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Immunohistochemical staining of Syndecan-1 (Sdc-1) core proteins in islet beta cells of formalin-fixed human pancreas V.2

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We use this protocol and it's working

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Abstract

Paraffin sections (4 μm thickness) of formalin-fixed human pancreases were treated with heat/citrate buffer for antigen retrieval. Sdc-1core proteins were detected immunohistochemically using rat anti-mouse CD138 (Sdc-1) mAb (BD Biosciences), with horseradish peroxidase-conjugated rabbit anti-rat Ig (Dako). Background staining was checked using the corresponding isotype control Ig instead of the primary antibody. 3-amino-9-ethylcarbazole (AEC) was used as the chromogen. For morphometry, stained sections were imaged using a light microscope with attached camera (Olympus BX41). Image J software with color deconvolution plugin was used for the quantitative analysis of the % of islet area stained.

Troubleshooting

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 × 90% ethanol (250 ml), 1 × 70% ethanol (250 ml).

2. Prepare acetate buffer components:

(i) 0.1N acetic acid: 290 µl glacial acetic acid in 50 ml deionized water

(ii) 0.1M sodium acetate: 410 mg anhydrous CH₃COONa in 50 ml deionized water.

Prepare 0.1M acetate buffer (pH 5.2) by mixing 10.5 ml 0.1N acetic acid and 39.5 ml 0.1M sodium acetate.

3. Prepare stock solution of 3-amino-9-ethylcarbazole (AEC; chromogen, 8 mg/ml: 40 mg AEC in 5 ml N-N-dimethyl formamide; protect from light and refrigerate at 4°C.

4. Prepare M.O.M. diluent: 200 µl M.O.M. protein concentrate stock solution (M.O.M immunodetection kit) in 2.5 ml phosphate-buffered saline (PBS) for use either as a blocking step to minimize non-specific Ig binding or for diluting antibodies.

5. Mabs and pAbs:

rat anti-mouse CD138 (anti-Sdc1) mAb, BD Bioscience #553712

horseradish peroxidase (HRP)-conjugated rabbit anti-rat Ig, Dako #PO450 (alternative HRP-rabbit anti-rat IgG, Sigma #A5795)

Rat IgG_{2aK}, BD Biosciences #555841

6. Other reagents:

Hydrogen peroxide (30% w/w), Chem-Supply Pty Ltd (Australia) #HA154-500M

Methanol, Merck #106009

3-Amino-9-ethylcarbazole (AEC), Sigma-Aldrich #A5754

Animal free blocker, Vector Labs #SP-5030

Stock protein concentrate, M.O.M immunodetection kit, Vector Labs # PK-2200

N-N-dimethyl formamide, Sigma #D158550

Glycergel mounting medium, Dako #C0563

- 1 See Guidelines, "Before starting".
- 2 Deparaffinize slides in each xylene for 1 min. 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.
- 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).
- 4 Block endogenous peroxidase activity by incubating sections in 3% hydrogen peroxide in methanol (25 ml 30% H₂O₂ + 225 ml methanol).
- 5 Wash 2 × 2 min in 250 ml phosphate-buffered saline (PBS) followed by wash in running tap water for 5 min.
- 6 Prepare citrate buffer, pH 6 for antigen retrieval. Dissolve 1.05 g Citric acid in 500 ml deionized water and pH using 2 - 10 M NaOH.
- 7 Transfer slides to 250ml citrate buffer and heat in microwave (1600 watt) for 2 min on High power followed by 2 × 6 min on Low power. Allow the slides to cool on the bench for 30 min. Wash slides in 250 ml PBS, 3 × 10 min.
- 8 Wipe around sections using tissue. To block non-specific binding of Ig, apply animal free block (diluted to 20% v/v with deionized water) to tissue sections and incubate for 10 min at room temperature.
- 9 Tip off excess block in Step 7, wipe around sections using tissue and incubate with 50 µg/ml anti-Sdc1 mAb (or 50 µg/ml rat IgG_{2aκ} as isotype control; diluted in M.O.M. diluent), 125-150 µl/section at room temperature for 1 hour.
- 10 Wash off primary antibody with PBS and transfer slides to slide container with 250 ml PBS. Wash 2 × 2min.
- 11 Wipe around sections using tissue and incubate with 26 µg/ml secondary HRP-rabbit anti-rat Ig (Dako; or alternative 30 µg/ml, Sigma) , 150 µl/section, for 30 min at room temperature.



- 12 Wash off secondary antibody with PBS and transfer to slide container with 250 ml PBS. Wash slides 2 × 2min.
- 13 Prepare AEC working solution: 4.75 ml acetate buffer (see Guidelines), 0.25ml AEC stock solution and 25 µl 3% H₂O₂. Filter using a disposable 0.2 µm filter. Use within 2 hours of preparation, refrigerate for short-term storage. Protect from light.
- 14 Wipe around sections using tissue and cover the sections with AEC solution for 30 min at room temperature.
- 15 Wash off AEC solution with deionized water and transfer slides to slide container with 250 ml deionized water. Wash 3x in 10 min.
- 16 Lightly counterstain with Gill's hematoxylin, wash in deionized water (2 x) and briefly dip in ammonium water (100 µl ammonia in 250 ml deionized water), 2 × 2 sec. Wash in deionized water (2x in 250 ml) and coverslip using glycergel mounting medium.
- 17 Image sections using a light microscope with camera attachment. Use Image J software with color deconvolution plugin to determine % of islet area stained.