Protocol for anti-En Ab immunofluorescent staining of mouse frozen sections

A. Tissue preparation

I. Embryos

1). Remove embryos in cold PBS.
2). Fix embryos in 4% paraformaldehyde (PFA) on ice on the following schedule*
E9.5-10.5 20 min
E10.5-18.5 30 min
(Puncture forebrain vesicle and chop tail at end if possible to improve fixative and wash penetration).
3). Wash embryos for 2X 15 min in cold PBS.

II. Adult brains/postnatal organs

1). Perfuse animal with 4% PFA.
2). Dissect out the brains/organs.
3). Wash tissue for 2X 15 min in cold PBS.

4. Sink in 30% sucrose/PBS. For embryos younger than E18.5, sink first in 15% sucrose/PBS, then 30%.
5. Equilibrate tissue in OCT for 30'-1 hour prior to embedding/freezing.
6. Freeze embryos in OCT, section at 12-16 um.
7. Collect sections on coated/charged slides. Allow to air dry for at least 1 hour.
8. Sections can be stored at –20ºC for several weeks until ready to use.

B. Staining

1. Allow sections to come to room temperature (~30 min).
2. ** Post-fix for 5 min with 4% PFA (Important: Skip this step for sections from adult tissue and embryos older than E15.5, and proceed to blocking after briefly rinsing slides in PBS).
3. Wash 3x 5 min in PBS.
4. Block 30-60 min with 10% normal donkey serum/0.2% Triton X-100 in PBS.
5. Primary antibody (Rabbit anti-mouse Enhb1): 4 ºC overnight, in 1% serum/0.2% Triton X-100/PBS (Use at 1:50; the antiserum has already been diluted at 1:10).
6. Wash 3x 10 min in PBT
7. Secondary antibody (1:500 Donkey anti-rabbit): 1-2 hours at RT, with 1% serum/0.2% Triton X-100/PBS.
8. Wash 3x 10 min in PBT
9. Counterstain and coverslip with aqueous-based mounting media.

* The Enhb1 antibodies are very sensitive to fixation. Generally, short fixation works better.
** If sections will not stick to the slides when the post-fixation is skipped, try to fix with shorter time and with cold PFA.