AK247, AK249, AK250, AK280 and AK281 antibodies label mouse glucagon-secreting alpha cells by immunohistochemistry

Ezia Oppliger, Marie N. Schmid, Clément Bindschaedler, Margaux Gosetto, Clément Poncet, Maxime Volery, Serkan Berkcan, Daniel Gil, Emma Jaques, Zacharie El Matribi, Julien Ollivier, Sara Da Fonte, Nylsa Chammartin, Célia Lazzarotto, Alexandre P. Vaudano, Ezgi Gozlugol, Anthony Nemeth, Nina Payot, Khatiba Khatibi, Ali Sassi, Cyril Guilhen, Priscilla Soulie

Bachelor in Biomedical Sciences, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibodies AK247, AK249, AK250, AK280 and AK281 detect by immunohistochemistry the glucagon-secreting alpha cells in mouse pancreatic islets.

Introduction

Pancreatic islet cells are highly differentiated and can be characterized by the distinctive hormones which they produce: insulin for beta cells, glucagon for alpha cells, somatostatin for delta cells and pancreatic polypeptide for PP cells (Baskin, 2015). Five recombinant antibodies (AK247, AK249, AK250, AK280 and AK281) detect pancreatic glucagon-secreting alpha-cells by immunohistochemistry and one (AK251) does not.

Materials & Methods

Antibodies: ABCD_AK247, ABCD_AK249, ABCD_AK250, ABCD_AK251, ABCD_AK280 and ABCD_AK281 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/; Lima *et al.*, 2020) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker $(GGGGS)_3$ (see Table 1 for clone names and references). HEK293 suspension cells (growing in FreeStyleTM 293 Expression Medium; Gibco, #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 4 days.

Table 1: Clone number, reference and production yields for the antibodies used in this study.

ABCD	Clone	Reference	Yield (mg/L)
AK247	H4H10223P	Okamoto and Gromada, 2016	5
AK249	H4H10232P		<5
AK250	H4H10236P		100
AK251	H4H10237P		40
AK280	IBA009	Watson *et al*., 2014	70
AK281	IBA032		100

Antigen: All antibodies were originally raised in mice against human glucagon (Uniprot #P01275) (Okamoto and Gromada, 2016; Watson *et al*., 2014).

Protocol: Mouse pancreas was surgically removed, fixed in PBS + 4% paraformaldehyde during 2 hs at RT. Pancreas sections were performed at the Histology Core Facility of the Geneva medical school, Switzerland. First, pancreas samples were dehydrated in an automatic tissue processing machine (Histokinette, Leica) in successive baths of ethanol at growing concentrations (70% twice 2 hs, 90% once 1 h, 95% once 1 h, 100% three times 1 h), followed by incubation in a solvent bath (three times 1 h; HistoSav, Biosystems #39-0591-05) and in bathes of liquid paraffin (three times 1 h; Leica #39601006). Samples were then embedded in liquid paraffin, cooled until solidification, and sectioned into 5 µm thick slices. Sections were deparaffinized and rehydrated with a series of alcohol solutions of decreasing concentrations (5 min in each solution, 100%, 95%, 70% and 50%) and kept in phosphate buffer saline (PBS) 30 min. Antigen retrieval was performed on deparaffined sections immersed in citrate 10 mM in PBS, pH 6 and microwave heated (once 7 min at 650 W and twice 5 min at 350 W). After blocking the endogenous peroxidase for 10 min with hydrogen peroxide (35%) diluted in 100% methanol, slides were washed for 3 min in ethanol (100%) and for 10 min in PBS. Sections were incubated for 30 min at RT with the primary antibodies (1/10) in antibody dilution buffer (DAKO #S2022). After three washes (5 min) in PBS, slides were incubated 20 min at RT with biotin-coupled anti -rabbit IgG (DAKO #K5001). Slides were then washed two times for 5 min in PBS and incubated for 20 min at RT with horseradish peroxidase-coupled streptavidin. After two washes (5 min) in PBS, slides were incubated with DAB chromogenic substrate (DAKO #K5001) until signal formation. Slides were then washed two times for 5 min in PBS and counter-stained with hemalum for 2 min. Sections were mounted in aqueous mounting medium (Aquatex, Sigma- Aldricht #108562) and scanned with an Olympus VS120 microscope.

Results

Alpha cells of pancreatic islets were successfully stained by AK247, AK249, AK250, AK280 and AK281 antibodies directed against glucagon, showing a strong intracellular signal (Fig. 1). No staining was observed with the AK251 antibody. The staining observed with the AK247 antibody confirms previously published observations (Frances and Thorel, 2019). No staining was observed in pancreas sections in which the primary antibody was omitted (Fig. 1, negative control).

References

Baskin DG. A historical perspective on the identification of cell types in pancreatic islets of Langerhans by staining and histochemical techniques. J Histochem Cytochem. 2015; 63(8):543-558. PMID:26216133

Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. Nucleic Acids Res. 2020; 48(D1):D261-D264. PMID:31410491

Frances MP, Thorel F. The AK247 and AK248 antibodies label mouse glucagon-secreting alpha cells by immunofluorescence in histological frozen sections. Antibody Reports. 2019; 2(4).

Okamoto H, Gromada J. Anti-glucagon antibodies. USA; US20160075778, 2016.

Watson DE, Siegel RW, Jia N, Sloan JH. Anti-glucagon antibodies and uses thereof. USA; US20140335096, 2014

Conflict of interest

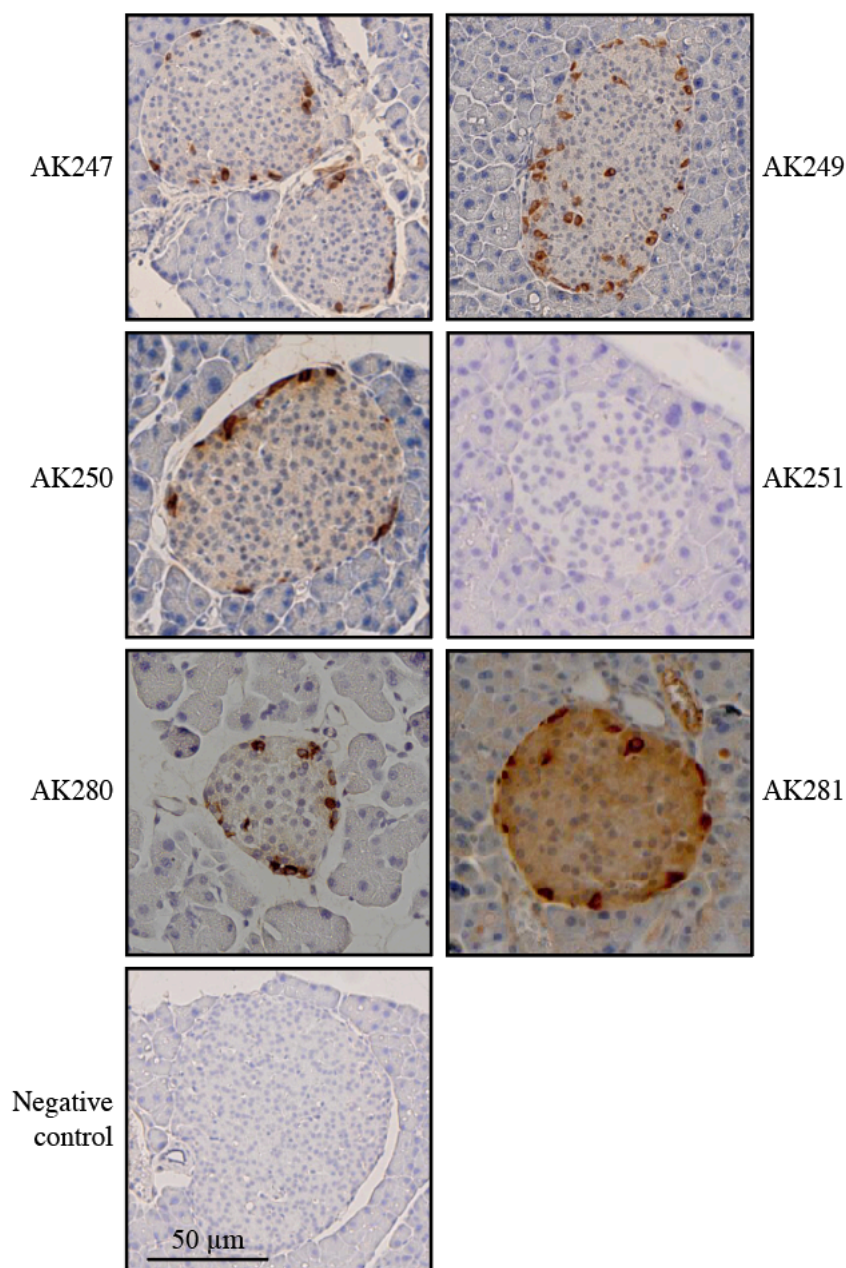The authors declare no conflict of interest.

Fig. 1. AK247, AK249, AK250, AK280 and AK281 antibodies bind specifically to pancreatic glucagon-secreting alpha cells, as detected by immunohistochemistry. No staining was observed with the AK251 antibody. No staining was observed when the primary antibody was omitted (negative control). Scale bar: 50 μm.