

Protocol for cre immunostaining of cryosections

1. Tissues are fixed for 3 hours in 4.0% paraformaldehyde (100 x volume of tissue) in 0.1M PBS on ice.
2. Tissues are washed 4-6 times in 0.1M phosphate buffered saline (PBS) over ~4 hours on ice.
3. Tissues are equilibrated in 30% sucrose in PBS overnight at 4 degrees C or until tissues settle to bottom of vial. (Note: some tissues high in fat or lung will never drop to bottom).
4. Tissue is frozen in OCT in cryostat chamber @ -20 to -35 degrees C, sectioned, mounted onto electrostatically-charged slides, and allowed to dry at room temperature (RT) for at least 30 min.
5. Sections are permeabilized for 1 hour at RT in 0.1% Triton X-100 in 10 mM PBS.
6. Sections are blocked for 1 hour RT in 5% normal donkey serum + 1.0% BSA in 10mM PBS.
7. Sections are incubated 6h-12h RT in a humid chamber in rabbit 1:1000 anti-cre (Novagen) diluted in 1.0% BSA + 0.1% Triton X-100 in 10 mM PBS.
8. Sections are washed 3 x 15 min. in 0.1% Triton X-100/PBS.
9. Sections are incubated 1h RT in Jackson Immunoresearch's "ML grade" donkey anti-rabbit IgG conjugated to CY3 (red fluorochrome) diluted 1:1000 in above dilution buffer.
10. Sections washed 3 x 5 min. in 0.1% Triton X-100/PBS.
11. Sections briefly rinsed in deionized water and mounted in Poly-AquaMount (Polysciences, Inc.) and viewed with either a conventional rhodamine filter cube (Mercury excitation) or the 543 HeNe line of a laser scanning confocal microscope.