For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-Insulin B chain (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M093-3	1C5A	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Insulin is polypeptide hormone at 5.8 kDa that is composed of one A-chain (21 amino acids) and one B-chain (30 amino acids). Insulin is synthesized as pro-insulin in β cell in Langerhans islet in pancreas and is converted Insulin by cleavage enzyme and is stored in β cell granules. Insulin secretion is urged by Glucose and Arginine and Glucagon and Tolbutamide and so on. Insulin urges the synthesis and storage of sugars, lipids, proteins, nucleic acids. And Insulin decreases the quantity of blood sugar.

SOURCE: This antibody was purified from hybridoma (clone 1C5A) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant human Insulin.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human Insulin B-chain (3.5 kDa) on Western blotting, Immunoprecipitation and Immunohistochemistry.

APPLICATIONS:

Western blotting; 1-5 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; 1 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 20 minutes each in 10 mM citrate buffer (pH 6.3)
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:

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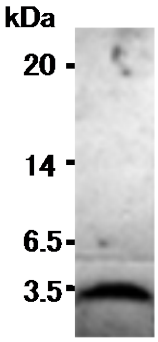
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Sample	Recombinant human Insulin	Not tested	Not tested
Reactivity on WB	+		

RELATED PRODUCTS:

M051-3 Anti-Insulin Receptor Substrate p53 (IRSp53) (Human) mAb (3F2)
M075-3 Mouse IgG1 (isotype control) (2E12)



Western blot analysis of Insulin B chain from recombinant human Insulin using M093-3.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Mix recombinant human Insulin in PBS with equal volume of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick Tris-Tricine gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)

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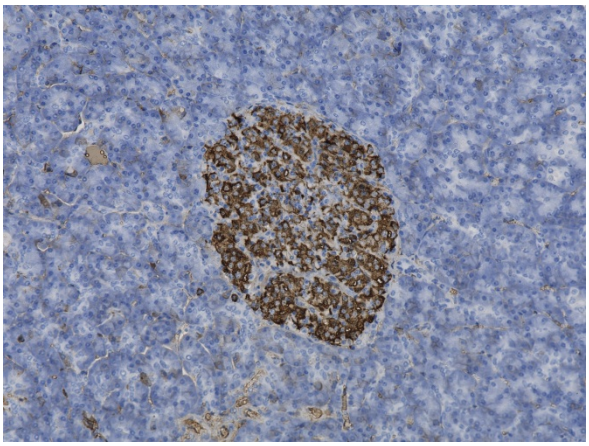
URLhttp://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
7) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 6 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Recombinant human Insulin)



Immunohistochemical detection of Insulin β chain in human pancreatic cancer tissue using M093-3.

Immunohistochemical staining for paraffin-embedded sections:

1) Deparaffinize the sections with Xylene 3 times for 3 minutes each.
2) Wash the slides with Ethanol 3 times for 3 minutes each.
3) Wash the slides with PBS 3 times for 3 minutes each.
4) Remove the slide from PBS and heat-treat with 10 mM citrate buffer (pH 6.3) for 20 minutes at 500W with microwave oven.
5) Let the slide cool down at room temperature in citrate buffer.
6) Remove the slide from citrate buffer and inactivate endogenous peroxidase with 3% H_2O_2 in PBS for 10 minutes.
7) Wash the slide 2 times in PBS for 3 minutes each.
8) Immerse the slide in blocking buffer [20 mM HEPES, 1% BSA, 135 mM NaCl] for 5 minutes at room temperature to block non-specific staining.
9) Incubate the section with the primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
10) Wash the slides 3 times in PBS for 3 min. each.
11) Incubate the section with Histostar (Ms + Rb) (MBL; code no. 8460) for 30 minutes at room temperature.
12) Wash the slides 3 times in PBS for 3 min. each.
13) Visualize by reacting for 3 minutes with Histostar DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
14) Wash the slides in water for 5 minutes.
15) Counterstain in hematoxylin for 5 minutes, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes.
16) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Human Pancreaic cancer tissue)