**Antibody Validation Experiment 2\_23\_23**

Using CyCIF antigen retrieval protocol V2 for both citrate and Tris-EDTA buffers.

Jason retrieved two slides in each condition on 2\_22\_23.

Pattern for each slide:

1. Stain with 3 dyes + DAPI
2. Bleach
3. Stain with 3 new dyes
4. Bleach

This means each slide can handle 6 dyes for a total of 12 dyes between 2 slides for each condition.

Here is the antibody breakdown for slide 1 in each condition:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Antibody 488 | Antibody 555/594/DY555 | Antibody 647 | Hoescht Applied? |
| Slide 1 cycle 1 | Villin | PDGF-555 | Glut-2 | X |
| Slide 1 cycle 2 | N/A | NHE3/SLC9A3-dy555 | Na/K/ATPase |  |
| Slide 2 cycle 1 | PCNA | Ecad-555 | N/A | X |
| Slide 2 cycle 2 | Ezrin | Chromogranin-594 | Gamma-Actin |  |

Positioned the antibodies by the following selection criteria:

1. A594 and DY555 must be cycle2 as they don’t bleach
2. Make known good antibodies last unless no available other dye.
3. Try to not put similar looking stains in adjacent channels, ie ecad and Na/K/ATPase

Bleach elaboration

When conducting the bleach cycle on each slide, I want a comparison to be made. This itself is not a publishable experiment, but it is a nice guiding point for that one. I might change my mind with publishable if multiple pieces of tissue exist on the same slide. Tissue microarrays are the best for it though, especially if on chambered slides.

1. Slide 1, 5 minutes in 4.5% H2O2 pH 10.
2. Image
3. Slide 1, 5 minutes MPCBA to finish off bleach
4. Image
5. Slide 2, 5 minutes MPCBA to bleach
6. Image
7. Repeat for next condition

Imaging Elaboration

All imaging performed on biotek citation 5

Entire tissue imaged at 20x NA 0.5

One tissue section will be imaged unless more sections are on slide. Then 2 will be imaged.

Staining Elaboration

1. All [C] will be 1:100 (maybe should fix ug/ul and not ratio)
2. Incubation time = 45 minutes
3. Dilution solution is intercept + 0.05% triton x100
4. All bleaches and stains done by hand and incubated in humidity chamber.
5. 150uL stain used.

Parameters

S1 C1 pH6

Approx. cords = 1310, -792, focus = 1368

Dapi:

5, 22

A488:

30, 22

A555:

800, 22

S1 C1 pH9

Approx. cords = 10637, -2103

Dapi

Same as above

488:

150, 22

555:

100, 22

647:

500, 22

S2 C1 pH6:

Approx. coord = 14549, 81

Dapi:

Same

A488:

30,22

A555:

300,22

A647:

100, 22

S2 C1 pH9

Approx. cords=9966, -568

Dapi

Same

A488:

150,22

A555:

100, 22

A647:

100, 22

S1 C2 pH 6:

A488:

200, 22

A555:

1000, 22

647:

125, 22

S1 C2 pH 9

A488:

200,22

A555:

70, 22

A647:

125, 22

S2 C2 pH 6

A488:

30, 22

A555:

500, 22

A647:

150, 22

S2 C2 pH 9

A488:

200, 22

A555:

50, 22

A647:

2150, 22