

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
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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 (<i>now also discontinued</i>)	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	

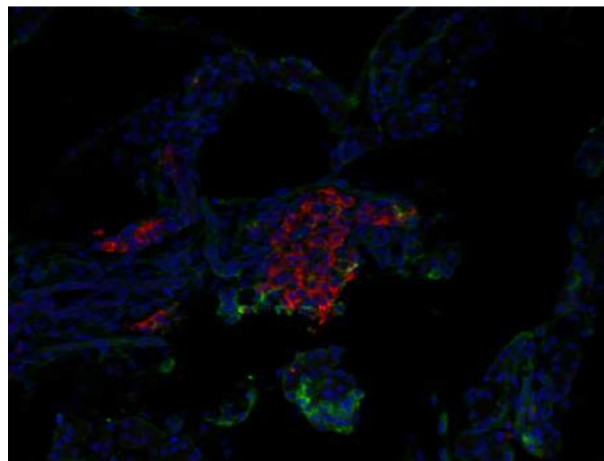
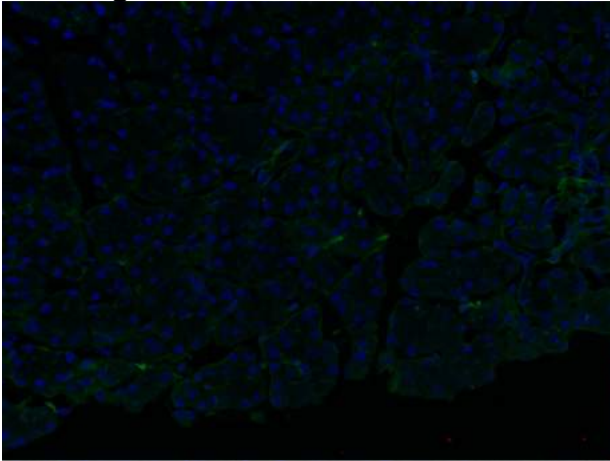
Dako A0564 GP INS Mouse Tissue (Wright Lab)

reference IF reagent; now discontinued as the “concentrated reagent”

Glucagon
Insulin
DAPI

Negative Control

1:1000



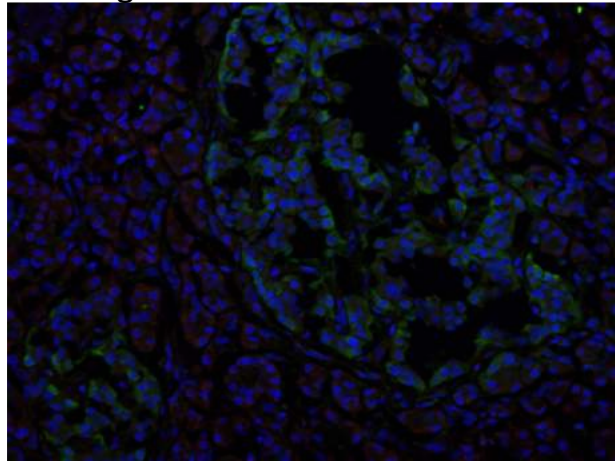
Discontinued as concentrate A0564.

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

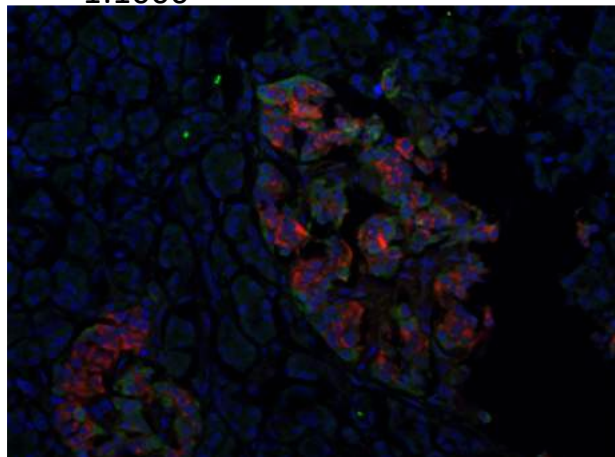
Sigma/Cell Marque 273A-14 RRID:AB_1158520 GP INS on Mouse Tissue
(Wright Lab) now discontinued

Glucagon
Insulin
DAPI

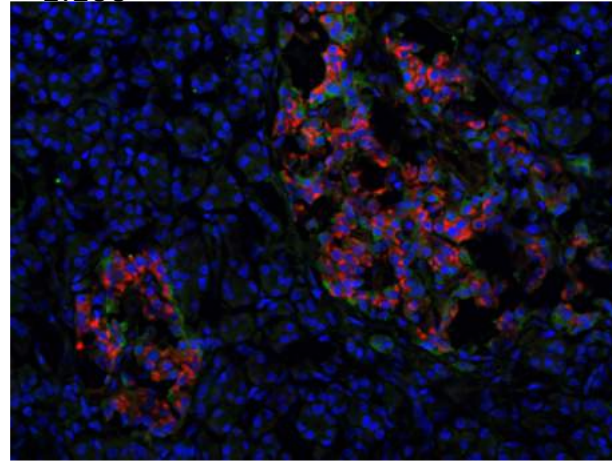
Negative Control



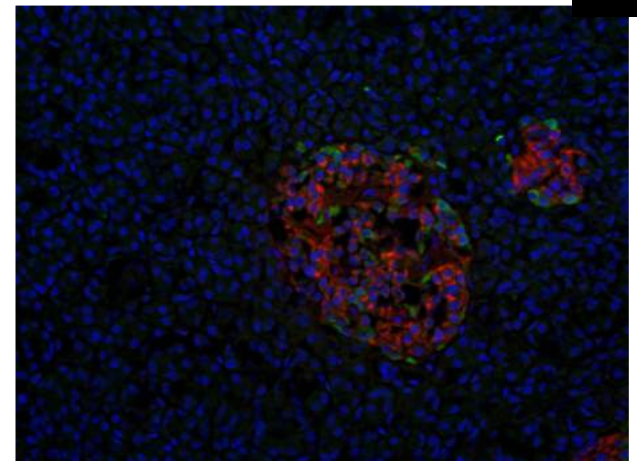
1:1000



1:100



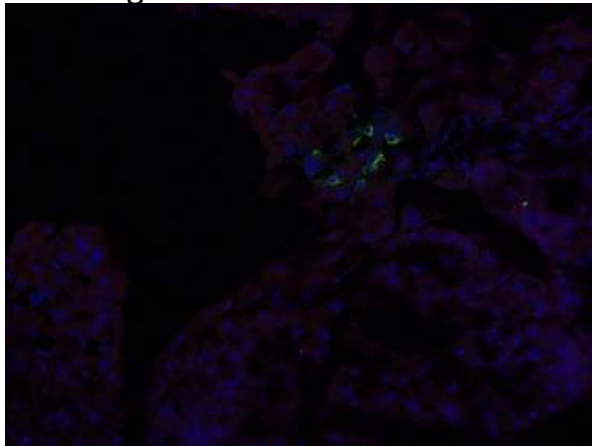
1:500



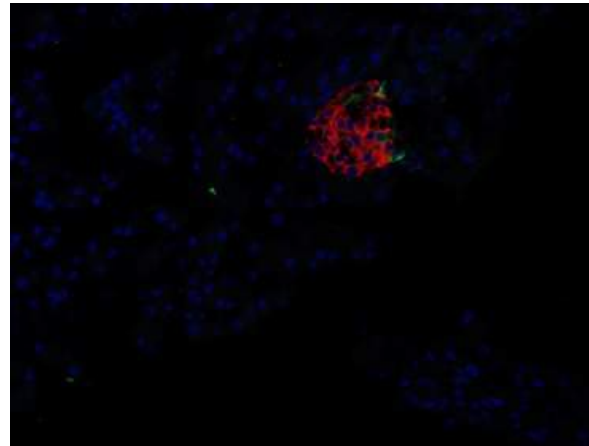
Sigma/Cell Marque 273A-14 RRID:AB_1158520 GP INS on Mouse Tissue
(Wright Lab) now discontinued

Glucagon
Insulin
DAPI

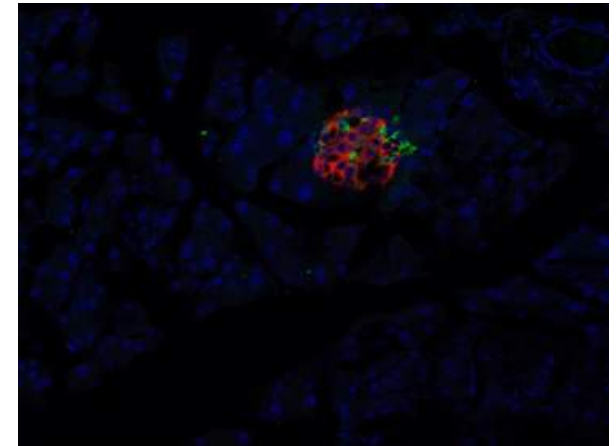
Negative Control



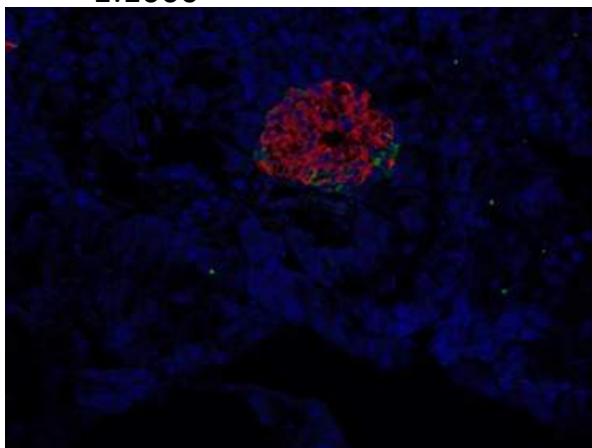
1:100



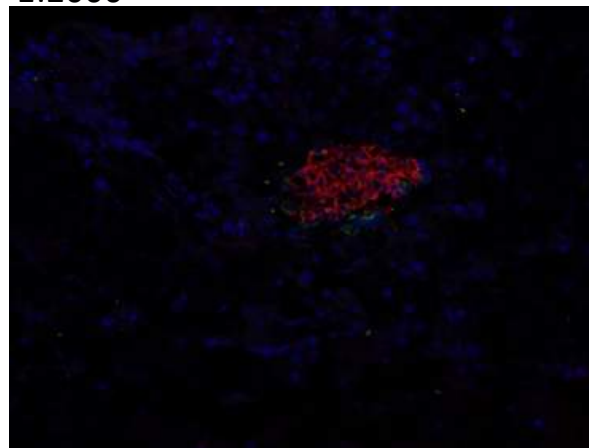
1:500



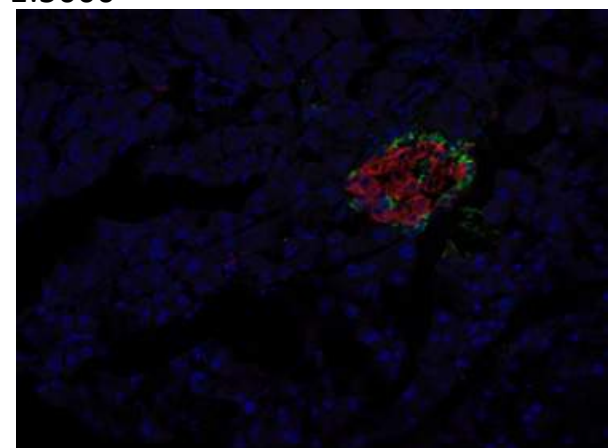
1:1000



1:2000



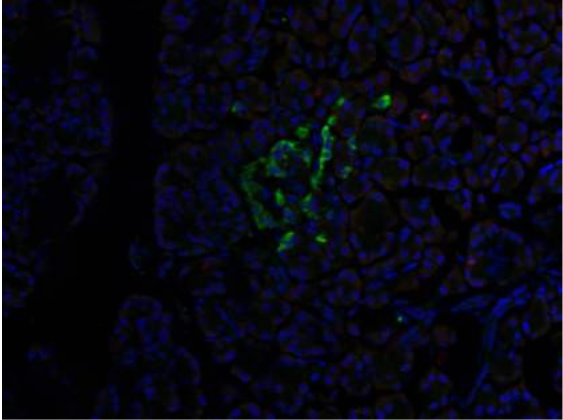
1:5000



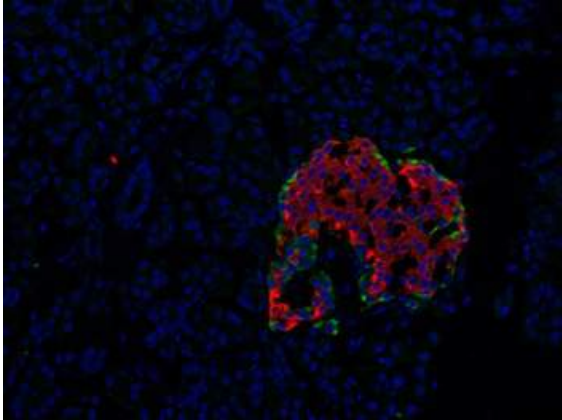
DSHB GS 9A8 RRID:AB_532383 Ms INS on Human Tissue (Wright Lab)

Glucagon
Insulin
DAPI

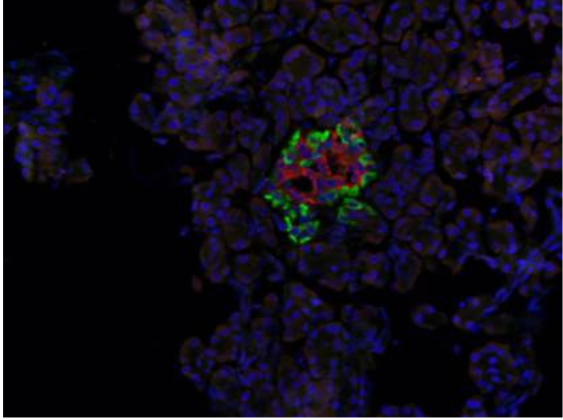
Negative Control



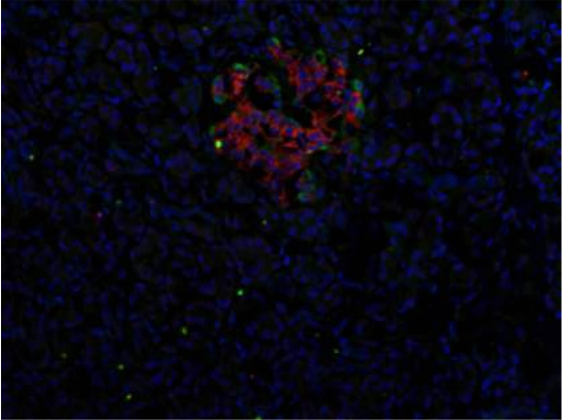
1:1000



1:100

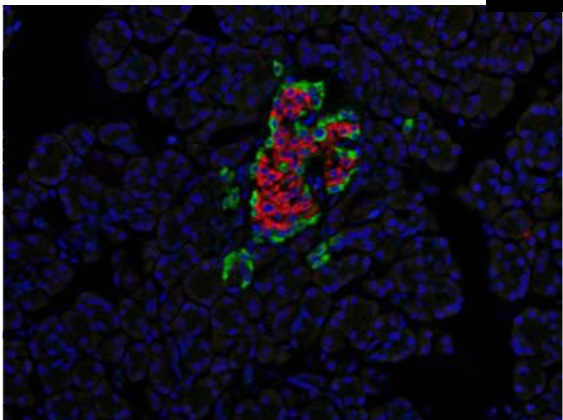


1:2500

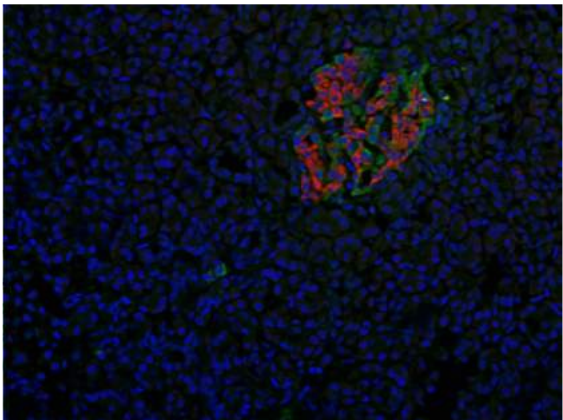


No signal on mouse tissues

1:500



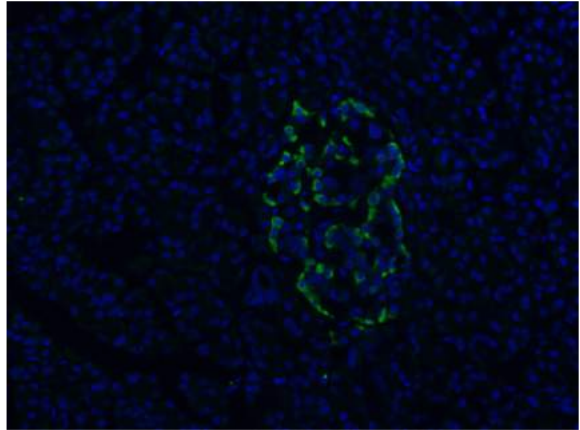
1:5000



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

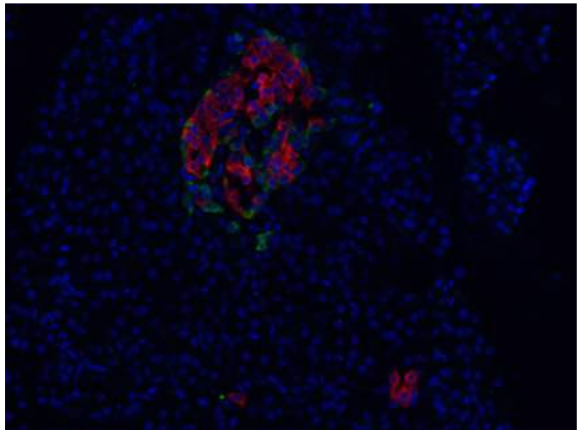
Glucagon
Insulin
DAPI

Negative Control



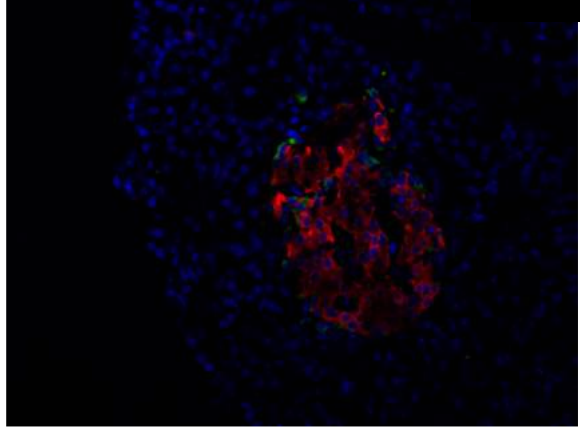
1:1000

1:100

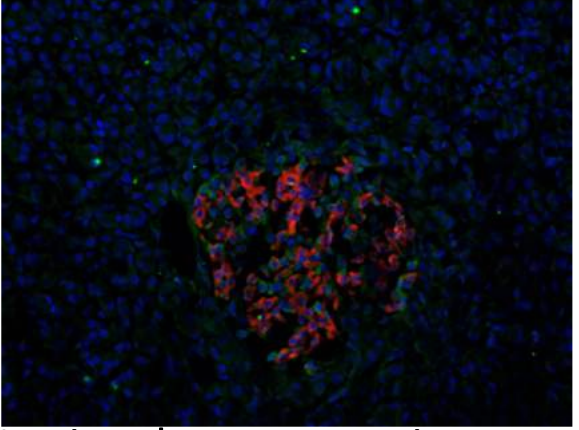
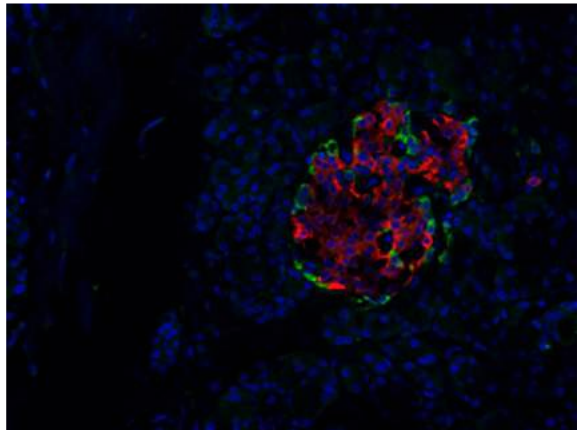


1:2500

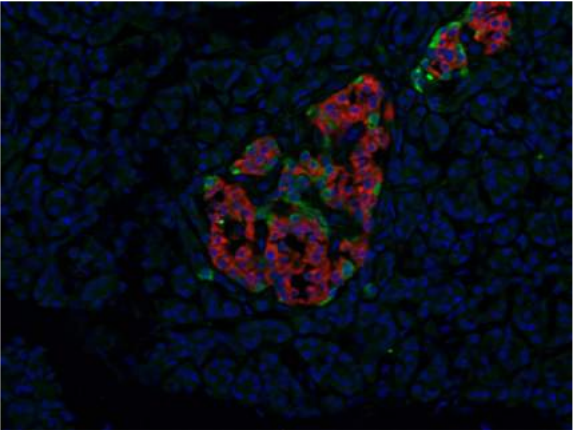
1:500



1:5000



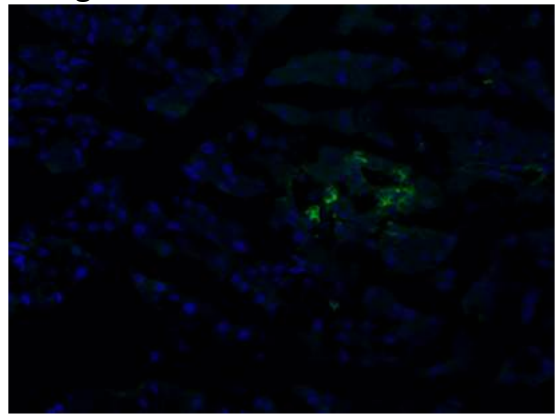
No signal on mouse tissues



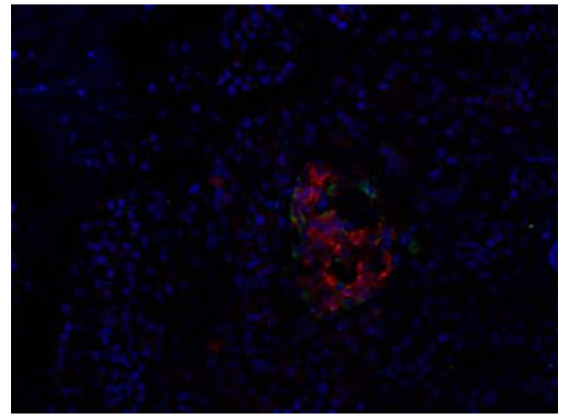
ThermoFisher 701265 RRID:AB_2532448 Rb INS on Human Tissue
(Wright Lab)

Glucagon
Insulin
DAPI

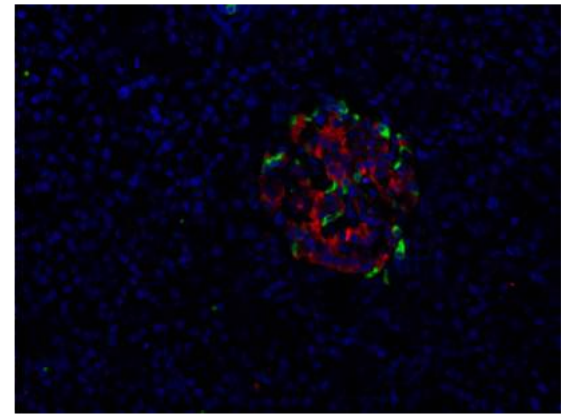
Negative Control



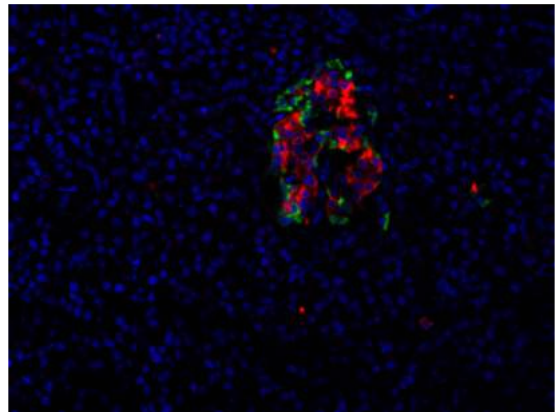
1:100



1:500



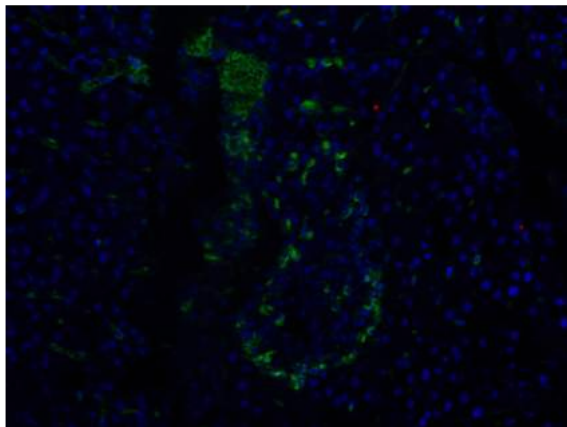
1:1000



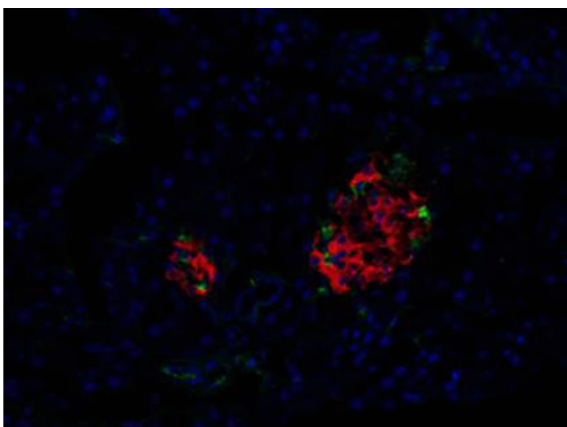
ThermoFisher 701265 RRID:AB_2532448 Rb INS on Mouse Tissue
(Wright Lab)

Glucagon
Insulin
DAPI

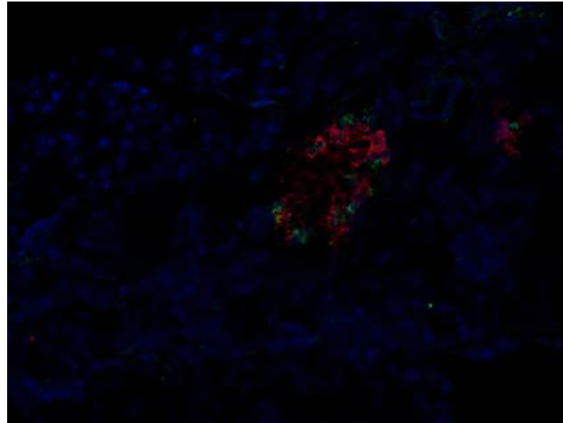
Negative Control



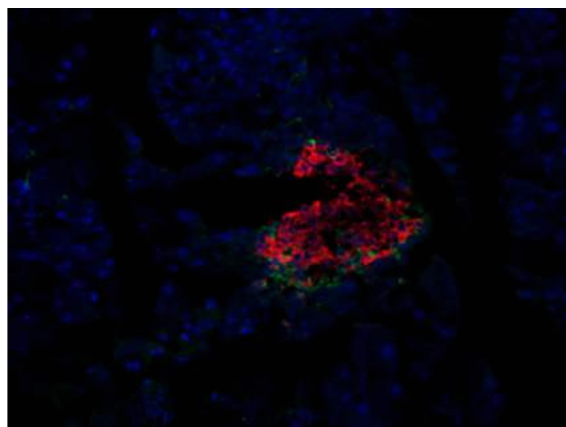
1:1000



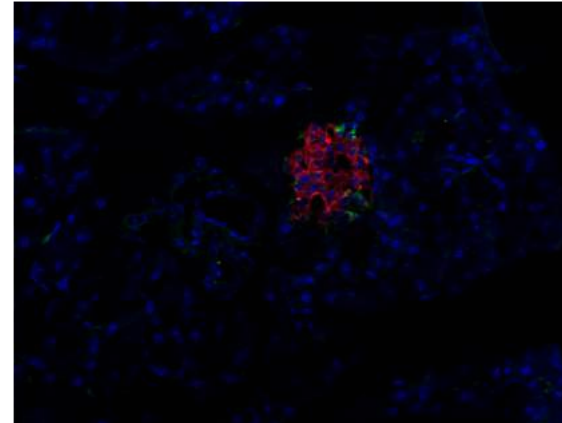
1:100



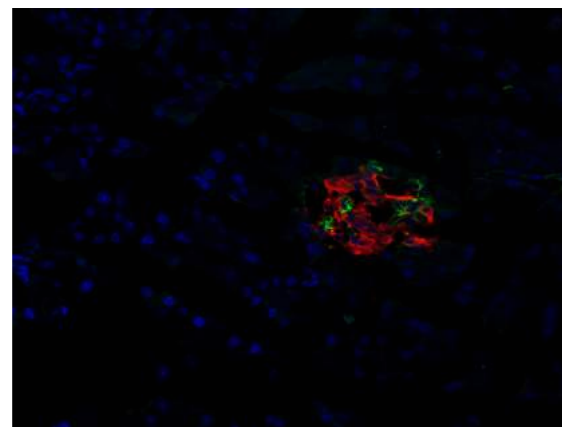
1:2500



1:500

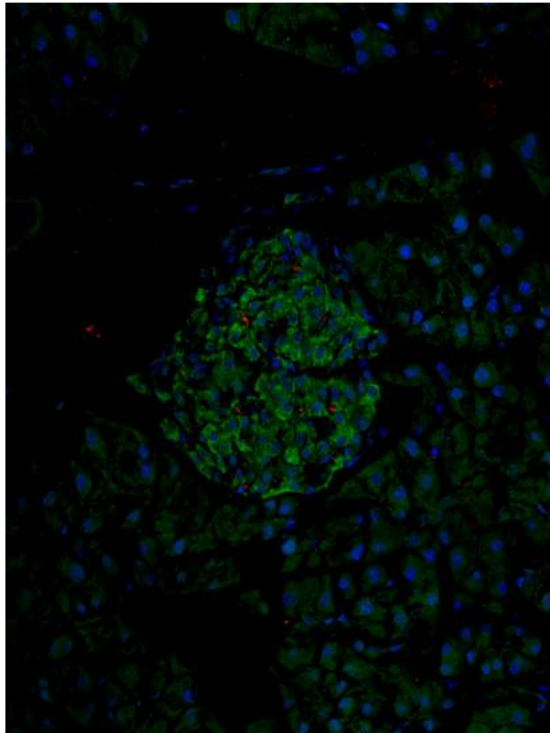


1:5000

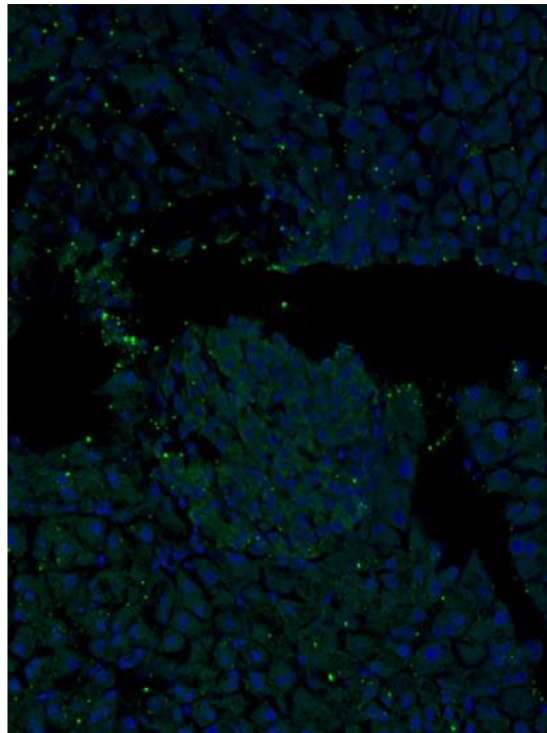


Genetex GTX27842 RRID:AB_371884 GP INS on Mouse Tissue (Wright Lab)

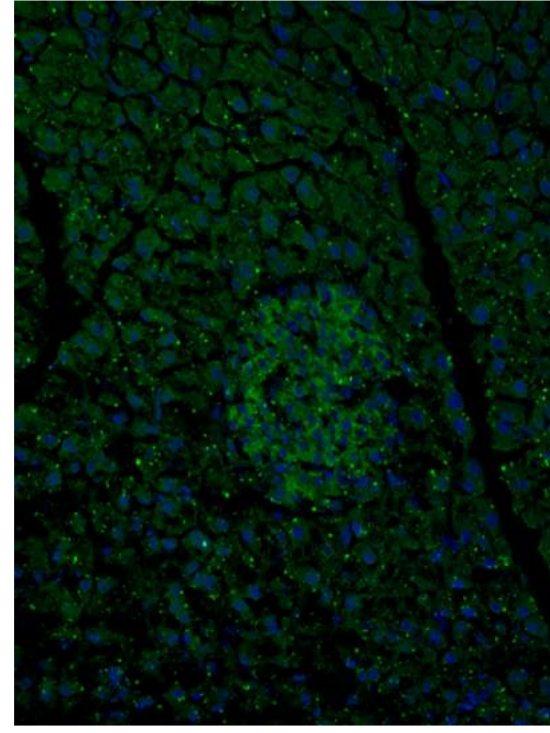
Positive control Slide
(Sigma/Cell Marque GP Insulin 1:500)



Genetex GTX27842 GP INS
1:500



Genetex GTX27842 GP INS
1:1000



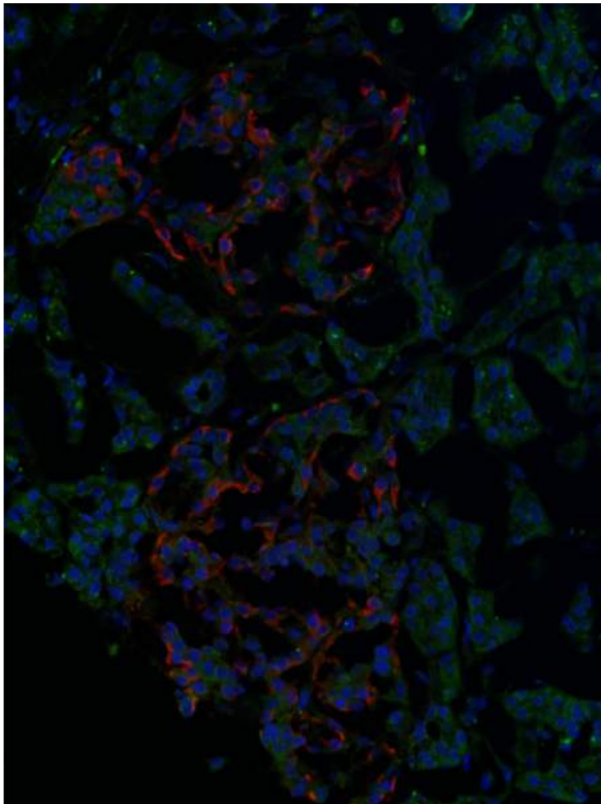
Insulin
DAPI

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)

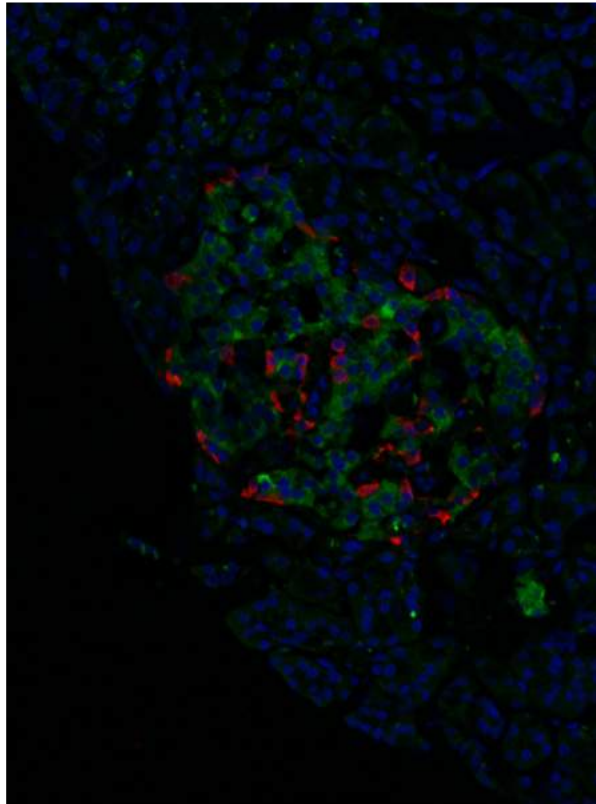
Glucagon
Insulin
DAPI

Negative Control

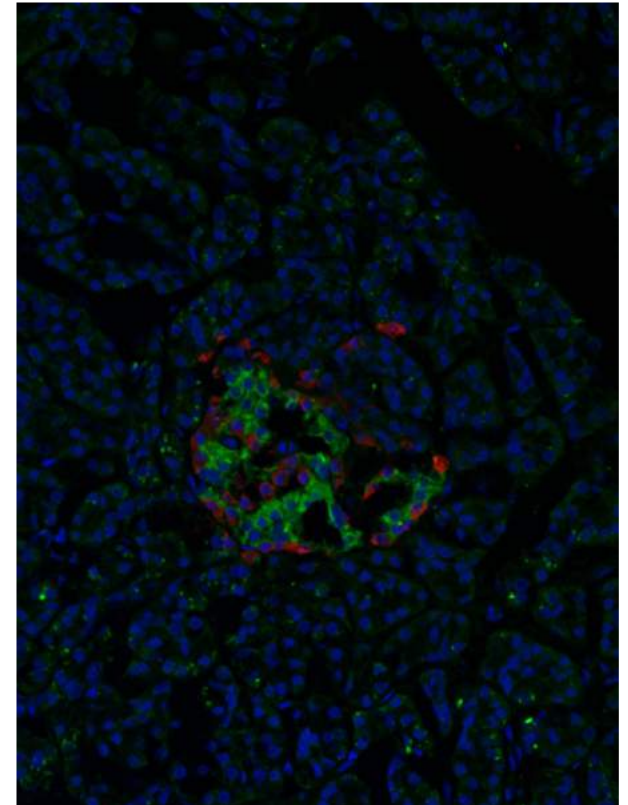


No signal on mouse tissue

Abcam 7842 1:100



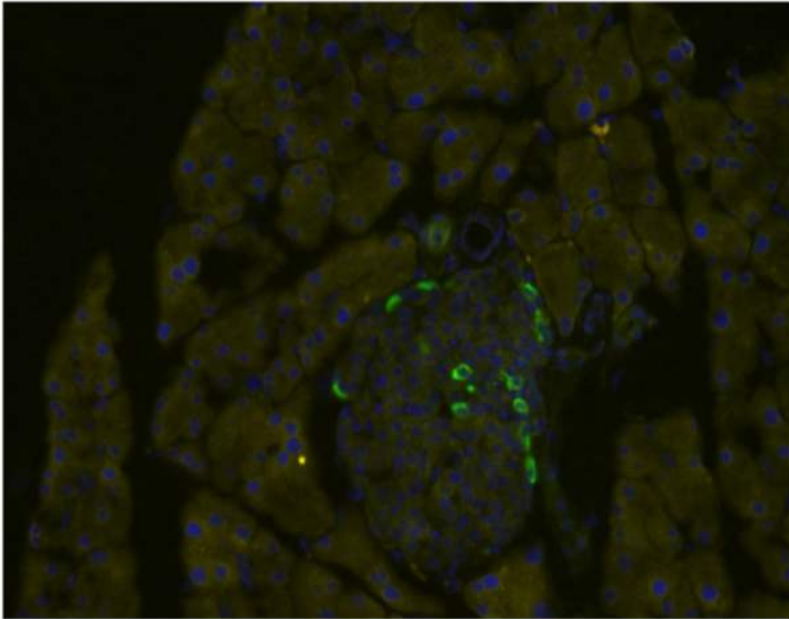
Abcam 7842 1:200 (long exposure)



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

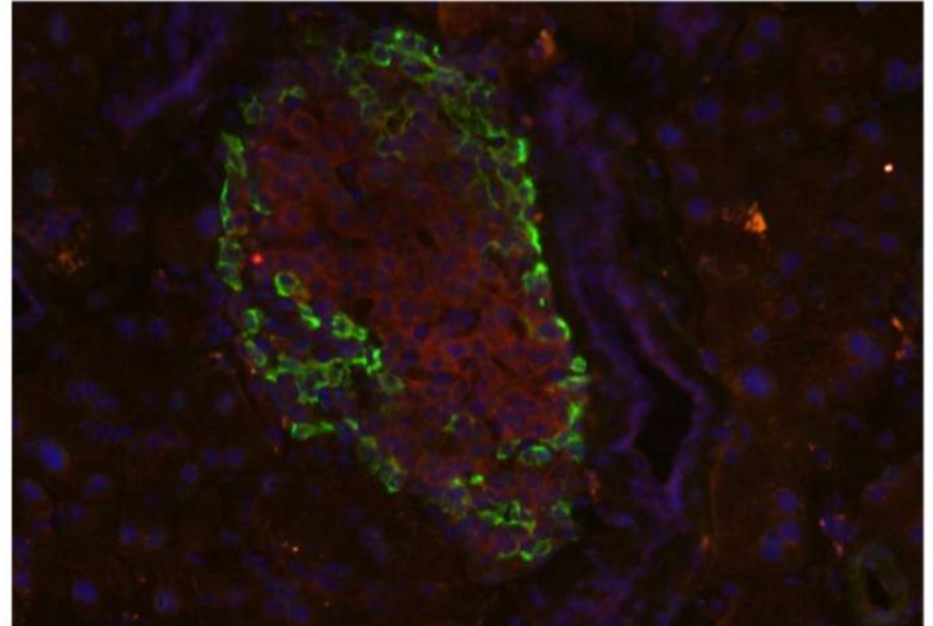
Glucagon
Insulin
DAPI

Negative Control



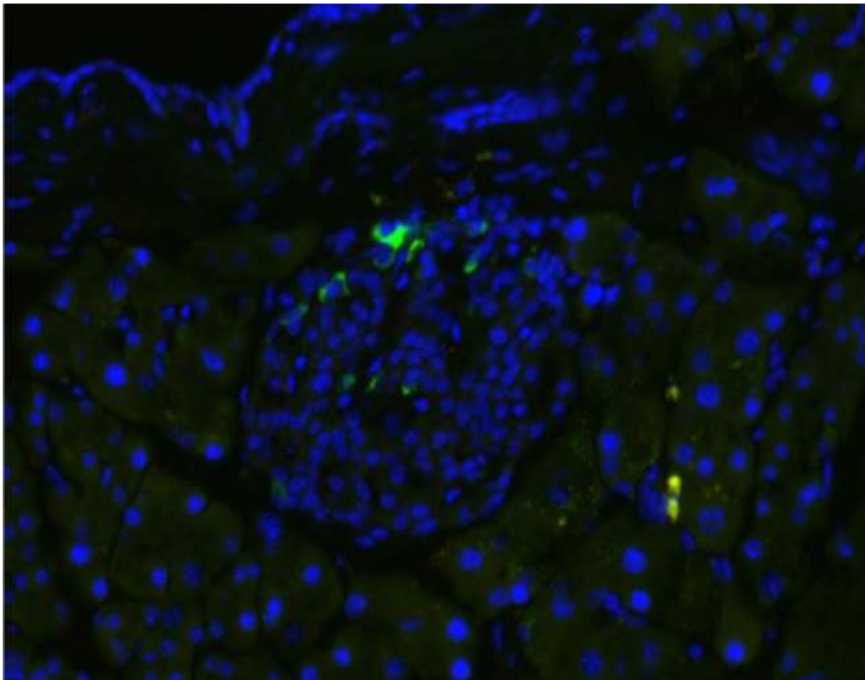
No signal on human tissue

1:200

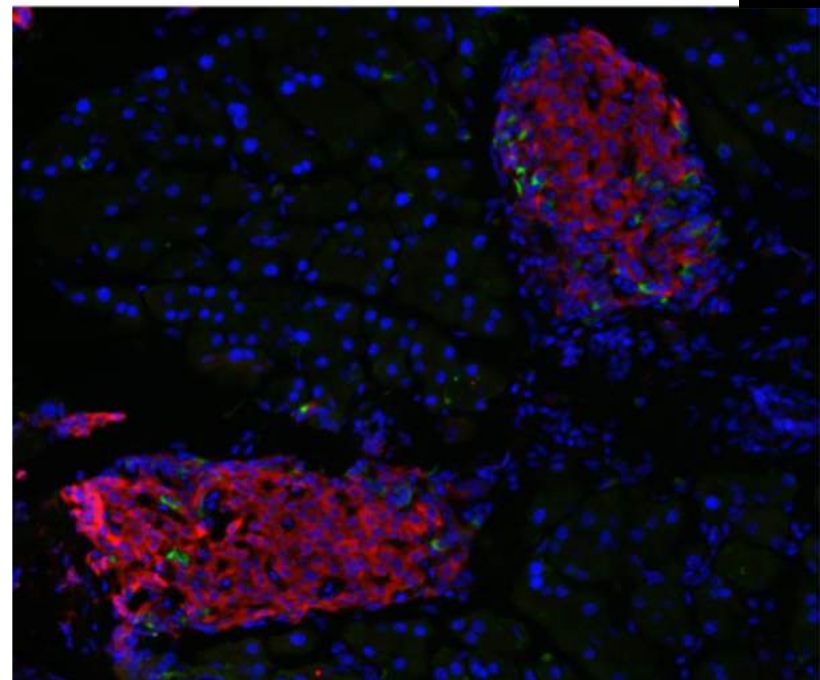


Thermofisher PA5-85595 RRID_2792735 Rb C-Peptide on mouse Tissue
(Dean Lab)

Negative Control



1:1000



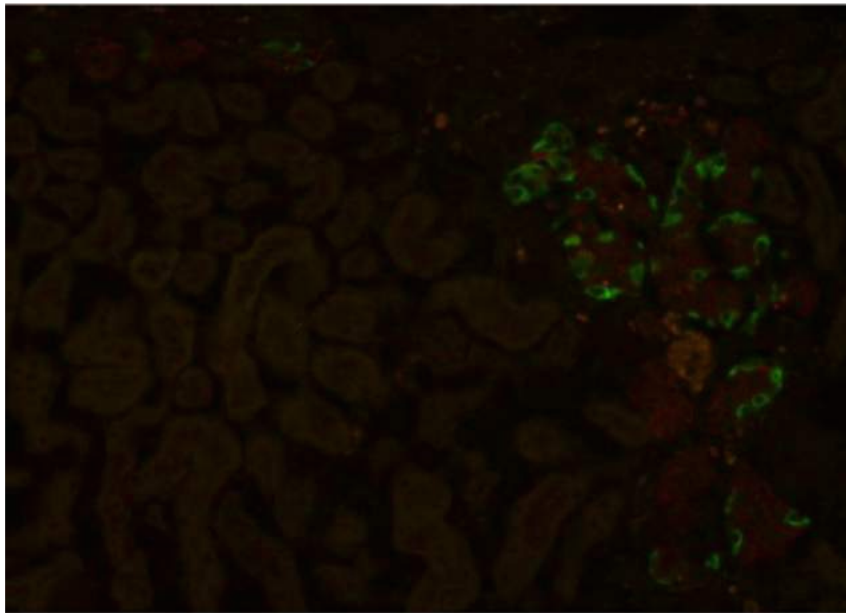
Glucagon
Insulin
DAPI

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

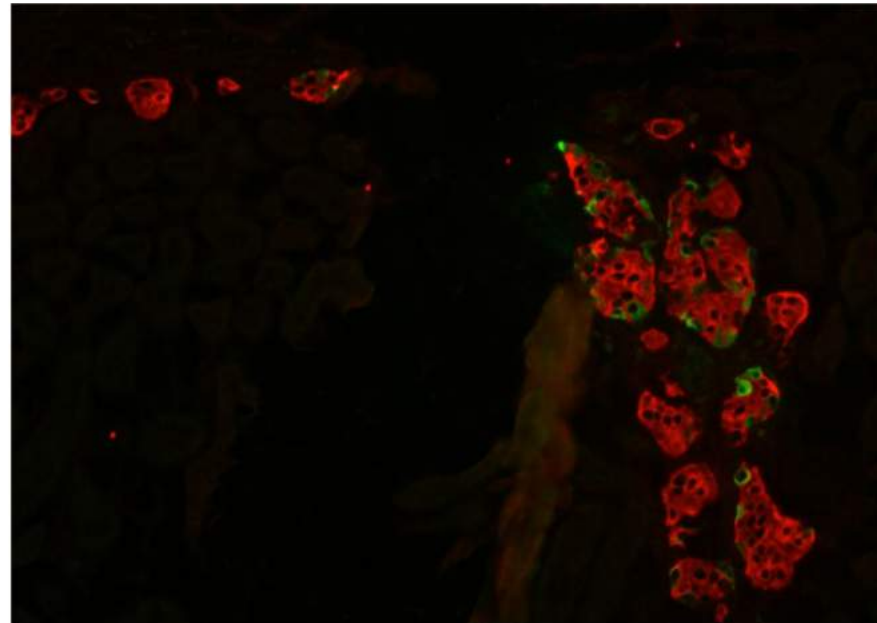
(Dean lab)

Glucagon
Insulin

Negative Control



1:1000



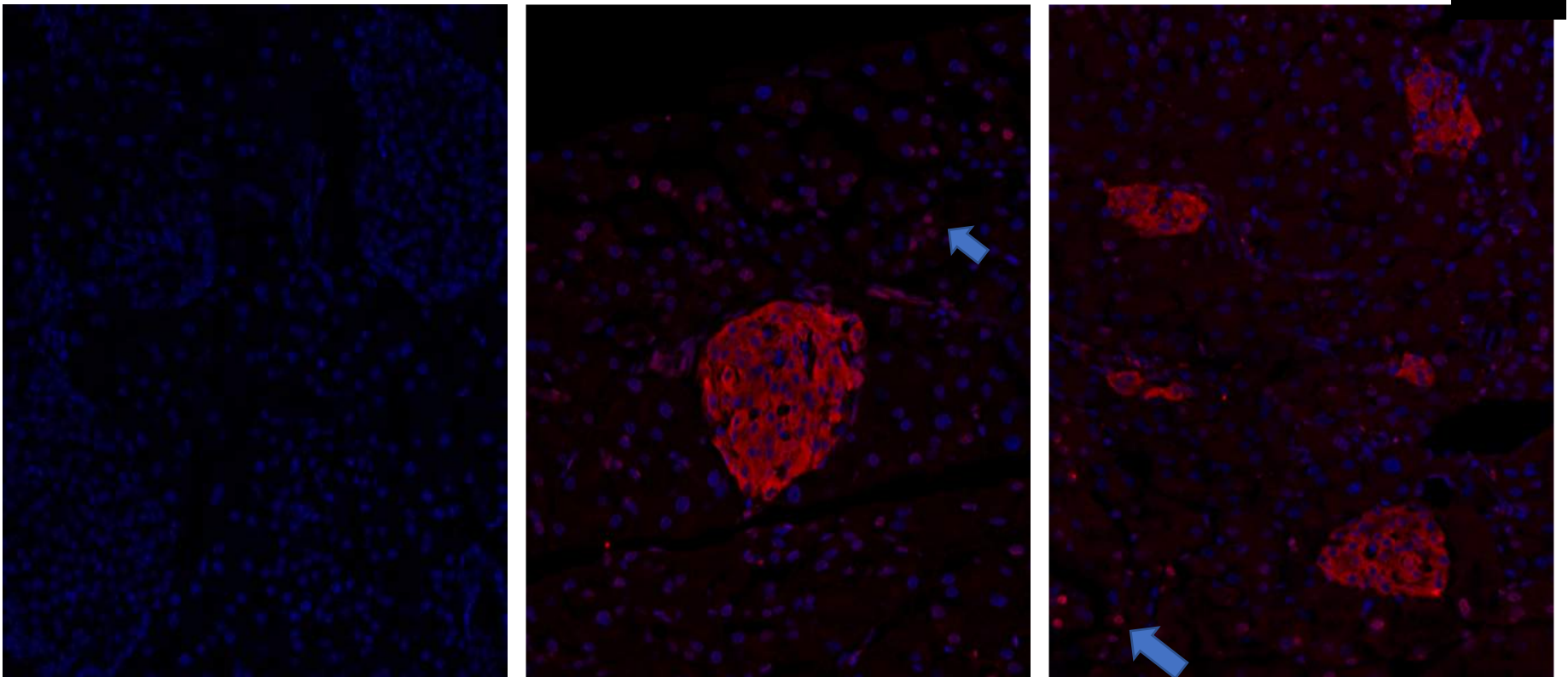
ABClonal A2090 RRID:AB_2764110 Rb INS on Mouse Tissue (Wright Lab)

Negative Control

1:500

1:2000

Insulin
DAPI



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

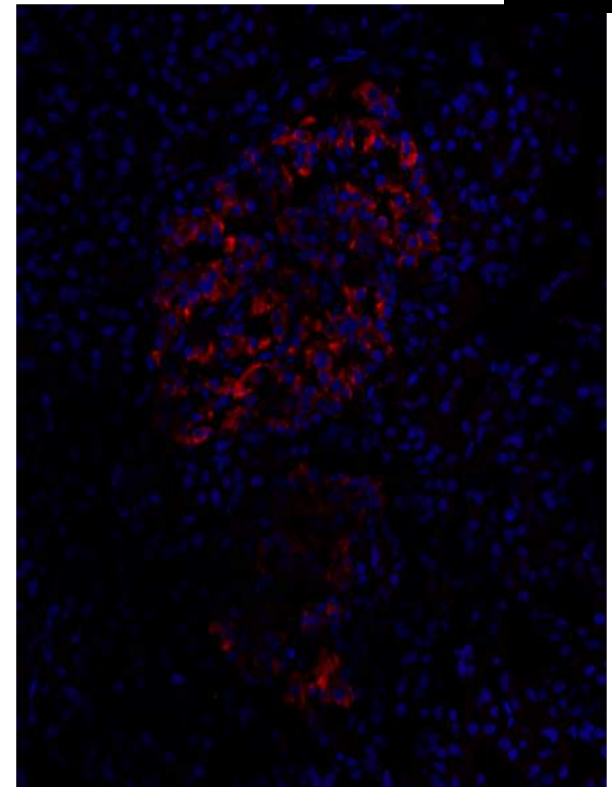
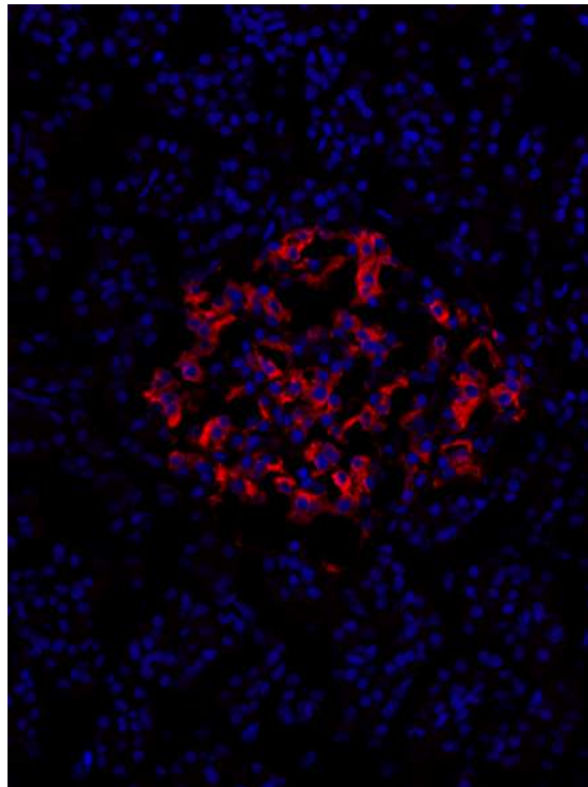
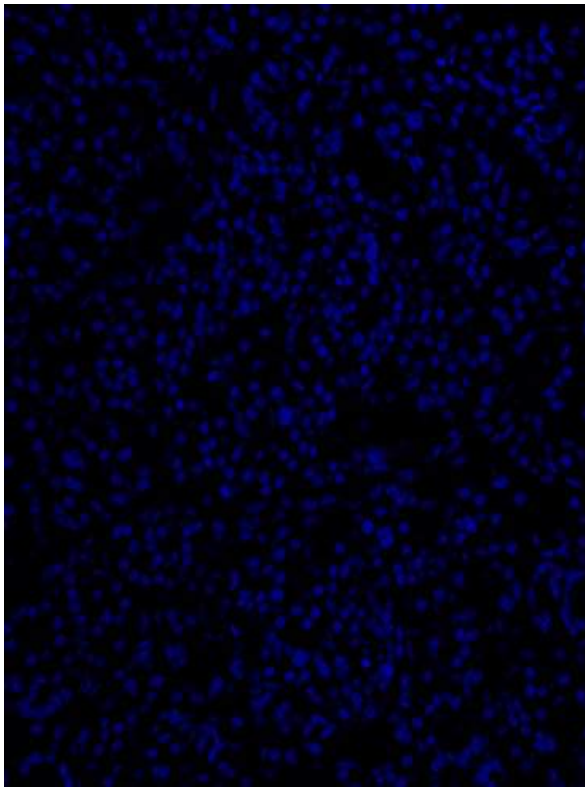
ABClonal A2090 RRID:AB_2764110 Rb INS on Human Tissue
(Wright Lab)

Negative Control

1:500

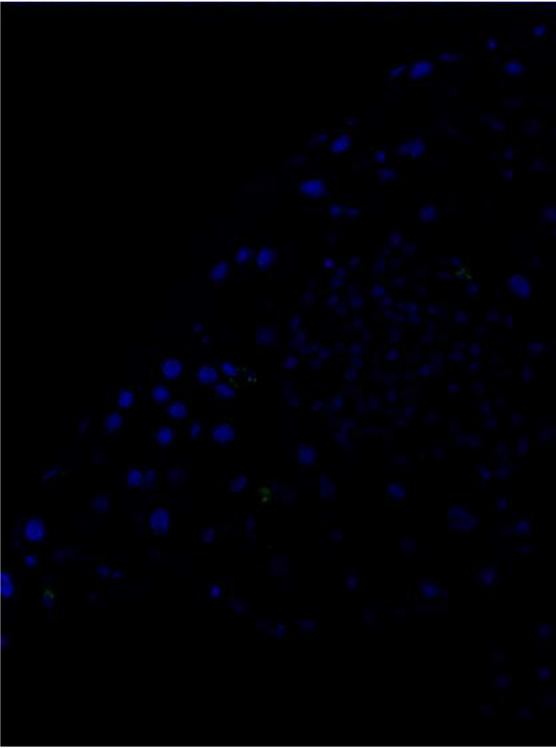
1:2000

Insulin
DAPI

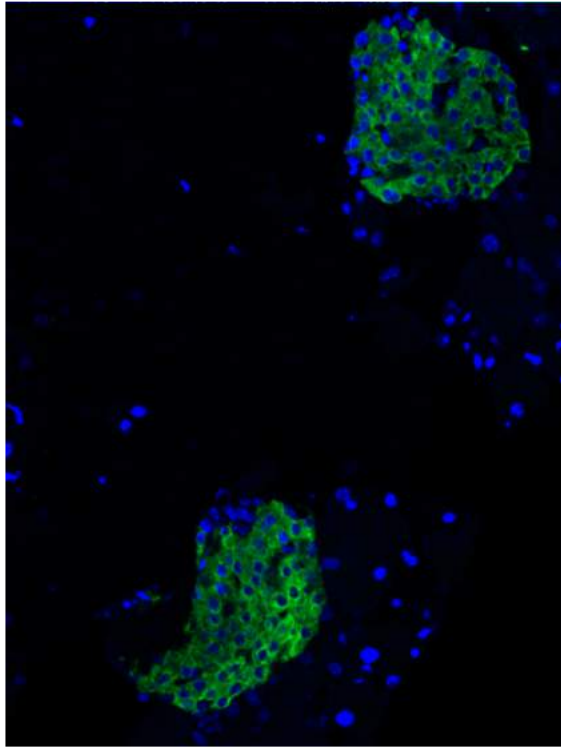


Cell Signaling 3014 RRID:AB_2126503 Rb INS on Mouse Tissue
(Wright Lab)

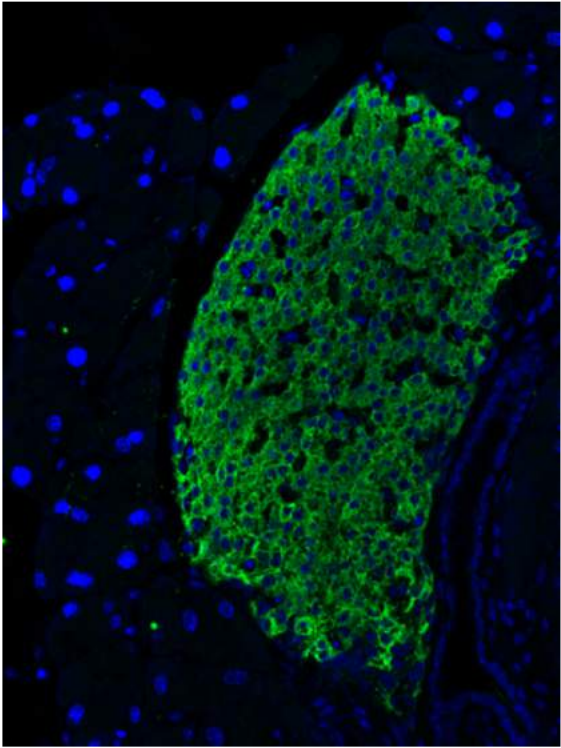
Negative Control



1:500



1:1000



Insulin
DAPI

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

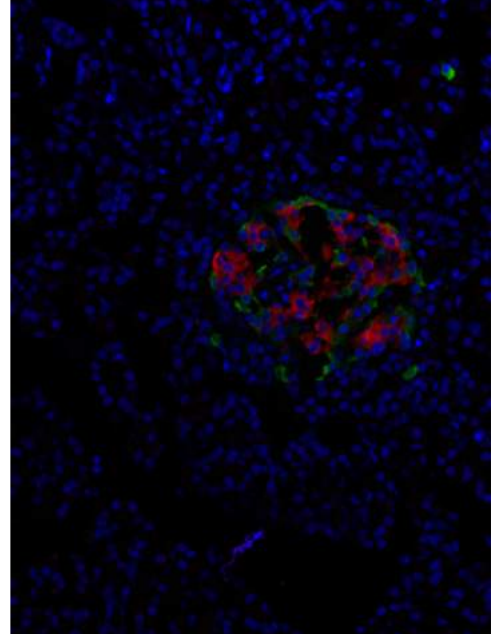
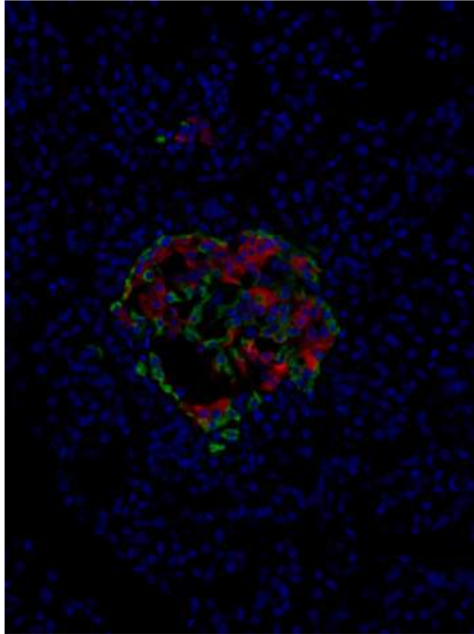
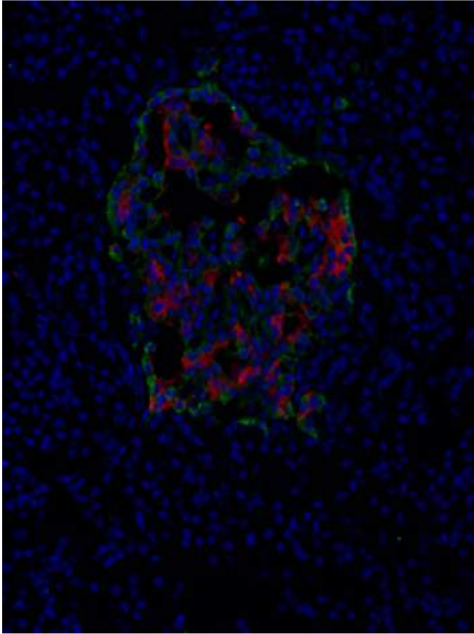
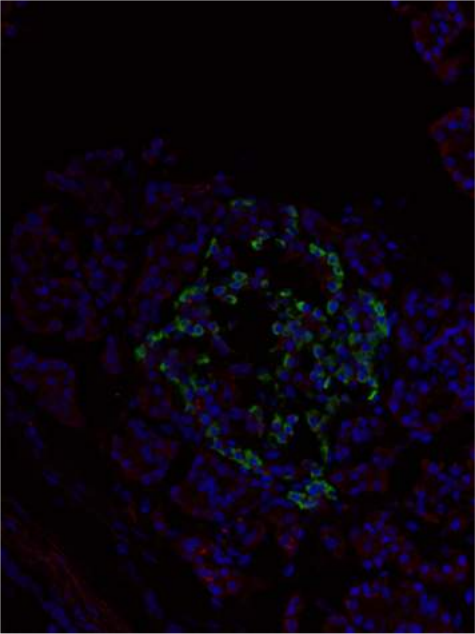
Glucagon
Insulin
DAPI

Negative Control

1:500

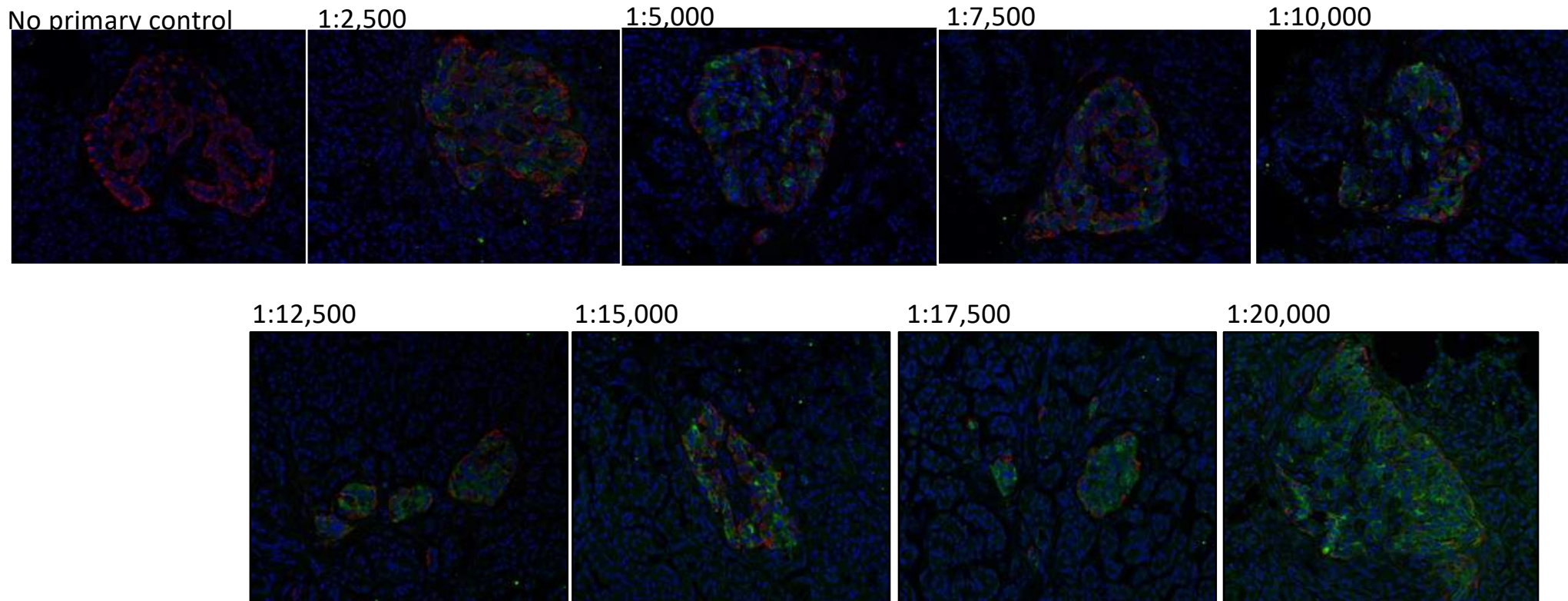
1:1000

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

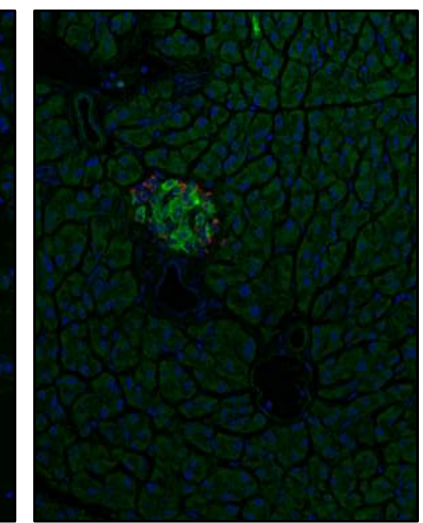
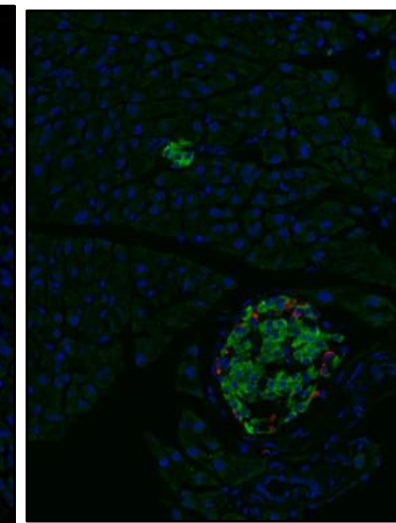
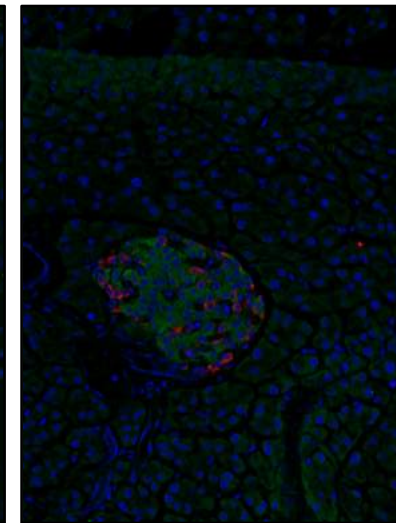
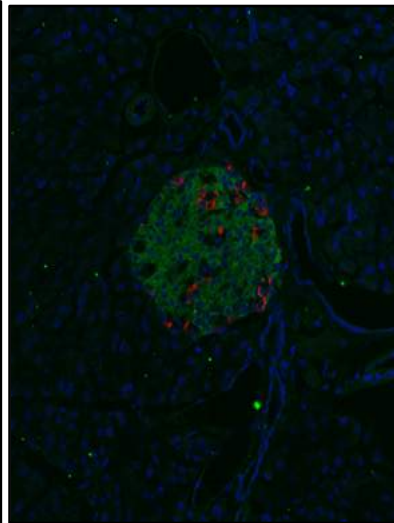
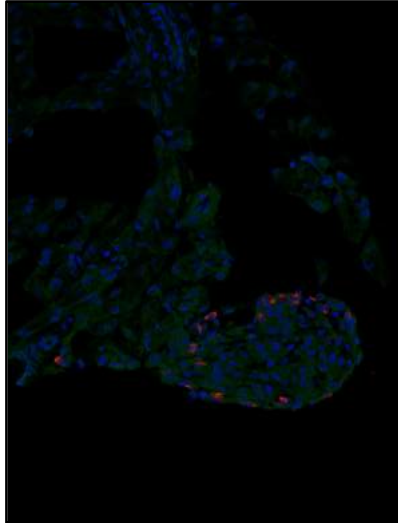
No primary control

1:2,500

1:5,000

1:7,500

1:10,000



Fitzgerald 20-IP35 GP anti-porcine INS RRID:AB_231771 GP INS

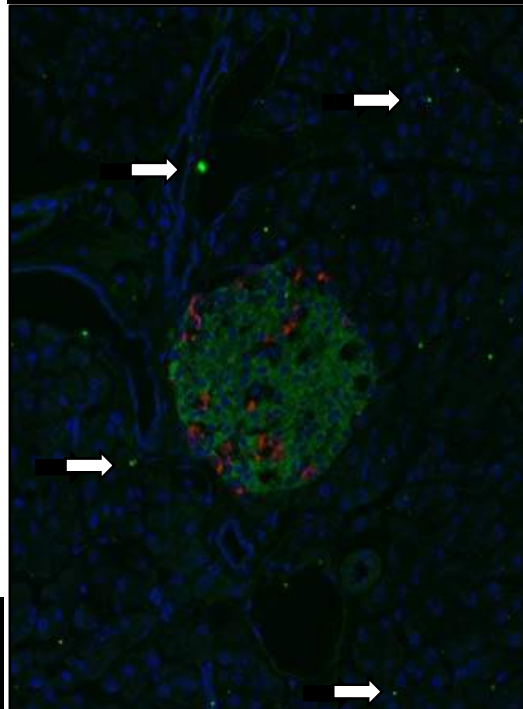
Quirk 1: Particulates in shipped reagent (Wright Lab)



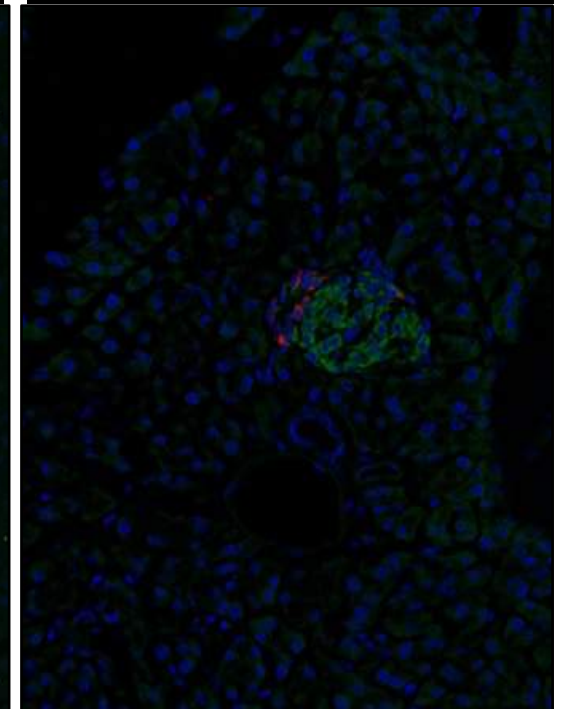
- This antibody, as shipped, has significant particulates.
- Particulates are removed by centrifugation.
- The “spun down” supernatant has been used through 3 freeze/thaw cycles without particulates re-appearing.
- **Particulate Removal: Centrifuge at least 10 min, ~12,000 x g, RT, then discard pellet**

GP INS (Fitzgerald) 1:5000
Rb Glucagon 1:500
DAPI

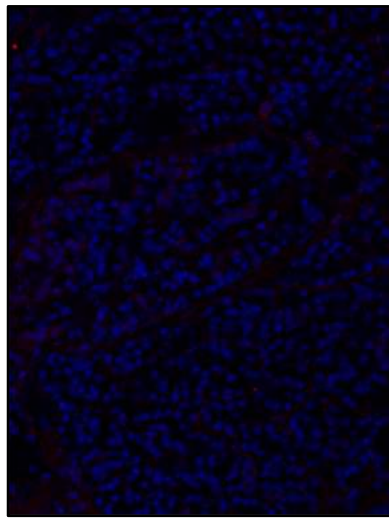
Antibody as shipped, particulates indicated by arrows.



Antibody after centrifugation and 3 freeze-thaw cycles – note lack of particulates.

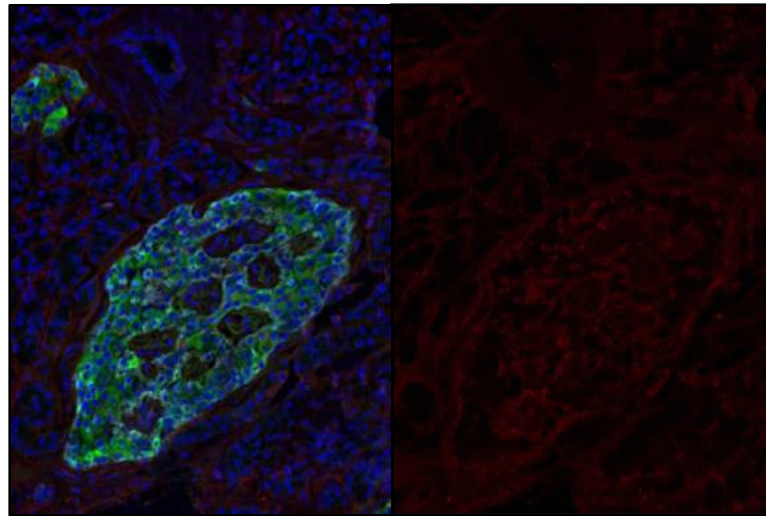


Fitzgerald 20-IP35 GP anti-porcine INS
RRID:AB_231771 GP INS Quirk 2: Cross-reaction
with anti-Sheep secondary antibody
(Wright Lab)



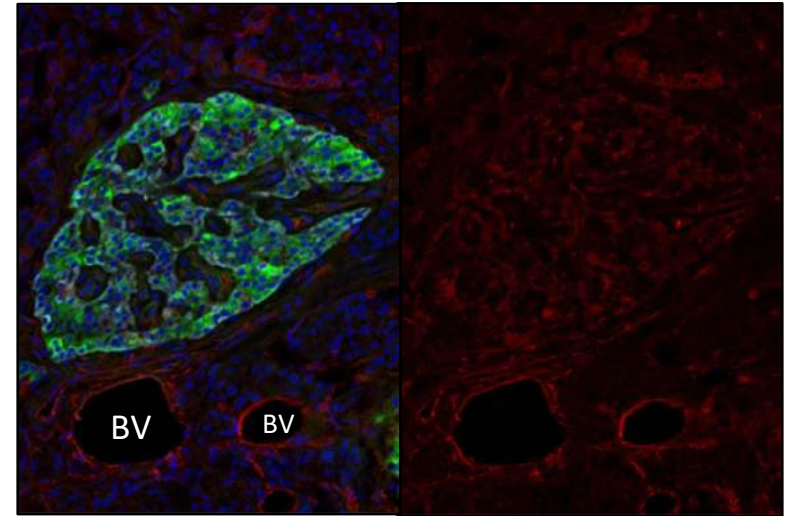
No primary AB, Dnky
anti-GP IgG AF488
No primary AB, Dnky
anti-SH IgG AF594
DAPI

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.