

## ***Staining Protocol for MCF-10A Frozen Sections***

### *Notes:*

- *Nuclear fast red provides better staining when complete dehydration is desired*
- *Toluidine blue can provide better contrast for hydrated sections*
- *Neither stain will bind Matrigel tightly (compared to eosin, for example)*
- *Other stains have not been tested but might work well (methylene blue, for example)*

- 1) Fix 8  $\mu\text{m}$  frozen sections in 75% ethanol for 30 sec. Move slides directly from  $-80^{\circ}\text{C}$  to ethanol (do not allow slides to warm to room temperature).
- 2) Transfer to distilled water for 30 sec.
- 3) Stain with a few drops of either nuclear fast red (Vector Laboratories #H-3403) or toluidine blue (VWR #34172-176; 0.01% (w/v) in PBS dropped to pH 5.5 with HCl) for 1 min.
- 4) Transfer to distilled water for 1 min.
- 5) Repeat Step 4.
- 6) For toluidine blue, stop and visualize.  
For nuclear fast red, dehydrate:
  - a. 75% ethanol for 30 sec.
  - b. 95% ethanol for 30 sec.
  - c. 100% ethanol for 30 sec.
- 7) For nuclear fast red, remove ethanol with a xylene dip for 1 min.
- 8) For nuclear fast red, air dry 5–10 min.