

The AE804 and AE806 antibodies label mouse insulin-secreting beta cells by immunofluorescence in histological frozen sections

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Abstract

The AE804 and AE806 antibodies detect the insulin-secreting beta 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-insulin AE804 and AE806 antibodies to recognize mouse pancreatic beta-cells by immunofluorescence in histological frozen sections.

Materials & Methods

Antibodies: ABCD_AE804 and ABCD_AE806 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 CG7C7 (or mAb 126) and AE9D6 (or mAb 125) (Ewulonu *et al.*, 1990) joined by a peptide linker (GGGS)₄. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (120 and 20 mg/L respectively for AE804 and AE806) were collected after 4 days.

Antigen: The antibodies were originally raised against human insulin (Uniprot #P01308) in BALB/c mice (Ewulonu *et al.*, 1990). 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: AE804 and AE806 antibodies were used to recognize insulin 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 AE804 (1/1000) and AE806 (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 AE804 and AE806 antibodies specifically detect insulin (Fig. 1, in green) in murine frozen sections of pancreas.

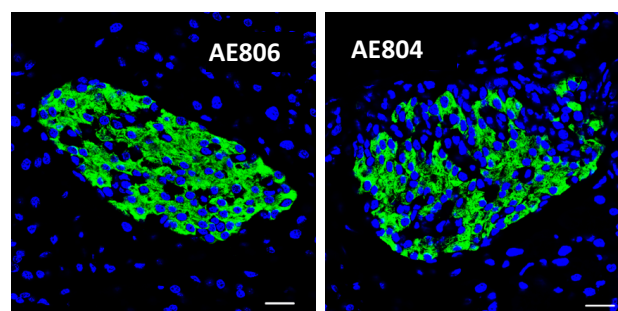


Fig. 1. AE804 and AE806 antibodies recognize insulin in mouse pancreatic tissue. Representative confocal image of mouse pancreatic tissue (anti-insulin in green; DAPI in blue). Scale bar: 20 μm.

References

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Conflict of interest

The authors declare no conflict of interest.