Immunohistochemical staining of heparanase (Hpse) in islets of formalin-fixed human pancreas

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Abstract

Paraffin sections (4 µm thickness) of formalin-fixed human pancreases were treated with heat/citrate buffer for antigen retrieval. Hpse was detected immunohistochemically using HP130 mouse anti-human Hpse mAb (Insight), with biotinylated anti-mouse IgG (1/250) (PK-2200, Vector Labs) and avidin-biotin-complex (ABC reagent; PK-2200, Vector Labs). 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. Stained sections were imaged using a light microscope with attached camera (Olympus BX41).

Citation: Lora Starrs,Debra Brown,Sarah Popp,Andrew Ziolkowski,Charmaine Simeonovic Immunohistochemical staining of heparanase (Hpse) in islets of formalin-fixed human pancreas. **protocols.io**

dx.doi.org/10.17504/protocols.io.kv4cw8w

Published: 22 Nov 2017

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 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 CH_3COONa 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, PK-2200, Vector) 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:

HP130 mouse anti-human heparanase mAb, Insight Biopharmaceuticals #INS-26-1-0000-21 (alternative: HPA1 rabbit anti-human heparanase, Insight Biopharmaceuticals #INS-26-2-0000)

Biotinylated anti-mouse IgG, M.O.M immunodetection kit, Vector Labs # PK-2200 (alternative: HRP-swine anti-rabbit Ig, Dako #PO217)

Mouse $IgG1_K$, eBioscience # 14-4714-85 (alternative Rabbit IgG, Southern Biotech, #0111-01)

6. Other reagents:

Hydrogen peroxide (30% w/w), Chem-Supply Pty Ltd (Australia) #HA154-500M
Methanol, Merck # CAS-No. 67-56-1

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

Avidin-biotin complex (ABC), M.O.M immunodetection kit, Vector Labs # PK-2200

N-N-dimethyl formamide, Sigma #D158550

Glycergel mounting medium, Dako #C0563

Protocol

Step 1.

See Guidelines, "Before starting"

Step 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.

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.

Block endogenous peroxidase activity by incubating sections in 3% hydrogen peroxide in methanol (25 ml 30% H_2O_2 + 225 ml methanol).

Step 5.
Wash 2 x 2 min in 250 ml phosphate-buffered saline (PBS) followed by wash in running tap water for 5 min.

Step 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.

Step 7.
Transfer slides to 250ml citrate buffer and heat in microwave (1600 watt) for 2 min on High power followed by 2 x 6 min on Low power. Allow the slides to cool on the bench for 30 min. Wash slides in 250 ml PBS, 3 x 10 min.

Step 8.
Wipe around sections using tissue. To block non-specific binding of Ig, apply M.O.M. diluent to tissue sections and incubate for 5 min at room temperature.

Step 9.

Tip off excess block in Step 7, wipe around sections using tissue and incubate with 340 µg/ml HP130 anti-Hpse mAb (or 350 µg/ml mouse $IgG_{1\kappa}$ as isotype control; diluted in M.O.M. diluent), 125-150 µl/section at room temperature for 30 min.

(Alternative: 228 µg/ml HPA1 rabbit anti-human heparanase IgG or 227 µg/ml rabbit IgG as isotype control).

Step 10.
Wash off primary antibody with PBS and transfer slides to slide container with 250 ml PBS. Wash 2 x 2min.

Step 11.

Wipe around sections using tissue and incubate with 1/250 diluted secondary biotinylated-anti-mouse IgG (M.O.M. immunodetection kit), 150 µl/section, for 10 min at room temperature.

(Alternative: 37 µg/ml HRP-swine anti-rabbit Ig)

Step 12.
Wash off secondary antibody with PBS and transfer to slide container with 250 ml PBS. Wash slides 2 x 2min.

Step 13.

Wipe around sections using tissue and cover with Vectastain ABC reagent (M.O.M immunodetection kit), for 5 min at room temperature.

(alternative: delete Step 13 if HPA1 anti-heparanase pAb is used)

Step 14.

Wash off ABC reagent with PBS and transfer to slide container with 250 ml PBS. Wash slides 3x in 10 min.

(alternative: delete Step 14 if HPA1 anti-heparanase pAb is used)

Step 15.

Prepare AEC working solution: 4.75 ml acetate buffer (see Guidelines), 0.25ml AEC stock solution and 25 µml 3% H_2O_2. Filter using a disposable 0.2 µm filter. Use within 2 hours of preparation, refrigerate for short-term storage. Protect from light.

Step 16.

Wipe around sections using tissue and cover the sections with AEC solution for 30 min at room temperature.

Step 17.
Wash off AEC solution with deionized water and transfer slides to slide container with 250 ml deionized water. Wash 3x in 10min.

Step 18.

Lightly counterstain withMayer’s hematoxylin, wash in deionized water (2 x) and briefly dip in ammonium water (100 µml ammonia in 250 ml deionized water), 2 x 2 sec. Wash in deionized water (2 x in 250 ml) and coverslip using glycergel mounting medium.

Step 19.
Photograph sections using a light microscope with camera attachment.