Click-it Plus EdU imaging aorta staining protocol

Materials/Reagents

- Click-it Plus 647 kit (488 works well too) – (Invitrogen C10640)
- Silicon coated 35mm dishes
    - Sylgard 184 (Fisher Scientific# 50-366-794)—to make coated dishes
- Pins for aorta (FST# 26002-20)
- "Magic Block" - blocking/permeabilization buffer
    - 0.3% TritonX-100, 0.05% Tween-20, 3% Normal Donkey Serum in HBSS+/+
- 2% PFA
- HBSS+/+
- Fine dissecting tools
- Prepare 3% BSA in PBS (must be made FRESH every time)
- Prepare 0.5% Triton X-100 in PBS

Protocol

Aorta Dissection

- IP inject 150uL of 10uM of EdU
- 2 hours post-injection, sacrifice mouse
- Perfuse through left ventricle with 10mLs of 2%PFA
- Remove all internal organs to expose the aorta
- Under a dissecting microscope, carefully remove adventitia with fine dissecting scissors
- Dissect out the aorta and place in silicon coated dish filled with 2%PFA
- Restrain aorta with pin and finish cleaning off adventitia to the best of your capability
- Filet open aorta by cutting the aorta longitudinally, exposing the endothelium
- Pin aorta so the endothelium side is facing up
- Remove PFA and wash 3X with HBSS+/+ for 5min at room temp (RT) on an orbital shaker. Protect from light

EdU staining

- Block with 3% BSA –2X for 5 mins
- Incubate with 0.5% Triton X-100
- Wash with 3% BSA –2X for 5mins
- Prepare Click-it reaction mix in the following order:

Component	Reagent	Volume (uL)	Storage
D	1X Click-it reaction buffer*	1320ul	4C
E	Copper protectant	30ul	4C
C	AlexaFlour picolyl azide	3.75ul	4C

F	1X reaction buffer additive**	150ul	-20C

*prepare 1X: 4mL of click-I rxn buffer + 36mL dH2O
**prepare necessary volume of 1X component F by diluting 10X component F in dH2O

- Add ~1.5mL of Click-it reaction mix to 35mm silicon coated plate and incubate at RT for 30 mins on orbital shaker. Protect from light
- Wash with 3% BSA –2X for 5 mins
- Block with Magic Block for 1hr at RT on orbital shaker
- Make 1.5mL primary antibody cocktail in Magic Block per silicon coated plate. Incubate overnight at 4C
- Wash with 1X HBSS+/+ 3X at RT for 5mins on shaker
- Make 1.5mL secondary antibody cocktail in Magic Block per silicon coated plate. Incubate at RT for 1 hour on orbital shaker (our secondary's are made in donkey)
- Wash with 1X HBSS+/+ 3X at RT for 5 mins on shaker
- Mount on glass slides using prolong gold