

Tepass staining protocols

We have anti-Dlg, anti-spectrin, anti-cadherin, anti-crumb, anti-catenin, anti-armadillo

Tepass reports this staining protocol for all (our variations in parentheses):

1. Fix 5% paraformaldehyde (4% is what we have been using – 1 part:3 parts dilution)
2. Optional: Fix 5 min methanol (have not tried)
3. Wash 4X15 min PBT (use 350ul)
4. Block 1H PBT + 0.2% BSA, 5% goat serum (can go overnight)
5. Primaries 1:200, Secondary 1:400 (primaries potencies have been dropping – try 1:50 or 1:25, no background from monoclonals) (secondaries use 1:200, invariably)
6. Wash 3x5min in PBT, second wash in 1:1000 DAPI, break up ovaries in third wash
7. Wash 1x5min in PBT+50% glycerol
8. Mount

Block solution

1ml PBT
100ul 100x BSA
50ul GSA