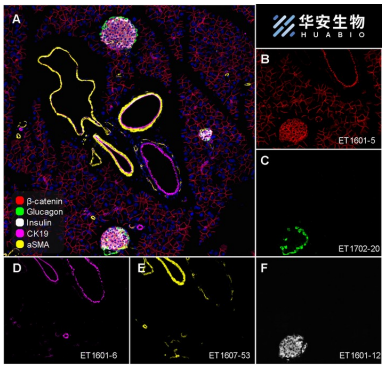

Fig4: Fluorescence multiplex immunohistochemical analysis of mouse pancreas (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-β-catenin (ET1601-5, Red), anti-Glucagon (ET1702-20, Green), anti-Insulin (HA750004, White), anti-CK19 (ET1601-6, Magenta) and anti-aSMA (ET1607-53, 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.

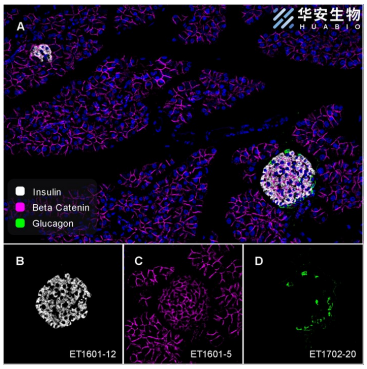

Fig5: Fluorescence multiplex immunohistochemical analysis of mouse pancreas (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Beta Catenin (ET1601-5, Violet), anti-Glucagon (ET1702-20, Green) and anti-Insulin (HA750004, White) on pancreas. HRP Conjugated UltraPolymer 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.

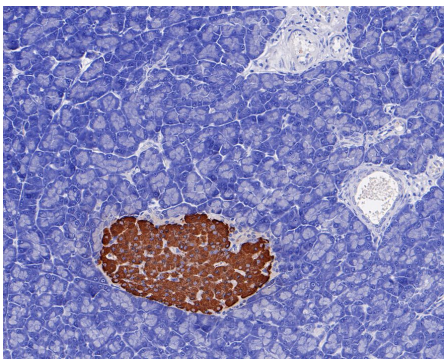

Fig6: Immunohistochemical analysis of paraffin-embedded rat pancreas tissue with Rabbit anti-Insulin antibody (HA750004) at 1/20,000 dilution.

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 (HA750004) 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.

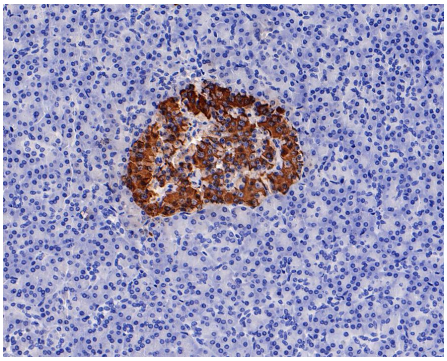

Fig7: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-Insulin antibody (HA750004) at 1/20,000 dilution.

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 (HA750004) 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.

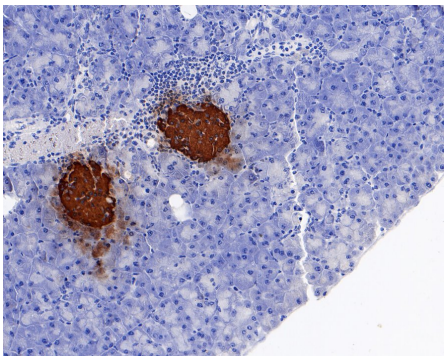

Fig8: Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue with Rabbit anti-Insulin antibody (HA750004) at 1/20,000 dilution.

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 (HA750004) 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.

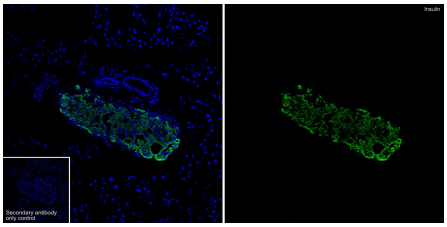

Fig9: Immunofluorescence analysis of frozen mouse pancreas tissue with Rabbit anti-Insulin antibody (HA750004) at 1/1,000 dilution.

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 (HA750004, green) at 1/1,000 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).

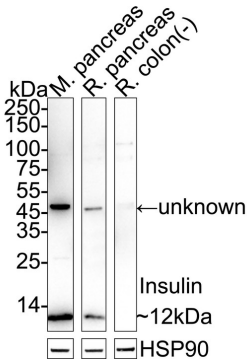

Fig10: Western blot analysis of Insulin on different lysates with Rabbit anti-Insulin antibody (HA750004) at 1/1,000 dilution.

Lane 1: Mouse pancreas tissue lysate
Lane 2: Rat pancreas tissue lysate
Lane 3: Rat colon tissue lysate (negative)

Lysates/proteins at 40 µg/Lane.

Predicted band size: 12 kDa
Observed band size: 12 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 (HA750004) 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.

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

Background References

1. Johansson U et al. Pancreatic Islet Survival and Engraftment Is Promoted by Culture on Functionalized Spider Silk Matrices. PLoS One 10:e0130169 (2015).
2. Hoelen H et al. Proteasomal Degradation of Proinsulin Requires Derlin-2, HRD1 and p97. PLoS One 10:e0128206 (2015).