Immunofluorescence staining of heparan sulfate (HS) in islet beta cells of formalin-fixed human pancreas and isolated islets

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Abstract

Paraffin sections of formalin-fixed human pancreas and isolated human islets were treated with 0.05% pronase for antigen retrieval, blocked with 2% bovine serum albumin (BSA; Sigma)/phosphate buffered saline (PBS), incubated overnight (4°C) with 10E4 (anti-HS) mAb (1/10; US Biological/Amsbio), washed and stained with AlexaFluor 488-goat anti-mouse IgM (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). The specificity of HS staining was checked on serial sections using IgMκ isotype control (BD Biosciences), instead of 10E4 mAb, together with anti-glucagon or anti-insulin antibody. Nuclei were stained with DAPI (0.2 µg/ml; Sigma). Sections were photographed using an automated Axio Observer inverted fluorescence microscope (Zeiss). Merged images were prepared using ZEN (version 2.3) software (Zeiss).

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Guidelines

10E4 anti-heparan sulfate (HS) mAb identifies highly sulfated HS localised in human beta cells but does not identify the less sulfated HS in alpha cells.

Reference:

Theodoraki A, Hu Y, Poopalasundaram S et al (2015) Mol Cell Endocrinol 399: 296-310.

Before start

Materials:

1. 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).

2. Prepare 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS).

3. Mabs and pAbs:
   10E4 (anti-HS) mAb, Amsbio #370255-1
   Goat anti mouse IgM AF488, Thermo Fisher #A21042
   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
   IgM_{κ}, BD Biosciences #550340

4. Other reagents:
   Pronase, Calbiochem #537088
   Bovine serum albumin,3Sigma #A3294
   DAPI, Sigma #D9524
   ProLong Diamond Antifade Mountant, Thermo Fisher #P36961

Protocol

Step 1.

See Guidelines, 'Before starting'.

Step 2.

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.

Step 3.

Wipe around sections with a tissue, encircle the sections using a diamond pencil and place in a slide container of tap water (250 ml).

Step 4.

Prewarm slide tray containing low level of water (to humidify) in 37°C incubator.

Step 5.

Prepare pronase (#537088 Calbiochem; for antigen retrieval to expose HS epitopes): 2.5 mg pronase in 5 ml de-ionized water.

Step 6.

Wipe around sections using tissue and cover each section with pronase solution. Return humidified slide tray to 37°C incubator for 10 min.

Step 7.
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.

Step 8.
Block sections with 2% bovine serum albumin (BSA) in PBS at room temperature for 30 min.

Step 9.
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.

Step 10.

Apply primary 10E4 anti-HS mAb, 100 µg/ml in 2% BSA/PBS, 125 µl/section. Incubate overnight at 4°C in a humidified tray (containing PBS).

Step 11.
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.

Step 12.

Apply secondary goat anti mouse IgM AF488 (Thermo Fisher #A21042), 20 µg/ml with 2% BSA/PBS), 125 µl/section, and incubate for 30 min at room temperature.

Step 13.

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.

Step 14.

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.

Step 15.
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.

Step 16.

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.

Step 17.

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

Step 18.

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.

Step 19.
Mount slides in ProLong® Diamond Antifade Mountant (Thermo Fisher #P36961).

Step 20.

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