

En Face Aorta Staining Protocol

Materials/Reagents

- 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

Protocol

- 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 endothelial side is facing up
- Remove PFA and wash 3X with HBSS+/- for 5min at room temp (RT) on an orbital shaker
- Remove HBSS and add 2mLs of Magic block to aortas (make sure the aortas are fully covered in magic block) and incubate for 1hr at RT on an orbital shaker
- Make primary antibody cocktail in 1.5mL of Magic block. Example:
 - (1:400) Rb anti-ERG (Abcam #ab92513)
 - (1:200) Rt anti-CD45 (eBioscience# 14-0459-82)
- Remove HBSS and add primary Ab cocktail to aorta and incubate overnight at 4C on an orbital shaker
- Following day, remove Ab and wash aorta 3X with HBSS+/- for 5 min at RT on orbital shaker
- Make secondary antibody cocktail in 1.5mL of Magic Block. Example:
 - (1:400) Donkey anti-Rb A568 (thermo# A10042)
 - (1:400) Donkey anti-Rt A488 (thermos#A21208)
- Add secondary Ab cocktail to aorta and incubate at RT for 1hr on orbital shaker
- Remove antibody and wash 3X with HBSS+/- for 5 min at RT on orbital shaker
- Mount aorta on slide with cover slide and prolong gold. Seal with nail polish.
- Image with confocal microscope.