Insulin Antibodies

- search for appropriate Ins antibodies to replace discontinued Dako insulin antibodies
- each slide coded as to lab performing the IF analysis
- PLEASE NOTE: Ins & Gcg color assignment varies (red vs. green) in some slides
- Please contact Chris Wright (cc. Brenda Jarvis) with questions or need for clarification

[chris.wright@vanderbilt.edu; brenda.jarvis@vanderbilt.edu]

Summary: For mouse tissue, there are 3 Rabbit, 1 Guinea Pig antibodies.
For human tissue, there are 5 Rabbit, 1 Mouse, 1 Rat, and 1 Guinea Pig antibodies.

Tabulated conclusions from our studies are in slide 2.

Brenda Jarvis, Elaina Ziehm (Chris Wright Lab)

Jade Stanley (Danielle Dean Lab)

Revised 3/20/2020

Summary – Immunofluorescence Data

on cryo-sections, indirect IF, without antigen Retrieval

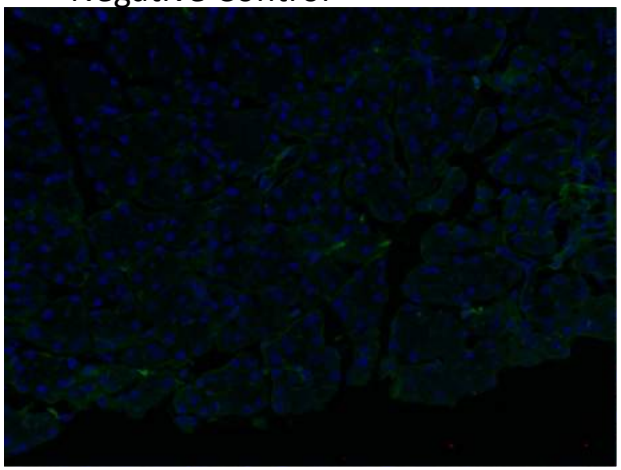
Antibody	Mouse	Tissue	Human	Tissue
Sigma/Cell Marque 273A-14 GP INS (***now also discontinued***)	1:5000	used to be great ++++	1:1000	used to be great ++++
DSHB GS 9A8 Ms c-Peptide	FAIL	no islet stain	1:1000	**great ++++**
DSHB GN ID4 Rat c-Peptide	FAIL	no islet stain	1:1000	**great ++++**
ThermoFisher 701265 Rb INS	1:5000	**great ++++**	1:1000	**great ++++**
ThermoFisher PA1-26938 GP INS	FAIL	No islet stain	FAIL	No islet stain
Genetex GTX27842 GP INS	FAIL	Stains islet; particulate contaminant - 2 different Lot #	FAIL	Stains islet; particulate contaminant - 2 different Lot #
Cell Signaling 3014 Rb INS	1:1000	**great ++++**	1:1000	**great ++++**
ABClonal A2090 Rb INS	FAIL	stains islet; also stains acinar nuclei (moderate signal)	1:1000	**great ++++** **no acinar BG in human**
Thermofisher PA5-85595 RRID_2792735 Rb C-peptide	1:1000	**great ++++**	1:1000	**great ++++**
Abcam 7842 GP INS	1:100-1:200	inconsistent / weak signal and inter-lab variability	1:100	inconsistent / weak signal and inter-lab variability
Abcam 195956 GP INS	FAIL	no islet stain	FAIL	no islet stain
Fitzgerald 20-IP35 GP anti-porcine INS	1:7500	**Great ++++ note 2 Quirks**	1:10,000	**Great ++++ note 2 Quirks**
Fitzgerald 20-IP30 GP anti-human INS	Not tested		Not tested	

Dako A0564 GP INS Mouse Tissue (Wright Lab)

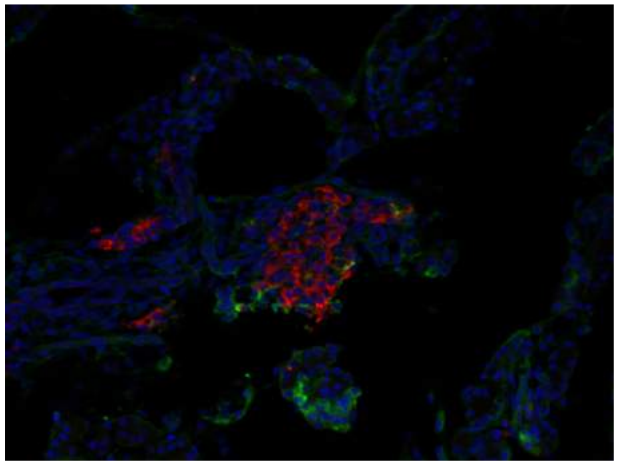
reference IF reagent; now discontinued as the "concentrated reagent"

Glucagon
Insulin
DAPI

Negative Control



1:1000



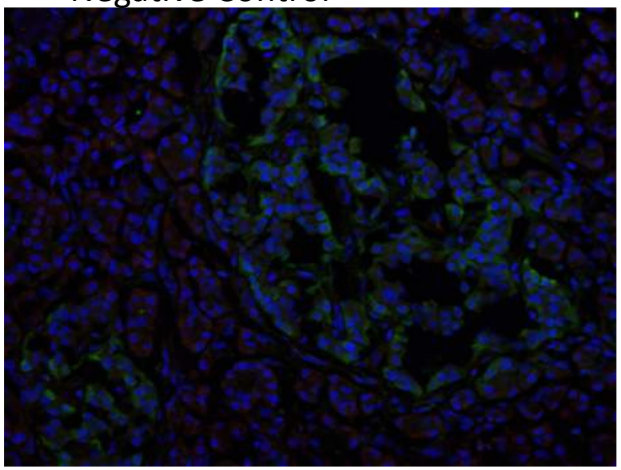
Discontinued as concentrate A0564.

Still available as diluted, "ready-to-use" version – maybe very limited supply

Sigma/Cell Marque 273A-14 RRID:AB_1158520 GP INS on Mouse Tissue (Wright Lab) now discontinued

Glucagon
Insulin
DAPI

Negative Control



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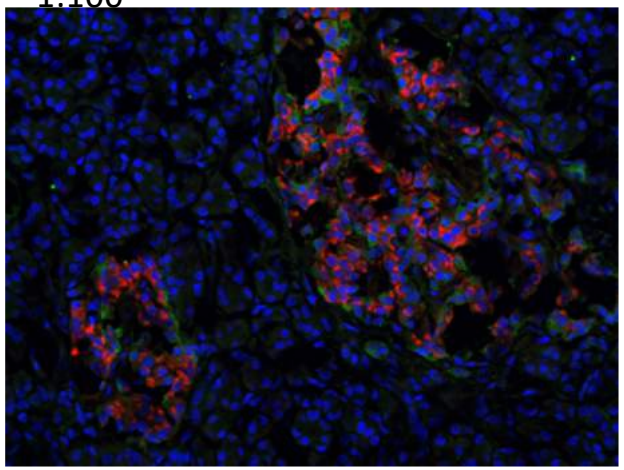

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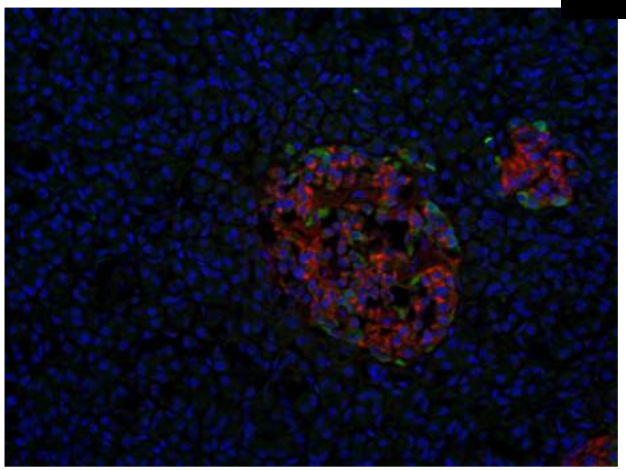

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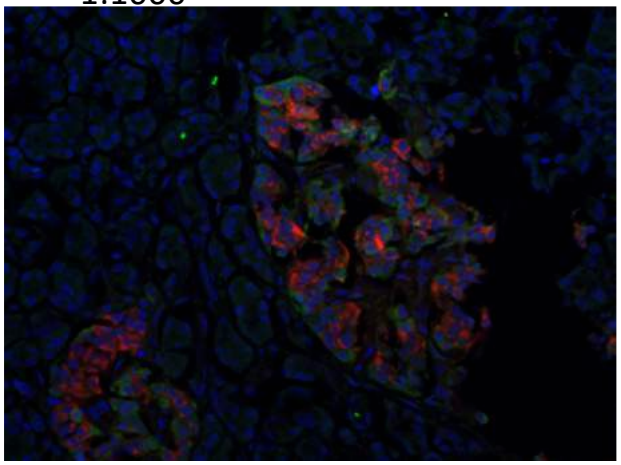
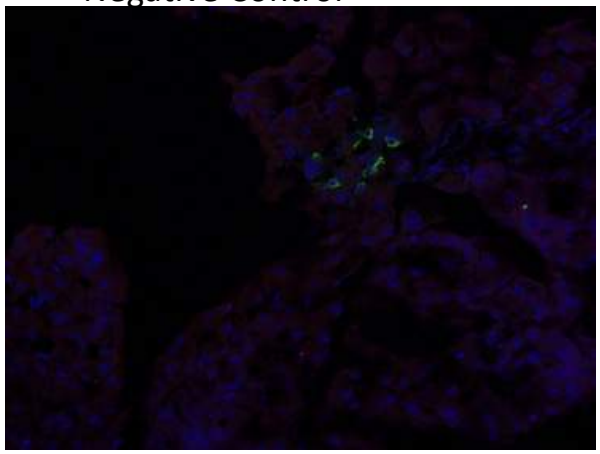


Sigma/Cell Marque 273A-14 RRID:AB_1158520 GP INS on Mouse Tissue (Wright Lab) now discontinued

Glucagon
Insulin
DAPI

Negative Control



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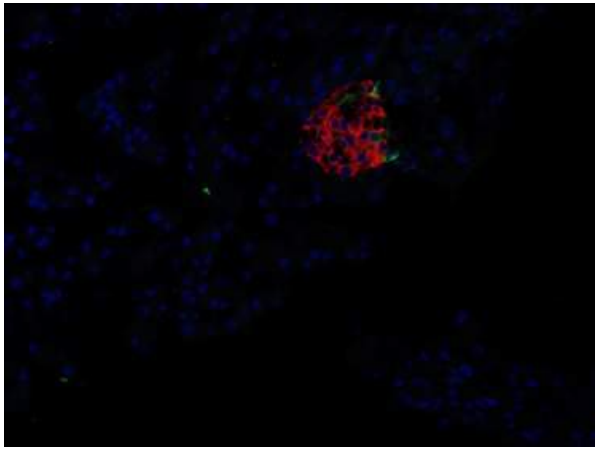

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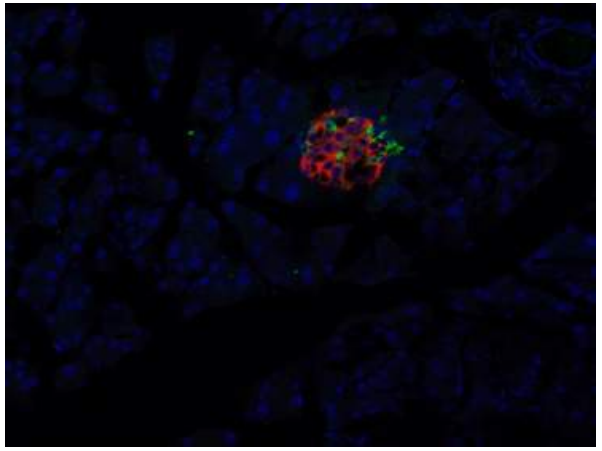
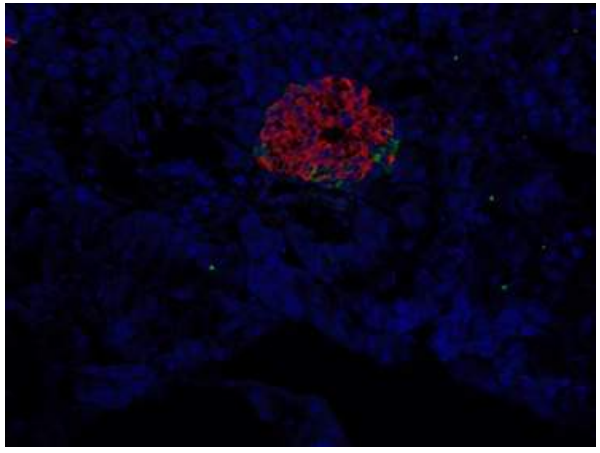


1:1000



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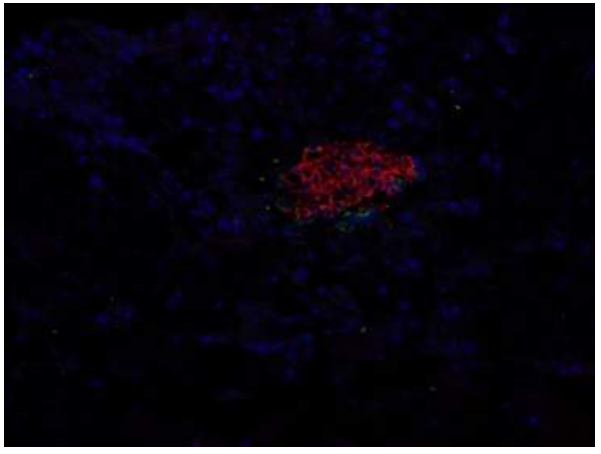
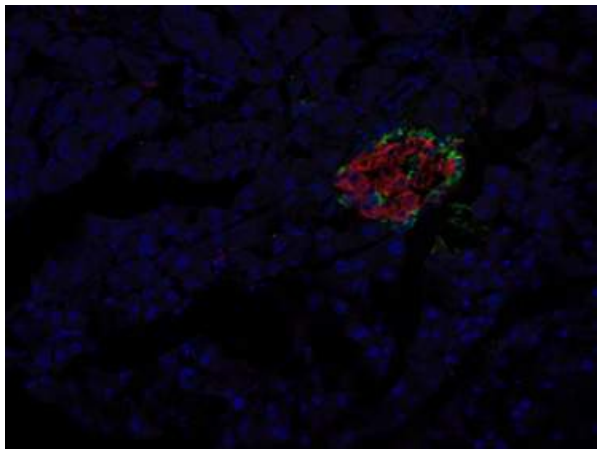


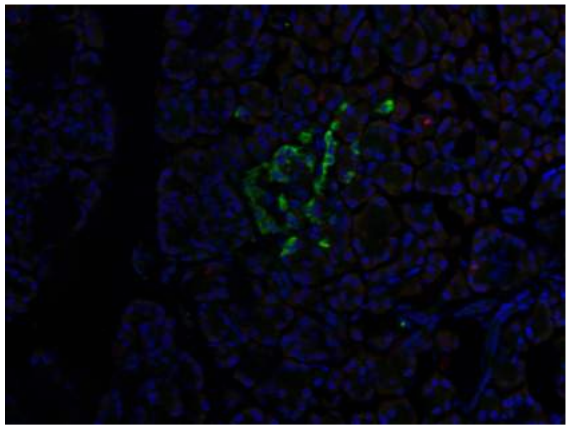
1:5000



DSHB GS 9A8 RRID:AB_532383 Ms INS on Human Tissue (Wright Lab)

Glucagon
Insulin
DAPI

Negative Control



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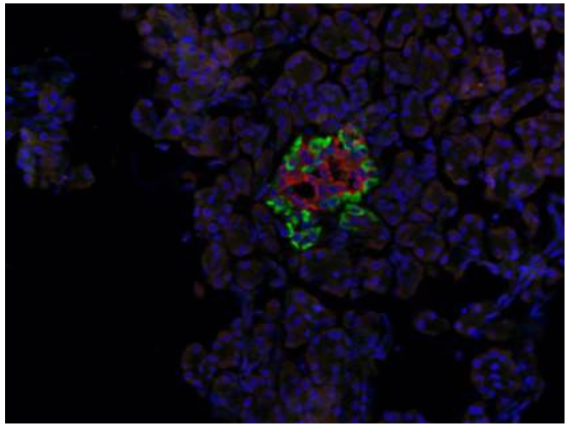

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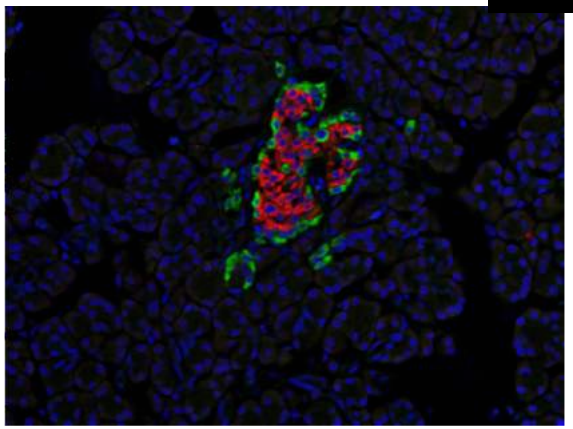

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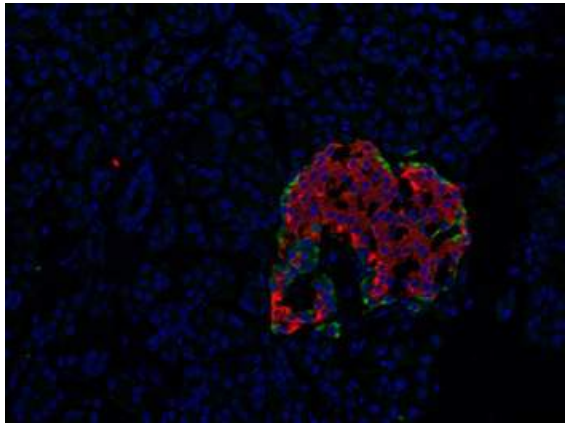

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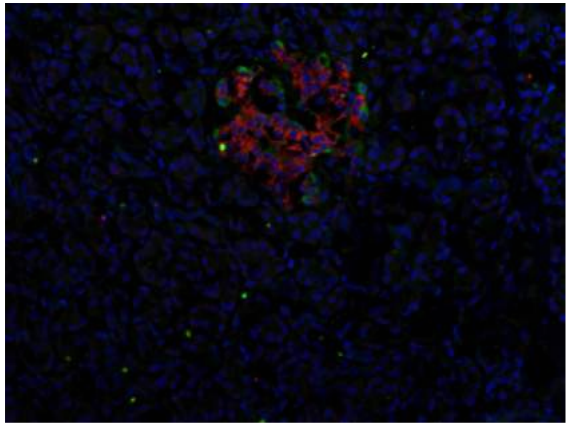

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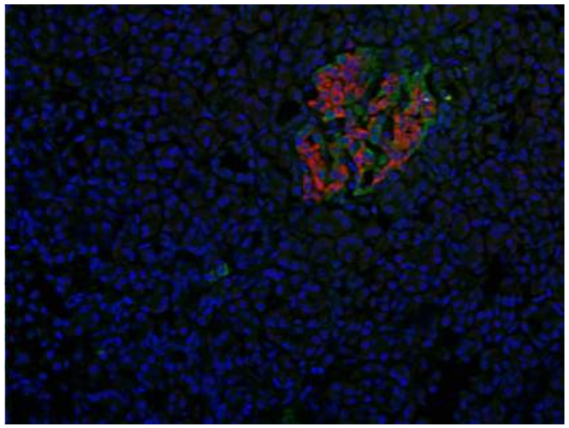
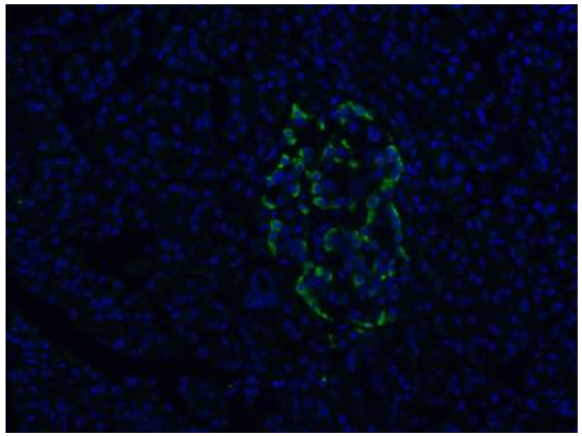


No signal on mouse tissues

DSHB GN ID4 RRID:AB_2255626 Rat INS on Human Tissue (Wright Lab)

Glucagon
Insulin
DAPI

Negative Control



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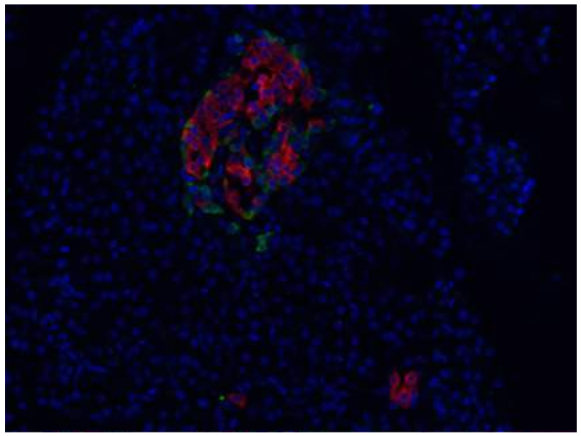

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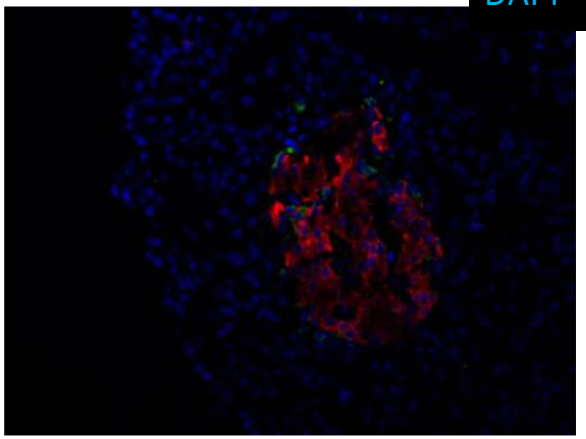

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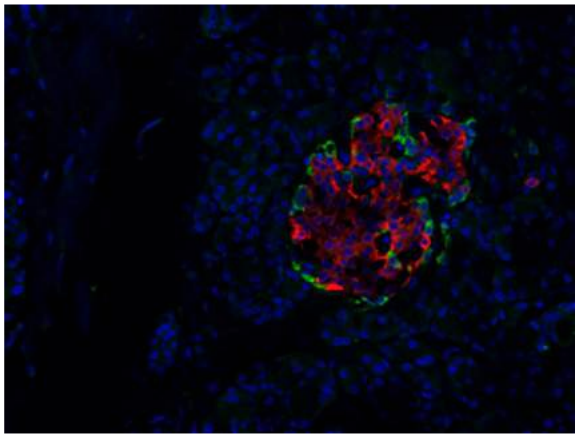

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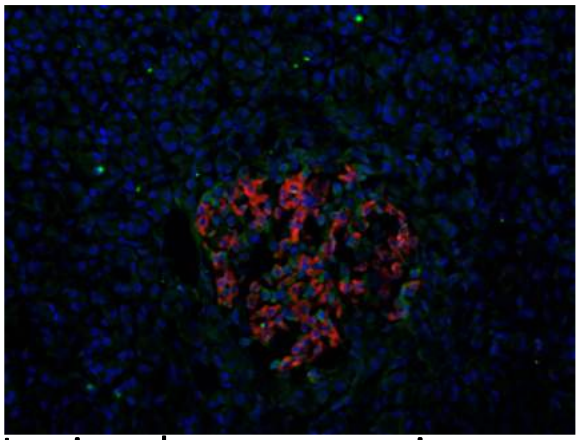

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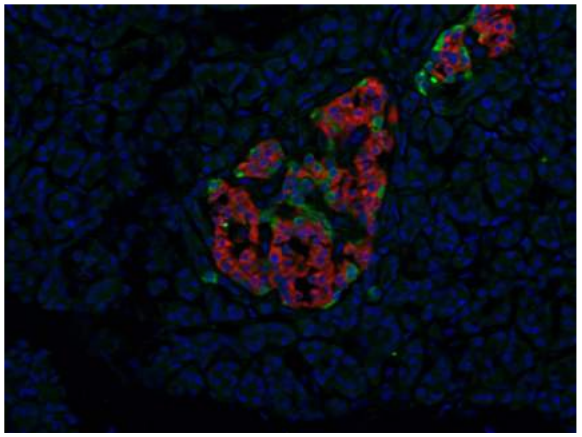
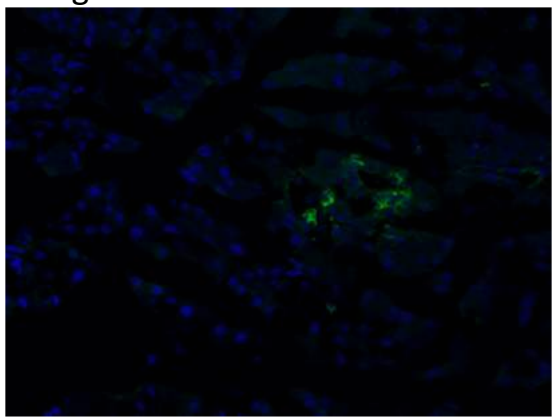


No signal on mouse tissues

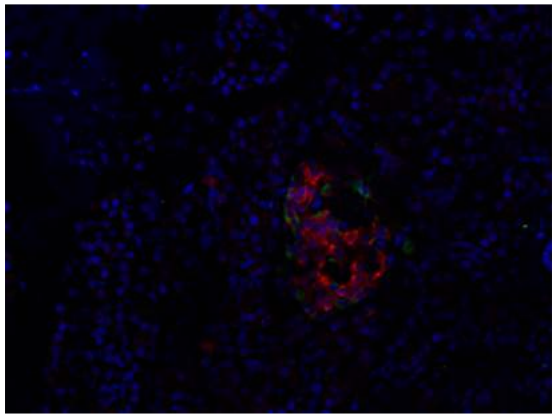
ThermoFisher 701265 RRID:AB_2532448 Rb INS on Human Tissue (Wright Lab)

Glucagon
Insulin
DAPI

Negative Control



1:100



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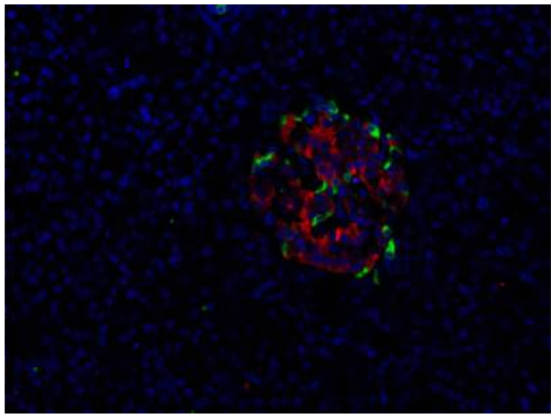

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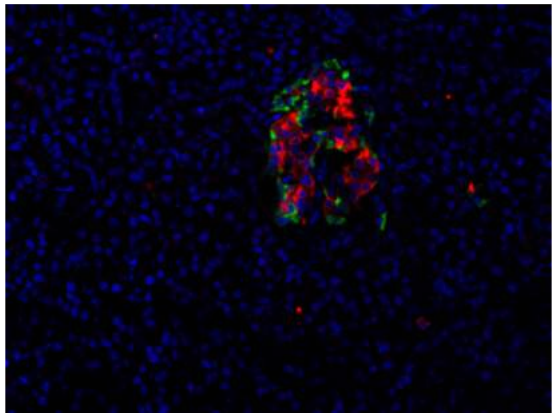
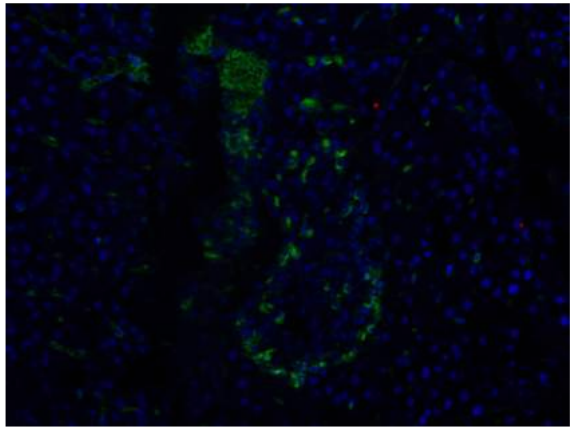


ThermoFisher 701265 RRID:AB_2532448 Rb INS on Mouse Tissue (Wright Lab)

Glucagon
Insulin
DAPI

Negative Control



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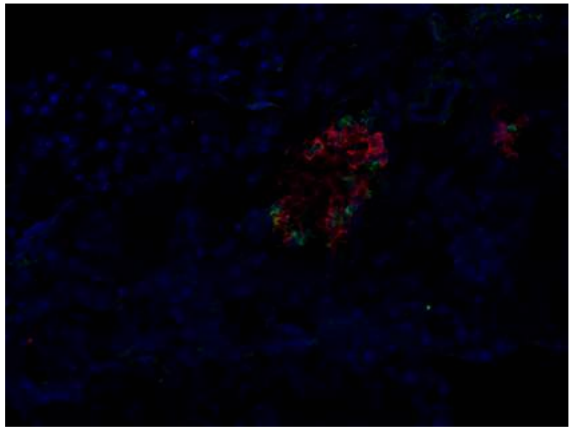
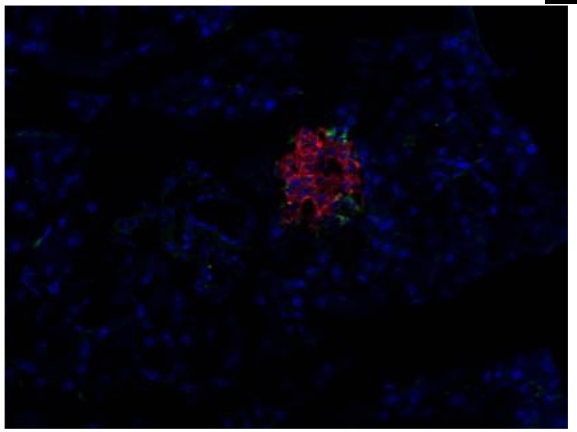
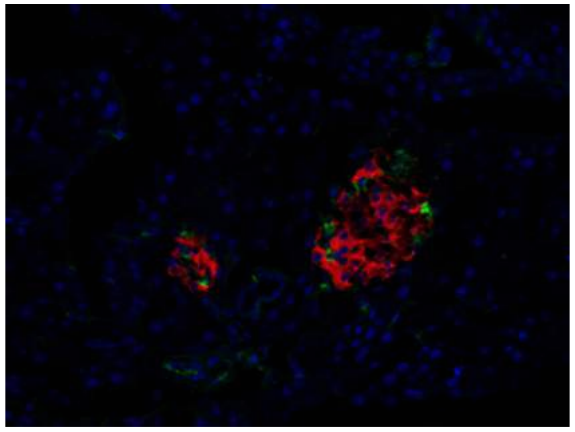


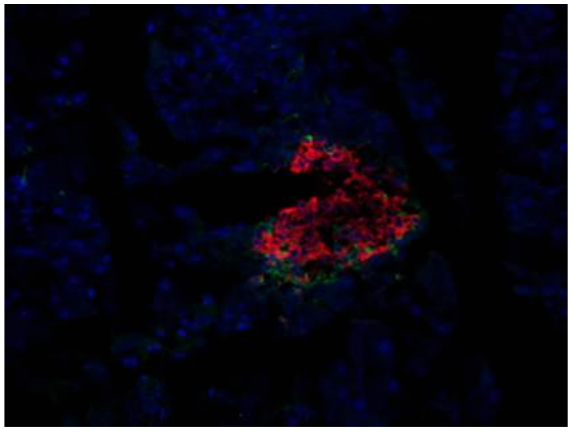
1:500



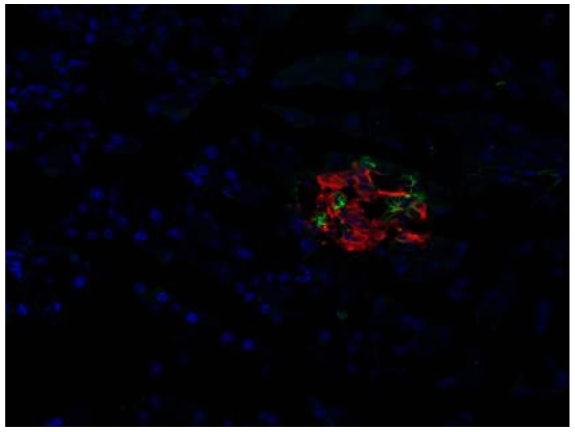
1:1000



1:2500

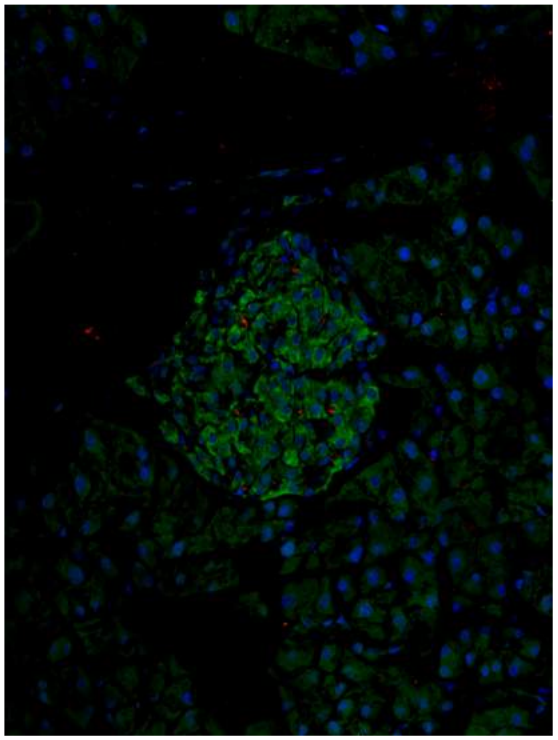


1:5000

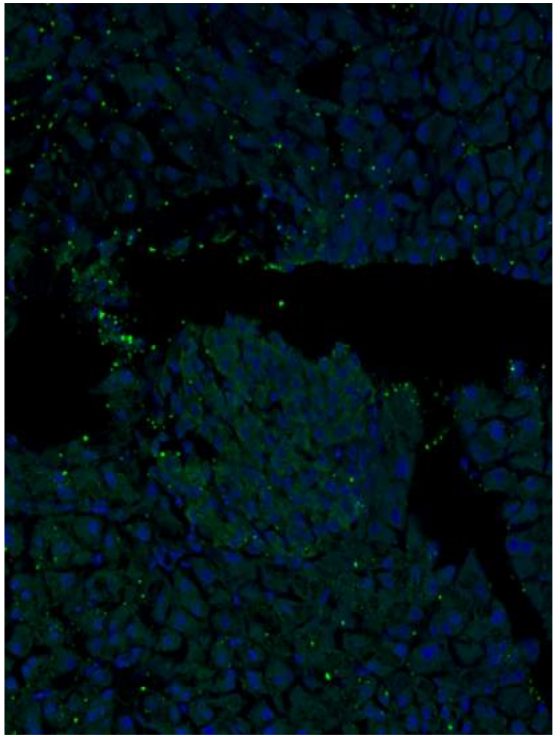


Genetex GTX27842 RRID:AB_371884 GP INS on Mouse Tissue (Wright Lab)

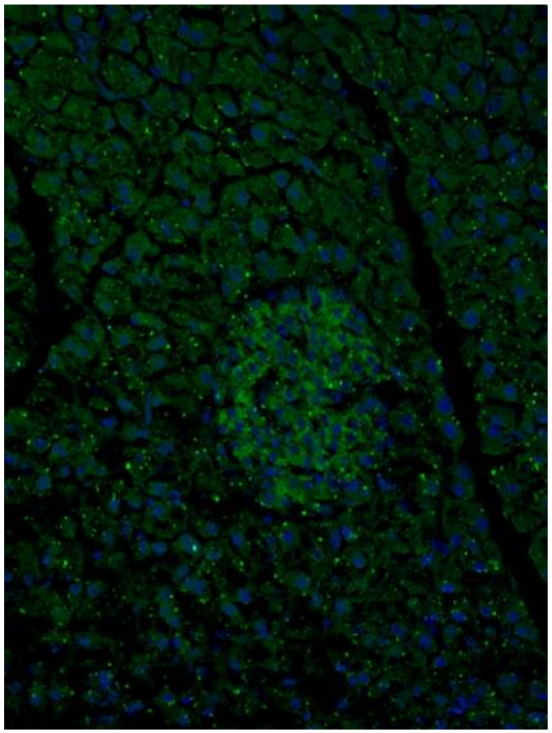
Positive control Slide
(Sigma/Cell Marque GP Insulin 1:500)



Genetex GTX27842 GP INS
1:500



Genetex GTX27842 GP INS
1:1000



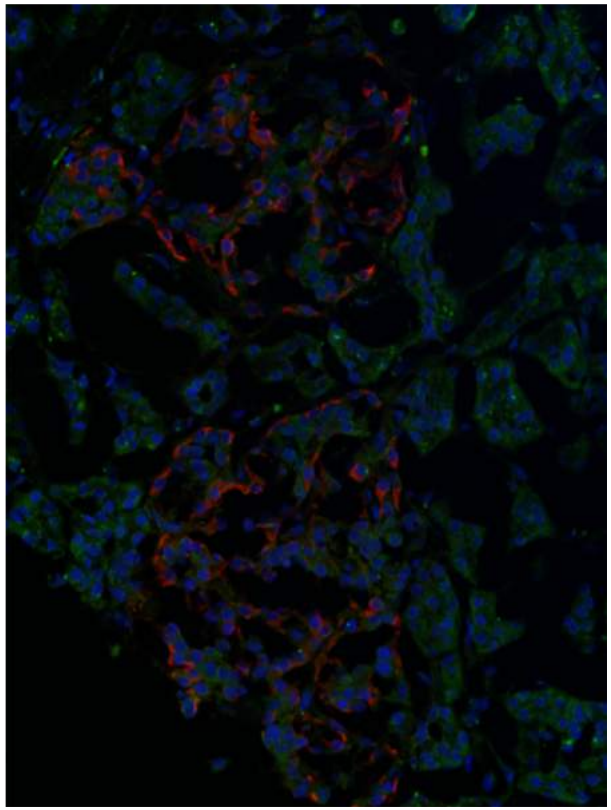
Insulin
DAPI

Identical slides run in the same experiment; only difference between the slides was the primary antibody. Two different lot numbers of GTX27842 purchased contained contaminating particulate matter of some sort.

Abcam 7842 RRID:AB_306130 GP INS on Human Tissue (Wright Lab)

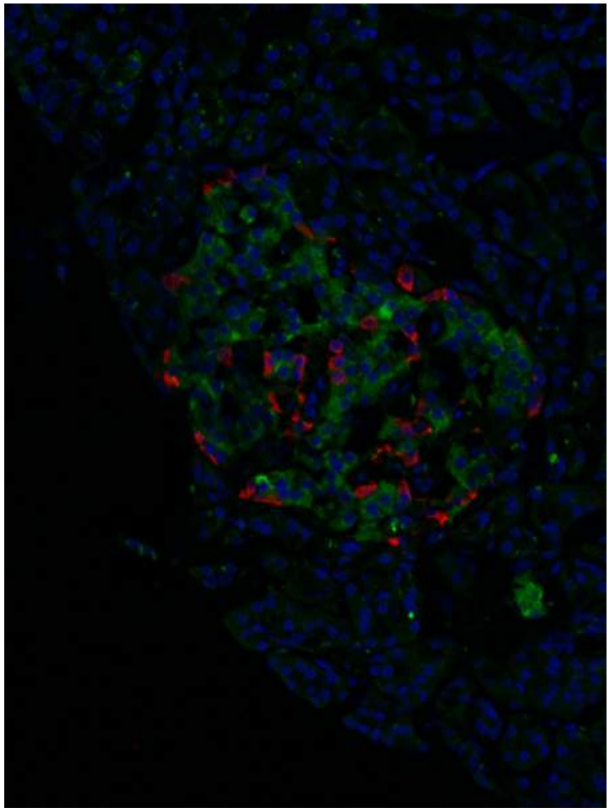
Glucagon
Insulin
DAPI

Negative Control

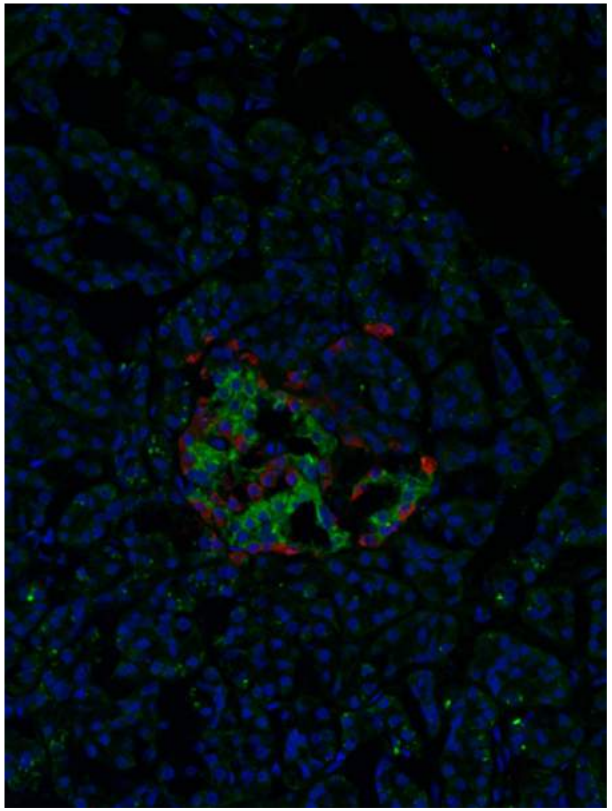


No signal on mouse tissue

Abcam 7842 1:100



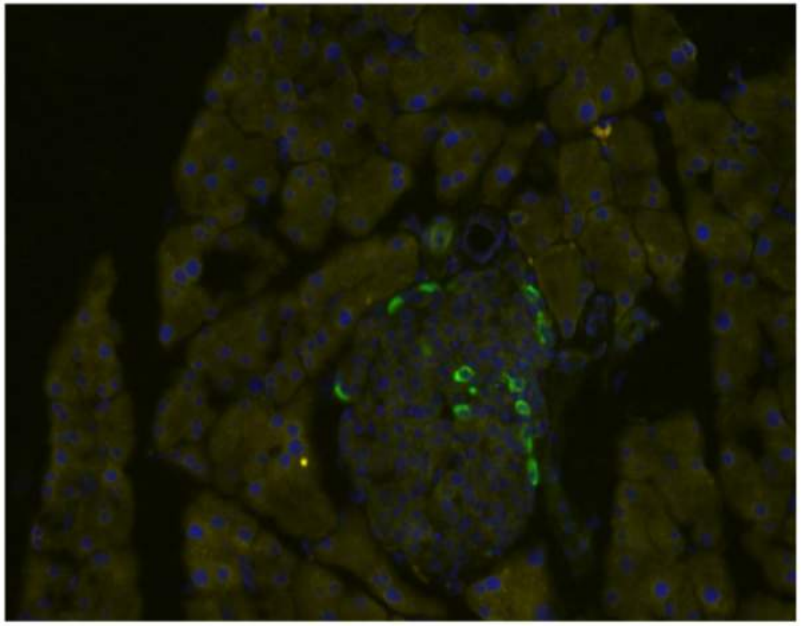
Abcam 7842 1:200 (long exposure)



Abcam 7842 RRID:AB_306130 GP INS Mouse Tissue (Dean lab)

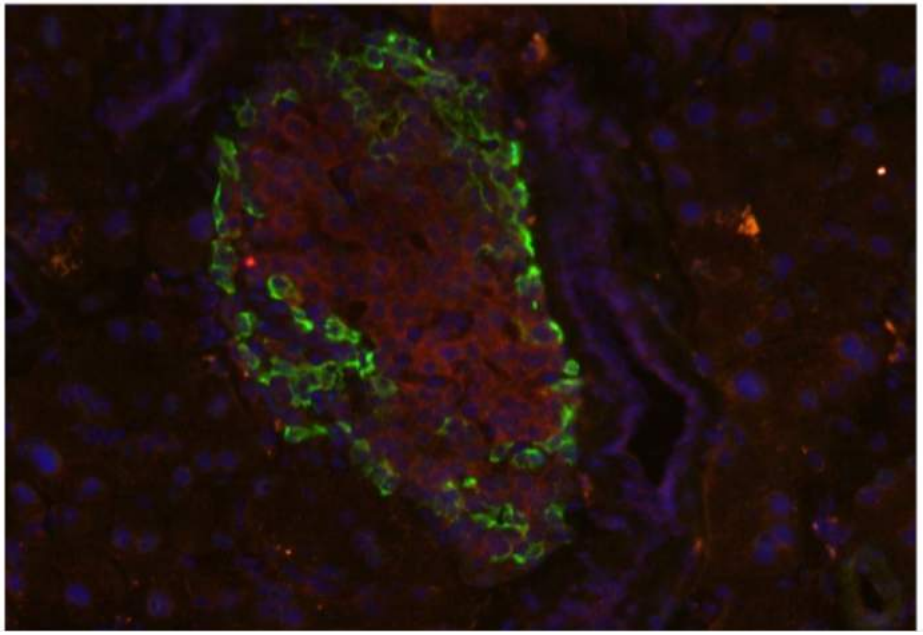
Glucagon
Insulin
DAPI

Negative Control



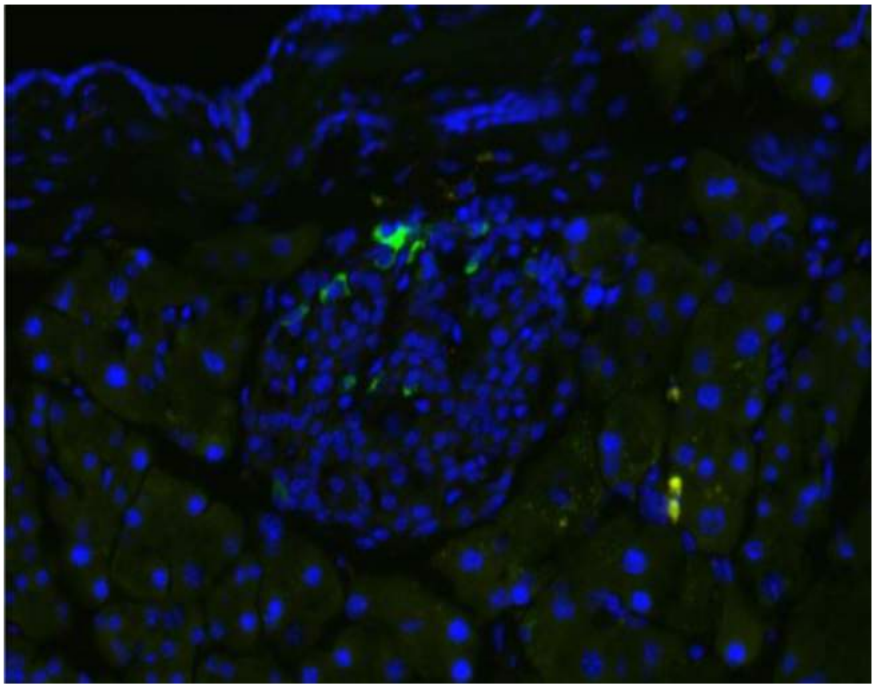
No signal on human tissue

1:200

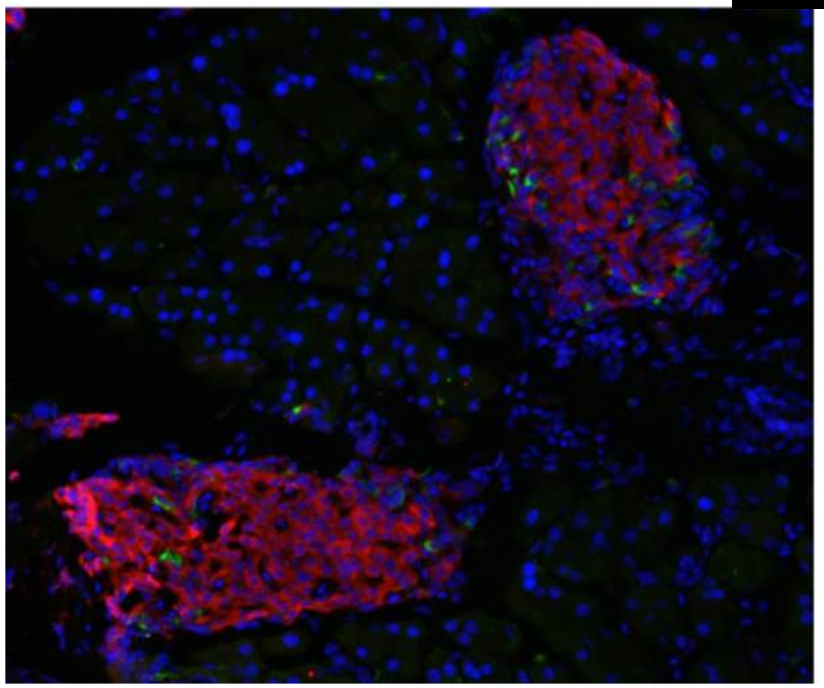


Thermofisher PA5-85595 RRID_2792735 Rb C-Peptide on mouse Tissue (Dean Lab)

Negative Control



1:1000

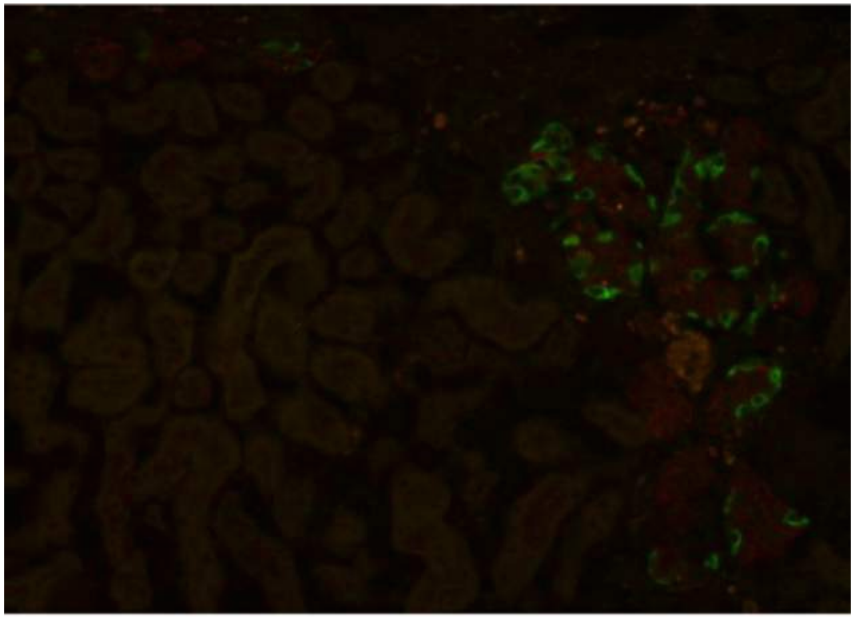


Thermofisher PA5-85595 RRID_2792735 Rb C-Peptide
human Islets injected under mouse kidney capsule

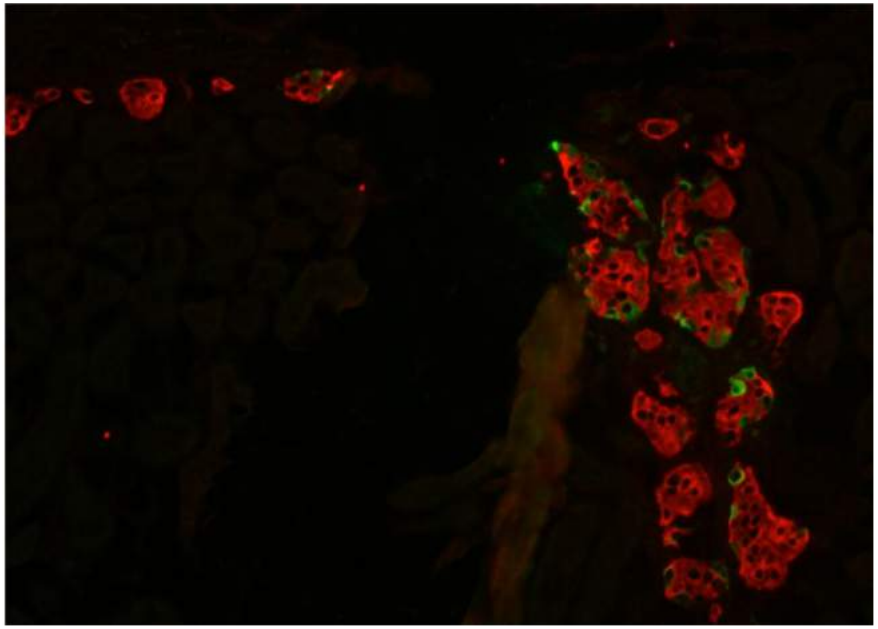
(Dean lab)

Glucagon
Insulin

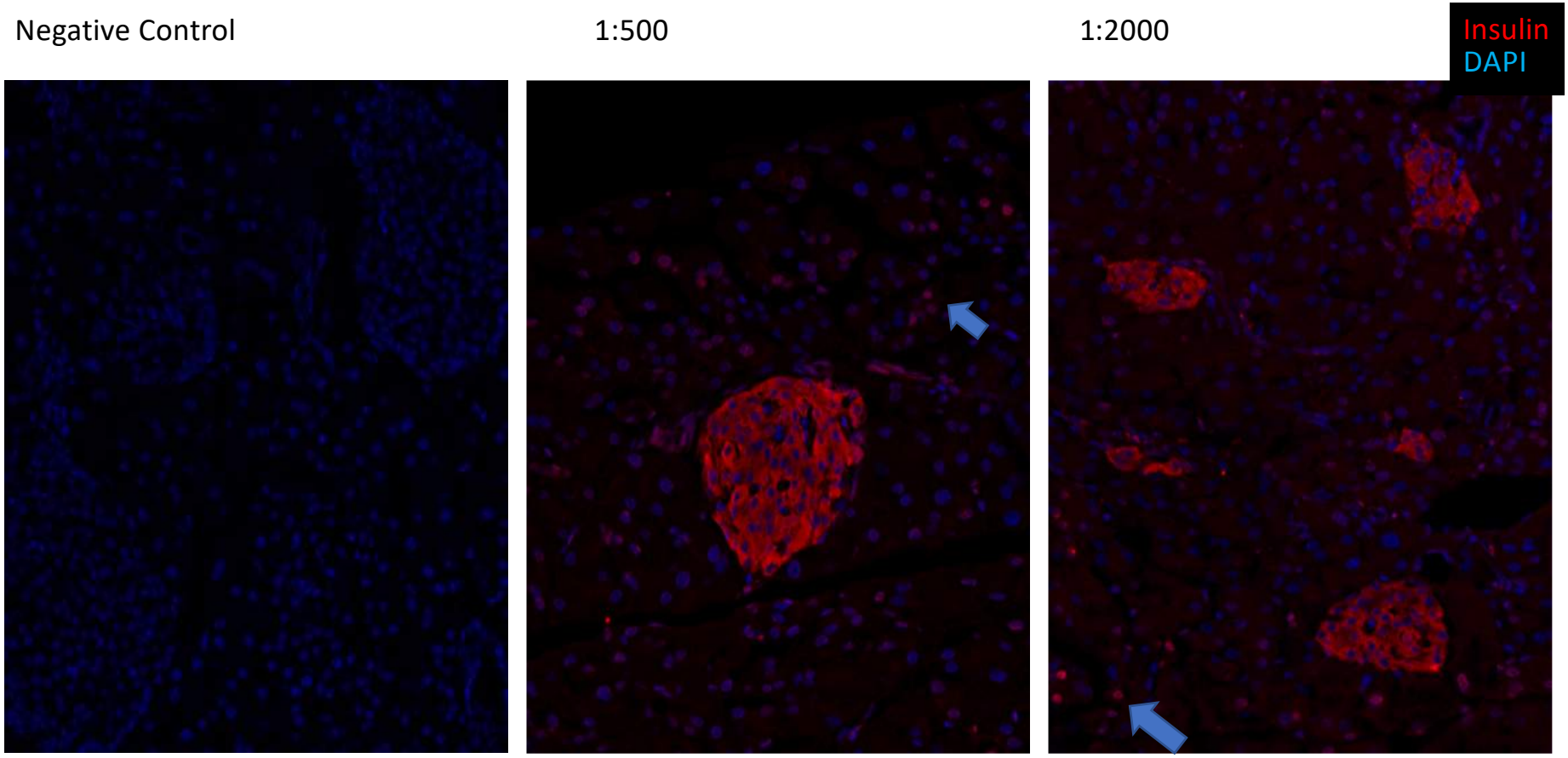
Negative Control



1:1000

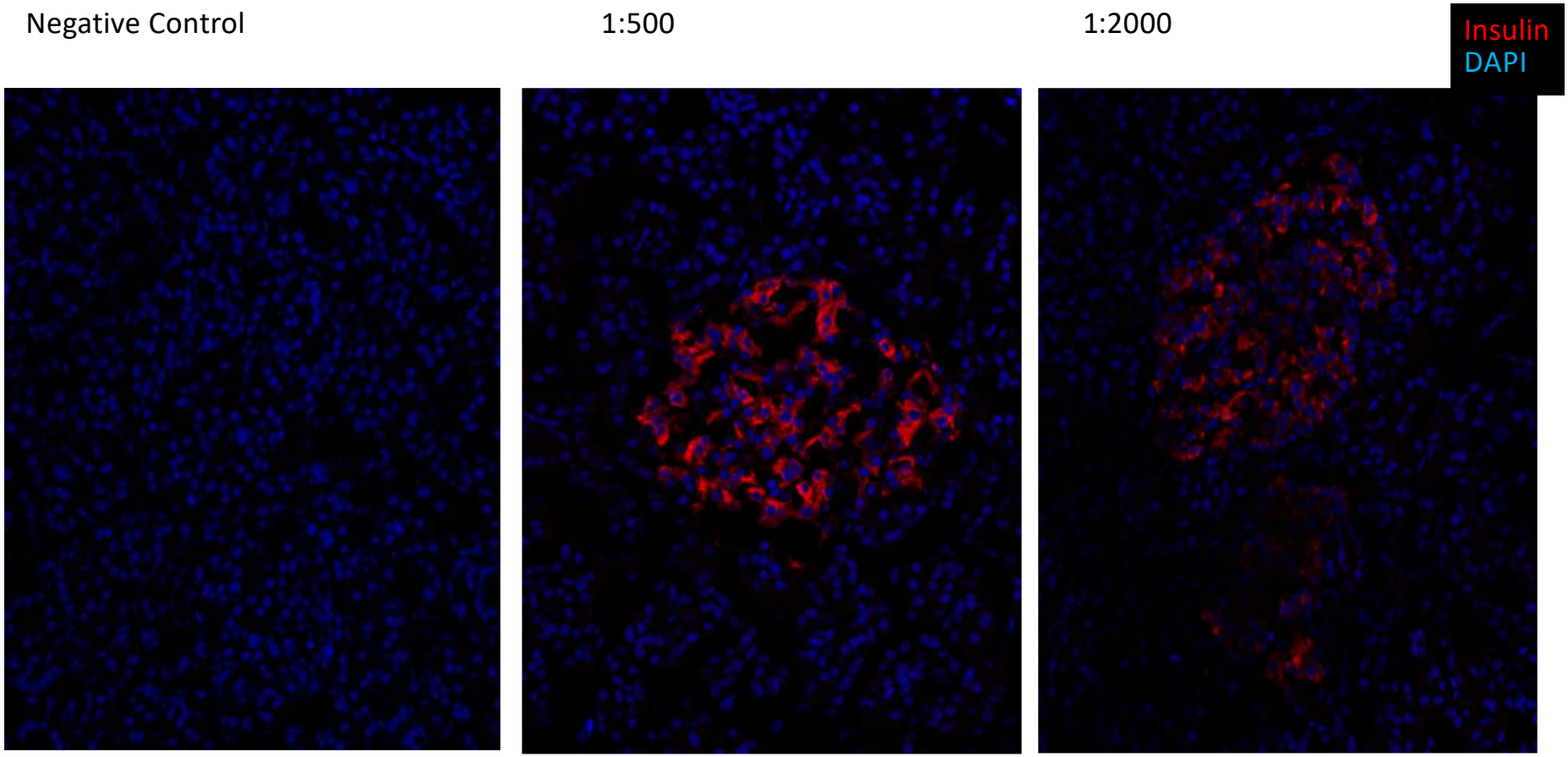


ABClonal A2090 RRID:AB_2764110 Rb INS on Mouse Tissue (Wright Lab)

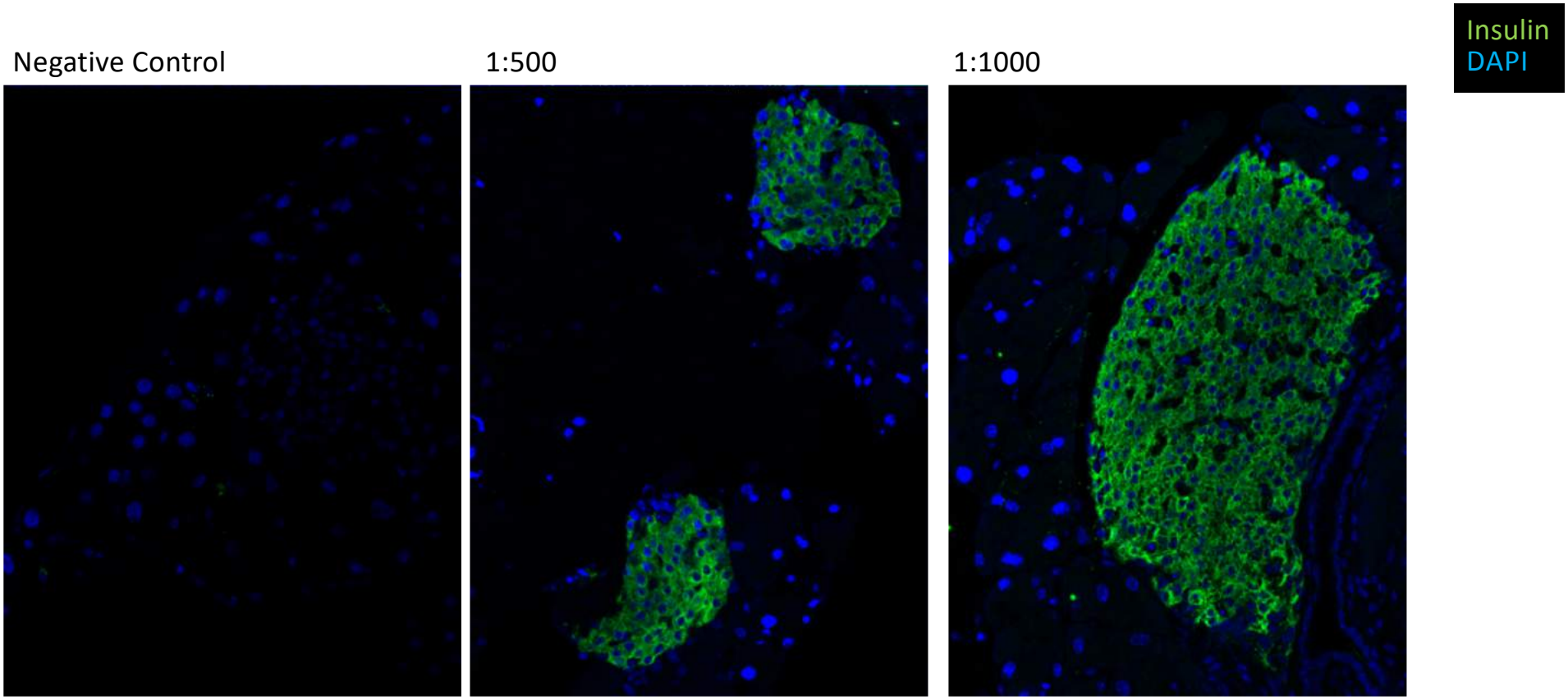


Blue arrows note the acinar nuclear background seen in every dilution tested.

ABClonal A2090 RRID:AB_2764110 Rb INS on Human Tissue (Wright Lab)



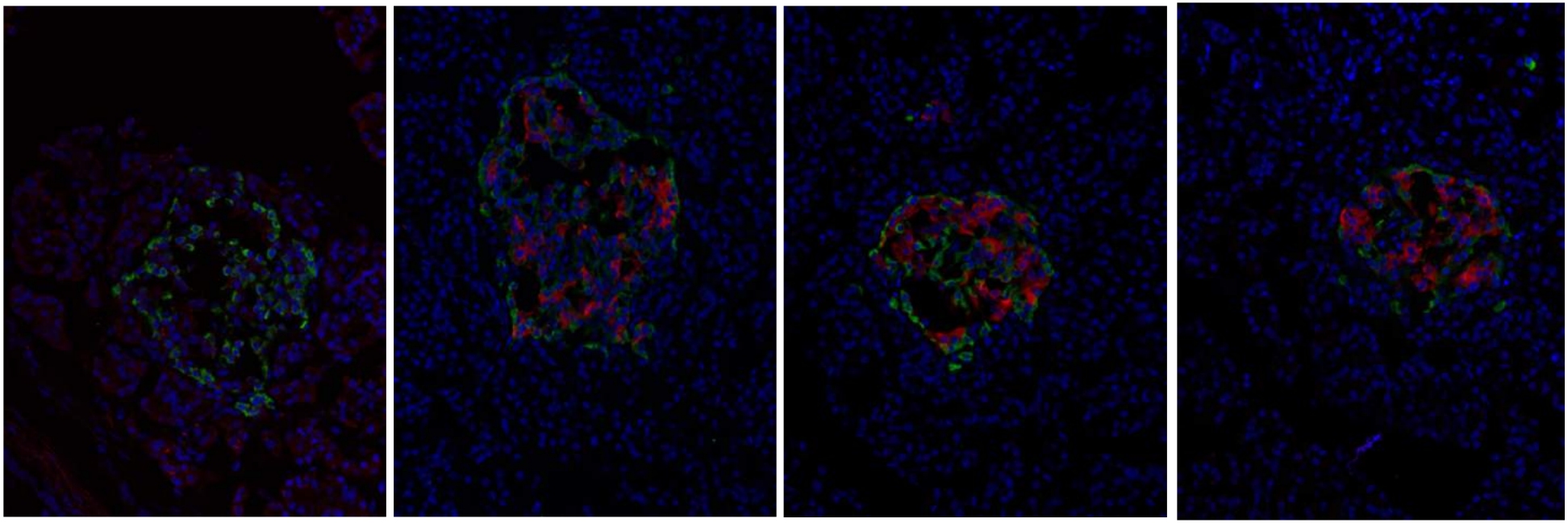
Cell Signaling 3014 RRID:AB_2126503 Rb INS on Mouse Tissue (Wright Lab)



Cell Signaling 3014 RRID:AB_2126503 Rb INS on Human Tissue (Wright Lab)

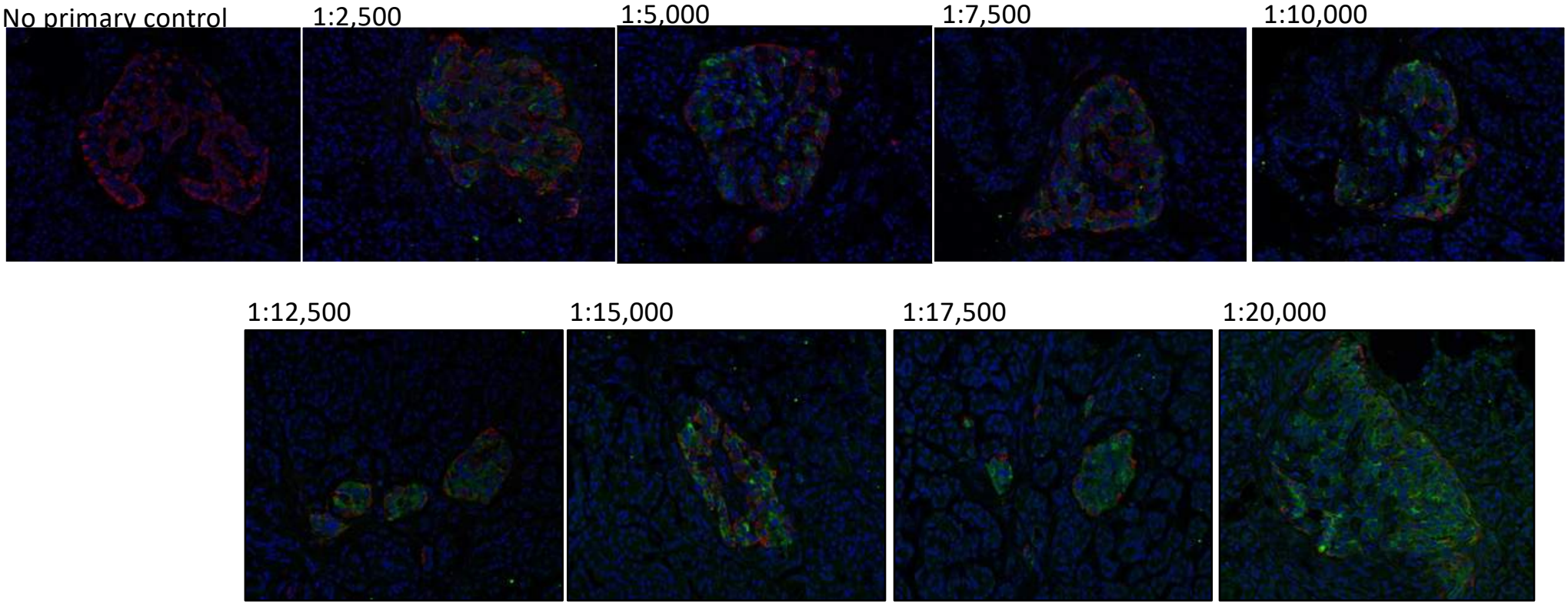
Glucagon
Insulin
DAPI

Negative Control | 1:500 | 1:1000 | 1:2000



Fitzgerald 20-IP35 GP anti-porcine INS RRID:AB_231771 GP INS on Human Tissue (Wright Lab)

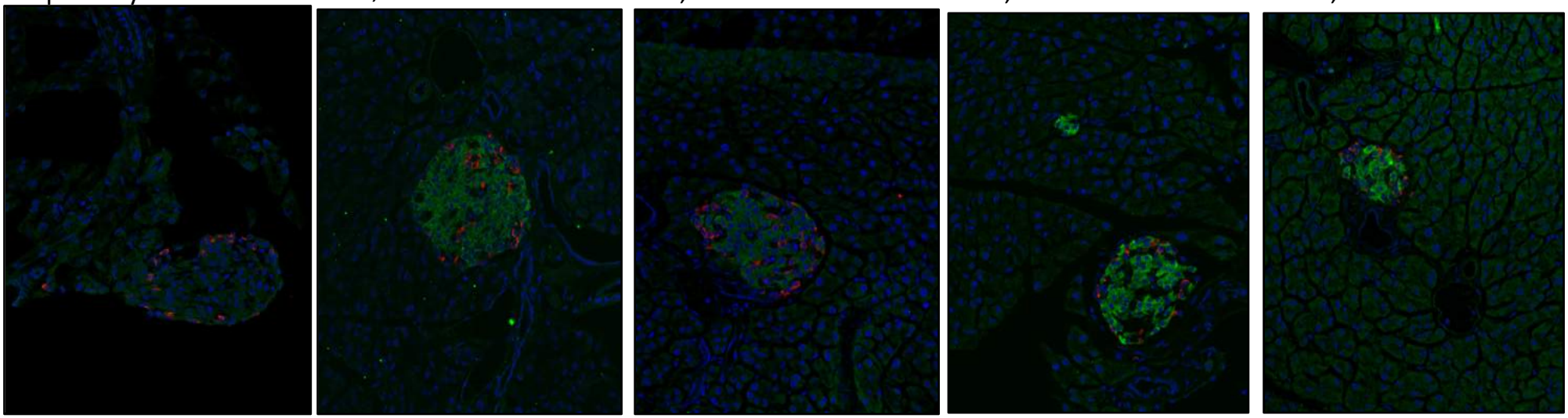
GP INS (Fitzgerald)
Rb Glucagon 1:500
DAPI



Fitzgerald 20-IP35 GP anti-porcine INS RRID:AB_231771 GP INS on Mouse Tissue (Wright Lab)

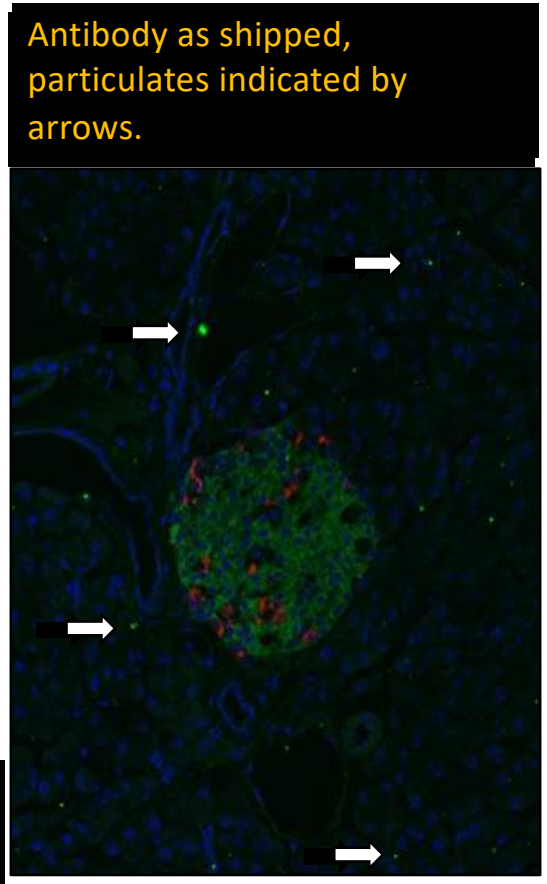
GP INS (Fitzgerald)
Rb Glucagon 1:500
DAPI

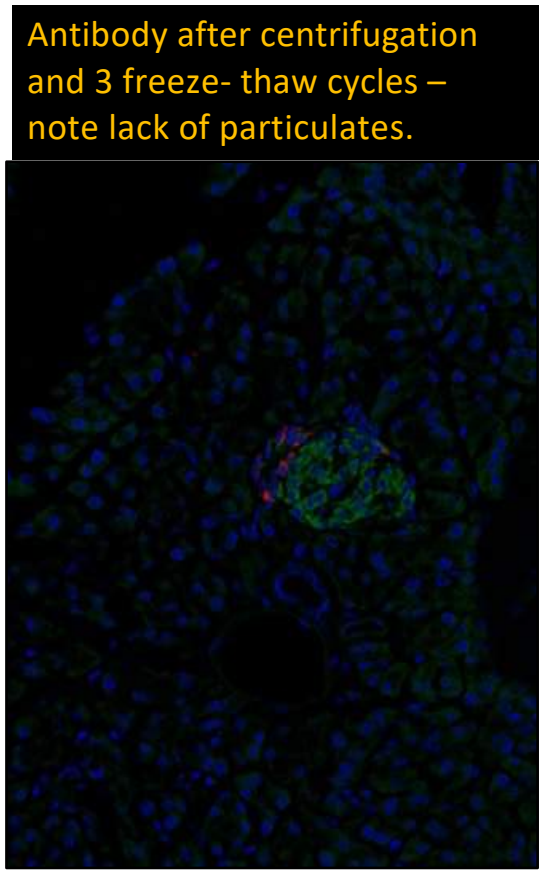
No primary control | 1:2,500 | 1:5,000 | 1:7,500 | 1:10,000



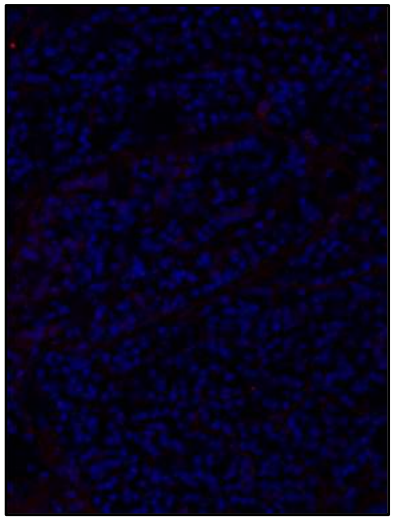
Fitzgerald 20-IP35 GP anti-porcine INS RRID:AB_231771 GP INS Quirk 1: Particulates in shipped reagent (Wright Lab)

- This antibody, as shipped, has significant particulates.
- Particulates are removed by centrifugation.
- The “spun down” supernatant has been used through 3 freeze/thaw cycles without particulates re-appearing.
- **Particulate Removal: Centrifuge at least 10 min, ~12,000 x g, RT, then discard pellet**



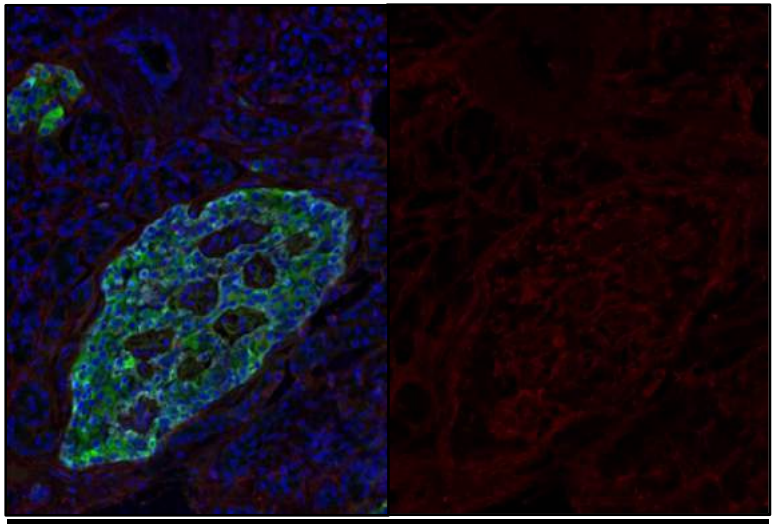


Fitzgerald 20-IP35 GP anti-porcine INS RRID:AB_231771 GP INS Quirk 2: Cross-reaction with anti-Sheep secondary antibody (Wright Lab)



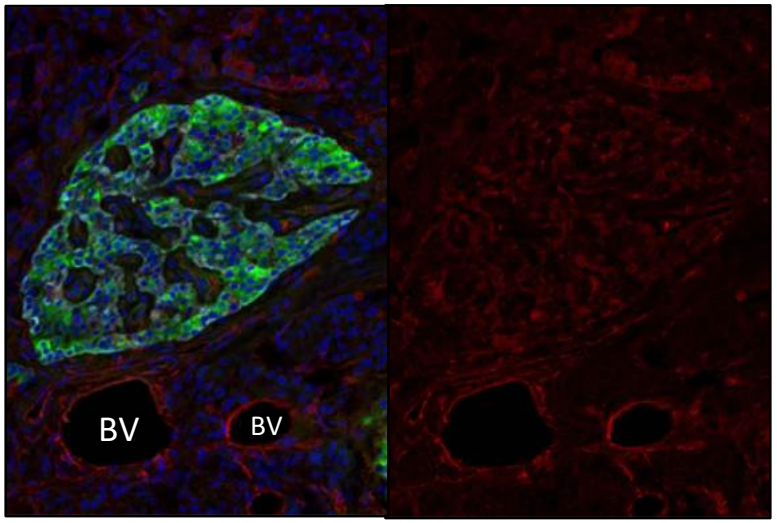
No primary AB, Dnky anti-GP IgG AF488
No primary AB, Dnky anti-SH IgG AF594
DAPI

High Non-specific Background



Gp INS + Dnky anti-GP IgG AF488 No primary, Dnky anti-SH IgG AF594
DAPI

Specific signal is obscured by background noise



Gp INS + Dnky anti-GP IgG AF488
Sh CD31 + Dnky anti- SH IgG AF594
DAPI

No cross reaction with goat, rabbit, mouse or rat IgG observed.