Billy Hinchen

Parhyale immunohistochemistry

Embryo Preparation

Embryos to be stained with beta-catenin can fixed via boiling: transfer the embryos to a 1.5 ml eppendorf tube and remove as much water as possible. Heat some water in a heat block to 95^{o}C and have some FASW on ice as well. Place the eppendorf, with the embryos in, into the heat block for 30 seconds then immediately to ice, adding some ice cold FASW as well. Under the stereoscope the chorion has moved far enough away from the embryo. Burst the chorion with a tungsten needle. Embryos can either be used now for staining or stored at -20^{o}C in methanol.

Day 1 (fixation and primary)

Unless otherwise stated the following protocol is carried out in 1.5ml eppendorf tubes at room temperature, c.a. 23^{o}C)

1. Wash 3 x 5mins PBS
2. [Wash 1 x 30mins 0.3% H_2O_2 in PBS (HRP only)]
3. Wash 2 x 15mins PBT (= PBS + 0.1% Triton X-100)
4. Block for 1 hour in PBS + 1% BSA + 5% NGS
5. Apply primary antibody made up in PBS + 1% BSA
6. Incubate over night at 4^{o}C on an orbital shaker

Day 2 I (secondary)

1. Wash 3 x 5mins PBT
2. Wash 4 x 15mins PBT
3. Apply secondary antibody made up in PBS + 1% BSA + 5% NGS
4. Incubate at room temperature for 1-2 hours
5. Wash 3 x 5mins PBS (in the dark if using fluorescence)

Day 2 II (visualization - fluorescence)

1. Clear and counterstain in 50% glycerol + DAPI (1:1000)
2. Wash 3x1 min PBS
3. Mount in VectaShield

Day 2 III (visualization - enzymatic development)

1. Incubate in 300µl DAB (or DAB + Ni) solution for 20mins
2. Transfer to a watchglass (in the DAB) under the stereoscope
3. Add 30µl of a 0.3% H_2O_2 solution (pre-made in PBS)
4. Follow the reaction for ca. 4mins
5. Stop the reaction by washing 2 x 1 min PT
6. Clear and counterstain in 50% glycerol + DAPI (1:1000)
7. Mount in 70% glycerol