Mar 29, 2021

Immunofluorescence staining of Collagen Type XVIII in islet beta cells of formalin-fixed mouse pancreas

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1 Works for me dx.doi.org/10.17504/protocols.io.btqynmxw

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SUBMIT TO PLOS ONE

ABSTRACT

Paraffin sections of formalin-fixed mouse pancreas were treated with 0.05% pronase for antigen retrieval, blocked with M.O.M Ig block in 2% bovine serum albumin (BSA; Sigma)/phosphate buffered saline (PBS), incubated overnight (4°C) with COL18A1 mAb (1/50; Santa Cruz), washed and stained with AlexaFluor 488-Donkey anti-mouse IgG (Thermo Fisher). The same sections were washed, incubated with rabbit anti-human glucagon IgG (Abcam) or guinea-pig anti-pig insulin Ig (Dako), washed and stained with Alexafluor 568-donkey anti-rabbit IgG or AlexaFluor 568-goat anti-guinea-pig IgG (Thermo Fisher). Background staining was determined using sections stained only with the secondary antibody. Nuclei were stained with DAPI (0.2 µg/ml; Sigma). Trueblack (Biotium) was applied to reduce autofluorescence. Sections were photographed using an automated Axio Observer inverted fluorescence microscope (Zeiss). Merged images were prepared using ZEN (version 2.3) software (Zeiss).

DOI

dx.doi.org/10.17504/protocols.io.btqynmxw

PROTOCOL CITATION

Sarah Popp, Charmaine Simeonovic 2021. Immunofluorescence staining of Collagen Type XVIII in islet beta cells of formalin-fixed mouse pancreas . **protocols.io** https://dx.doi.org/10.17504/protocols.io.btqynmxw

KEYWORDS

Collagen Type XVIII, mouse islets, mouse pancreas, Immunofluorescence

CREATED

Mar 29, 2021

LAST MODIFIED

Mar 29, 2021

PROTOCOL INTEGER ID

48632

BEFORE STARTING

Preparation:

Prepare graded alcohols and xylene for deparaffinizing tissue sections: 2 x xylene (250 ml/slide container), 2 x absolute ethanol (250 ml/slide container), 1 x 90% ethanol (250 ml), 1 x 70% ethanol (250 ml).

Prepare block: 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). M.O.M Ig block 1 drop Ig block to 1.25ml 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS).

Materials:

1. Antibodies:

Collagen Type XVIII (COL18A1) mAb, Santa Cruz #SC-32720
Donkey anti-mouse IgG AF488, Thermo Fisher #A21202
Polyclonal guinea pig anti-pig insulin, DAKO#A0564
Rabbit polyclonal anti-human glucagon, Abcam #ab133195
Goat anti-guinea pig IgG AF568, Thermo Fisher #A11075
Donkey anti-rabbit IgG AF568, Thermo Fisher #A10042

2. Other reagents:

Pronase, Calbiochem #537088
Bovine serum albumin, Sigma #A3294
M.O.M Ig block, VECTOR #MKB-2213-1
DAPI, Sigma #D9524
TrueBlack, 20X, Biotium#23007
ProLong Diamond Antifade Mountant, Thermo Fisher #P36961
1.5H cover glass (Marienfeld, #0107222, Lauda-Konigshofen, Germany)

1 Deparaffinize slides in each xylene for 1 min (see Guidelines). Rehydrate slides in graded alcohols beginning in absolute ethanol (10 dips)/ container of absolute ethanol), followed by 90% ethanol (10 dips) and 70% ethanol (10 dips). Wash well in running tap water for 5 min.

2 Wipe around sections using tissue and cover each section with pronase solution (2.5 mg pronase in 5 ml tap water (pH 7) i.e. 0.05% pronase) for antigen retrieval. Return humidified slide tray to 37°C incubator for 10 min.

3 Wash sections with phosphate-buffered saline (PBS), 3 x, then 3 x 5 min in slide container containing 250 ml PBS with agitation of slides at 0, 2, and 5 min of each wash.

4 Block sections with M.O.M Ig block/2% bovine serum albumin (BSA) in PBS at room temperature for 30 min.

5 Tip off block, wipe around sections and apply primary Col18A1 mAb, 4 µg/ml in 2% BSA/PBS, 125 µl/section. Incubate overnight at 4°C in a humidified tray (containing PBS).

6 Wash sections with PBS, 3 x, then 3 x 5 min in slide container containing 250 ml PBS with agitation of slides at 0, 2, and 5 min of each wash.

7 Apply secondary donkey anti mouse IgG AF488 (Thermo Fisher #A21202), 1 µg/ml with 2% BSA/PBS), 125 µl/section, and incubate for 30 min at room temperature.

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8 Wash sections with PBS, 3 x, then 3 x 5 min in slide container containing 250 ml PBS with agitation of slides at 0, 2, and 5 min of each wash.

9 Apply anti-insulin or anti-glucagon pAb:
(a) For insulin staining, apply polyclonal guinea pig anti-insulin (DAKO #A0564), 130 µg/ml in 2% BSA/PBS, 125 µl/section, and incubate for 30 min at room temperature.
(b) For glucagon staining, apply rabbit polyclonal anti-glucagon (Abcam #ab133195), 10 µg/ml in 2% BSA/PBS, 125 µl/section, and incubate for 30 min at room temperature.

10 Wash sections with PBS, 3 x, then 3 x 5 min in slide container containing 250 ml PBS with agitation of slides at 0, 2, and 5 min of each wash.

11 Apply secondary antibodies for anti-insulin or anti-glucagon pAb:
(a) For insulin staining, apply goat anti-guinea pig IgG AF568 (Thermo Fisher #A11075), 10 µg/ml dilution in 2% BSA/PBS, 125 µl/section, and incubate for 30 min at room temperature.
(b) For glucagon staining, apply donkey anti-rabbit IgG AF568 (Thermo Fisher #A10042), 4 µg/ml in 2% BSA/PBS, 125 µl/section, and incubate for 30 min at room temperature.

12 Wash sections with PBS, 3 x, then 3 x 5 min in slide container containing 250 ml PBS with agitation of slides at 0, 2, and 5 min of each wash.

13 Stain sections with DAPI (1 mg/ml in water; Sigma #D9524), 0.2 µg/ml in PBS for 2 min.

14 Wash sections with PBS, 3 x, then 1 x 5 min in slide container containing 250 ml PBS with agitation of slides (10 x) at 0, 2.5 min and 5 min.

15 Stain sections with 1x TrueBlack (Biotium #23007) in 70% EtOH, 125 µl/section for 30 sec.

16 Wash sections with PBS, 3 x, then 1 x 5 min in slide container containing 250 ml PBS with agitation of slides (10 x) at 0, 2.5 min and 5 min.

17 Mount slides in ProLong® Diamond Antifade Mountant (Thermo Fisher #P36961), using 1.5H cover glass (uniform thickness (170 microns) and low autofluorescence)

18 Image sections using an automated Axio Observer inverted fluorescence microscope (Zeiss). Prepare merged images using ZEN (version 2.3) software (Zeiss).

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