

# Schistosome Whole-Mount Tunnel

Jim Collins 12-15-15

Note: Using AbCam *in situ* BrdU-Red DNA fragmentation (TUNEL) assay Kit, cat no. ab66110.

- 1.) Separate, fix, and dehydrate parasites using procedures for *in situ* hybridization.
- 2.) Rehydrate parasites in PBSTx. Rinse 1x
- 3.) Treat with 20  $\mu\text{g/ml}$  ProK in PBSTx for 45 min. at RT.
- 4.) Post Fix 10 min in 4% Formaldehyde in PBSTx for 10 min.
- 5.) Rinse 1x in PBSTx.
- 6.) Incubate in "Wash Buffer" from kit.
- 7.) Make up "DNA Labeling Solution": 10  $\mu\text{l}$  "Reaction Buffer", 0.75  $\mu\text{l}$  "TdT Enzyme", 8 $\mu\text{l}$  "Br-UTP", 33.25  $\mu\text{l}$   $\text{H}_2\text{O}$ . Use 50  $\mu\text{l}$ / 2-3 male worms.
- 8.) Incubate at 37°C for 4 hours while shaking.
- 9.) Rinse 2x in PBSTx at RT.
- 10.) Block for 45 min in *in situ* FISH block for 45 min.
- 11.) Incubate in 100  $\mu\text{l}$  Antibody Solution (5  $\mu\text{l}$  Anti-BrdU-Red Antibody, 95  $\mu\text{l}$  "Rinse Buffer") overnight at 4°C.
- 12.) Rinse 2x in PBSTx. Then wash for 2 hours with PBSTx with 1  $\mu\text{g/ml}$  DAPI.
- 13.) Clear samples in 80% Glycerol and mount on slides in Vectashield.