**Human Islet Immunofluorescent Whole-Mount**

Transfer islets to epi tube or 15mL conical (depending on number).

Allow islets to settle (5-15 min on bench).

Aspirate media.

Wash with 1X PBS.

Fix with 4% PFA x1 hr at RT (leave sitting on bench).

- 100-500uL in epi tube, 4mL in 15mL conical

Aspirate PFA.

Block with 1X PBS + 10% FBS + 0.3% Triton (44.85mL 1X PBS + 5mL FBS + 150uL Triton) for 1 hr at RT (leave sitting on bench).

- 1mL in epi tube, 4mL in 15mL conical

Aspirate blocking solution.

Incubate with primary antibodies in blocking solution for 48hrs at 4oC (sitting in rack, no agitation).

- 50uL in epi tube, 200uL in 15mL conical

- anti-insulin 1:400, all others 1:100

Aspirate primary antibody solution.

Incubate with blocking solution for 2hrs at RT on bench.

- 1mL in epi tube, 4mL in 15mL conical

Incubate with secondary antibodies in blocking solution for 3hrs at RT or O/N at 4oC (sitting in rack, no agitation).

- 50uL in epi tube, 200uL in 15mL conical

- all 1:200

Aspirate secondary antibody solution.

Incubate with blocking solution for 2hrs at RT on bench.

- 1mL in epi tube, 4mL in 15mL conical

Incubate with DAPI in 1X PBS for 30min at RT on bench.

- 50uL in epi tube, 200uL in 15mL conical

Transfer islets to slide (10-20uL).

Add drop of mounting media, allow to mix with islets.

Coverslip.

\*\*can re-use antibody solutions if you are careful