

Whole-mount IHC protocol for zebrafish embryos

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1. Dechorionate embryos, if necessary, and fix in PFA at 4 °C for overnight.
2. Aspirate PFA and then wash embryos by PBST for 10 min X 3 times
3. Dehydrate embryos through a series wash of methanol/ PBST (25%→50%→75%→100% MeOH/PBST, 10 mins for each step)
4. Placed embryos in 100% methanol in the freezer (-20°C) for overnight.
Note: Embryos could be stored in 100% methanol for few months
5. The embryos are rehydrated through a series wash of methanol/PBST (100%→75%→50%→25% MeOH/PBST, 5 mins for each step)
6. Wash embryos by PBST for 5 min X 3 times
7. Wash the embryos with Tris buffer (150 mM Tris-HCl pH9.0) for 5 mins.
8. Equilibrate the embryos in Tris buffer (150 mM Tris-HCl pH9.0) at 70°C for 15 mins.
9. Wash the embryos with PBST for twice, 5 mins for each wash.
- **Optional: step 10-13 are for embryos of stage > 24hpf**
10. Rinse the embryos quickly with dH₂O twice on the ice.
11. Penetrate the embryos with Pre-chilled Acetone (-20°C) at -20°C for 20 mins.
12. Remove the acetone and rinse the embryos quickly with dH₂O twice for 5 min X 3 times.
13. Blocking the embryos with fresh prepared 10% NGS/2%BSA /PBT for 4 hrs at 4°C.
14. Incubate the embryo with the primary Ab (concentration varies with different Ab) diluted in 2% NGS/2%BSA /PBT at 4 °C for 1 days.
15. Wash the embryos with PBT for 30 min X 4 times.
16. Incubate the embryos with Secondary Ab diluted in 2% NGS/2%BSA /PBT for 3hrs at R.T. (in dark).
17. Wash the embryos with PBST for 5 min X 5 times.
18. Fixed the embryos again with 4% PFA for 20 mins at RT.
19. Wash the embryos with PBST for 5 min X 5 times.
20. Immerse in 100% glycerol/PBS for microscope observation (through a series glycerol/PBS wash 25% →50%→75%→100% glycerol/PBS , 20 mins each)

Reagent recipe

PBT	0.1 M phosphate buffer (Na ₂ HPO ₄ · 2H ₂ O + NaH ₂ PO ₄ · H ₂ O, pH7.3-7.4) + 0.8% Triton X100
PBST	PBS + 0.1% Tween20
Tris-HCl	150 mM Tris-HCl pH9.0
NGS	Normal goat serum
BSA	Bovine serum albumin

ABOUT



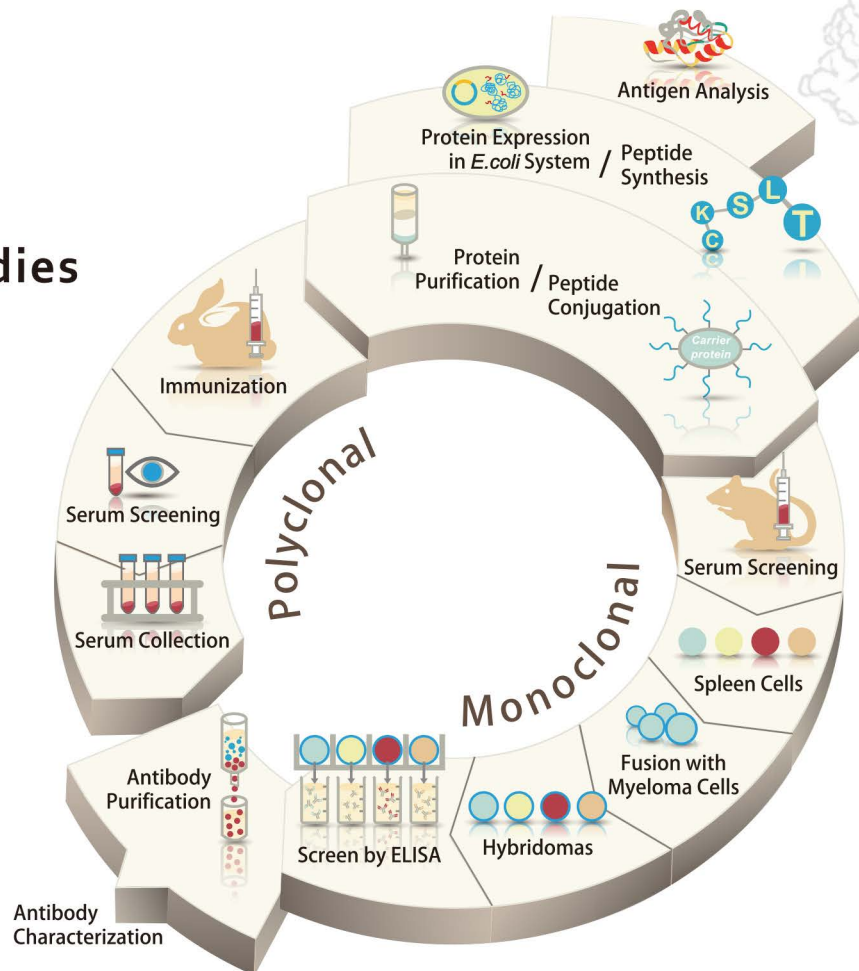
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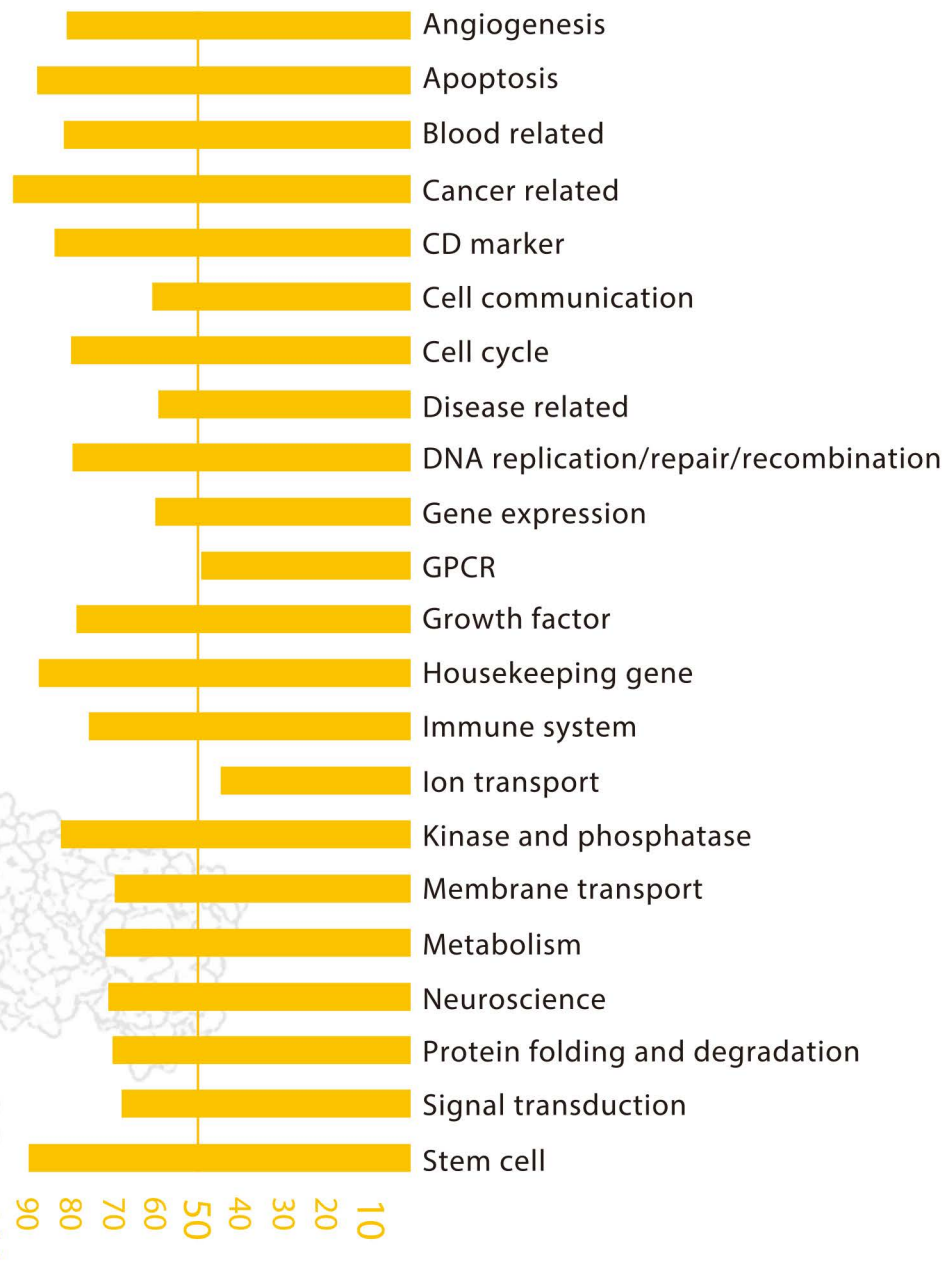
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