The AK247 and AK248 antibodies label mouse glucagon-secreting alpha cells by immunofluorescence in histological frozen sections

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Abstract

The AK247 and AK248 antibodies detect the glucagon-secreting alpha cells by immunofluorescence in mice pancreatic islets.

Introduction

Pancreatic islet cells are highly differentiated and can be characterized by the distinctive hormones they produce: insulin for beta cells, glucagon for alpha cells, somatostatin for delta cells and pancreatic polypeptide for PP cells (Baskin, 2015). Here, we describe the ability of the anti-glucagon AK247 and AK248 antibodies to recognize mouse pancreatic alpha-cells by immunofluorescence in histological frozen sections.

Materials & Methods

Antibodies: ABCD_AK247 and ABCD_AK248 antibodies (ABCD nomenclature, web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (www.unige.ch/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond, respectively, to the sequence of the variable regions of the clones H4H10223P and H4H10231P (Okamoto and Gromada, 2016) joined by a peptide linker $(GGGS)_4$. HEK293 suspension cells (growing in FreeStyleTM 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (10 mg/L) were collected after 4 days.

Antigen: The antibodies were originally raised against human glucagon (Uniprot #P01275) in humanized transgenic mice (Okamoto and Gromada, 2016). Mouse pancreas was fixed with 4% paraformaldehyde (Santa Cruz Biotechnology #sc-281692) in PBS for 1h and 30 min followed by an overnight dehydration in a 20% sucrose (Sigma-Aldrich #84100) solution in PBS. Pancreases were embedded in OCT (Tissue-Tek #4583) and cryo-sectioned at 10 µm-thickness.

Protocol: AK247 and AK248 antibodies were used to recognize glucagon in frozen sections of mouse pancreas. The immunofluorescence protocol was carried out at room temperature. After each incubation step, sections were washed 3 times for 5 min with PBS.

Frozen sections were rinsed with PBS before 20 min permeabilization with 0.1% TritonX-100 (AppliChem #A1388.0500) in PBS. Blocking was performed with 3% bovine serum albumin (Sigma-Aldrich #A3912-100G) and 0.1% Tween20 (AppliChem #A1389.0500) in PBS for 30 min. The sections were incubated for 2h with the recombinant antibodies AK247 (1/100) and AK248 (1/50). After washing, the sections were incubated for 45 min in PBS containing the secondary antibodies anti-rabbit Alexa 488 (1/500, Life Technologies #A11034) and DAPI (1/500, Life Technologies #D3571). Samples were washed and mounted on SuperFrost Plus slides (Thermo Scientific #J1800AMNZ) using a DAPI Fluoromount-G media (Southern Biotech #0100-20). All sections were examined with a confocal microscope (Leica TCS SPE).

Results

Both AK247 and AK248 antibodies specifically detect glucagon (Fig. 1, in red) in murine frozen sections of pancreas.

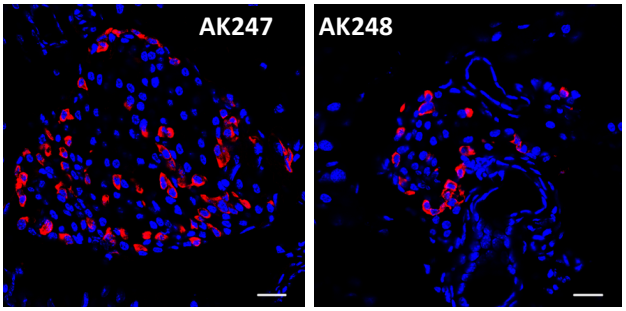

Fig. 1. AK247 and AK248 antibodies recognize glucagon in mouse pancreatic tissue. Representative confocal image of mouse pancreatic tissue (anti-glucagon in red; DAPI in blue). Scale bar: 20 µm.

References

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Conflict of interest

The authors declare no conflict of interest.