

Heat Induced Antigen Retrieval (HIAR)

(Source: Poh Kheng Loi, Institute of Neuroscience)

Some monoclonal Antibodies, such as anti-VegT (a.k.a “anti-spadetail”) and anti-Dlx3b require special pre-treatment of target tissues for optimal antigen preservation in whole mounts.

1. Incubate whole mount embryos in standard prehybridization buffer for *in situ* hybridization at 65° overnight, then proceed with standard antibody labeling protocol.

With HIAR, use 1:1000 for anti-VegT or 1:500 for anti-DLX3b; without HIAR, use 1:100 dilution for anti-VegT, or 1:50 for anti-Dlx3b. Dilution information for other Antibodies after HIAR treatment is currently not known or available.

Most other anti-zebrafish mABs work well using standard fixation and processing, i.e. use whole mounts or tissue sections fixed in 4% paraformaldehyde. For dilution information, please refer to antibody provider’s recommendations.