Schistosome Whole-Mount Tunel
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 µg/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 µl “Reaction Buffer”, 0.75 µl “TdT Enzyme”, 8µl “Br-UTP”, 33.25 µl H₂O. Use 50 µl/ 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 µl Antibody Solution (5 µl Anti-BrdU-Red Antibody, 95 µl “Rinse Buffer”) overnight at 4°C.
12.) Rinse 2x in PBSTx. Then wash for 2 hours with PBSTx with 1 µg/ml DAPI.
13.) Clear samples in 80% Glycerol and mount on slides in Vectashield.