

# Resource Summary Report

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

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### Proper Citation

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### Protocol Information

**URL:** <https://dx.doi.org/10.17504/protocols.io.kvycw7w>

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**Summary:** 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/Ambio), 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 IgM? isotype control (BD Biosciences), instead of 10E4 mAb, together with anti-glucagon or anti-insulin antibody. Nuclei were stained with DAPI (0.2 ?g/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).

**Associated Publications:** Simeonovic CJ, Popp SK, Starrs LM, Brown DJ, Ziolkowski AF, Ludwig B, Bornstein SR, Wilson JD, Pugliese A, Kay TWH, Thomas HE, Loudovaris T, Choong FJ, Freeman C, Parish CR (2018) Loss of intra-islet heparan sulfate is a highly sensitive marker of type 1 diabetes progression in humans. PLoS ONE 13(2): e0191360. doi: [10.1371/journal.pone.0191360](https://doi.org/10.1371/journal.pone.0191360)

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