Western Blotting of Zebrafish Embryos

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Reference: *The Zebrafish Book*, 5th Ed

Harvest embryos, the number of which depends on their stage at harvest. For example, 3 dpf: 50 – 100 and 24 hpf: 100 – 150).

Dechorionate embryos either manually or using Pronase (1 mg/ml; 5 – 10 min for 24 hpf or 10 – 20 min for 3 dpf).

Transfer the dechorionated embryos to cold PBS with 1 mM EDTA, remove yolk, transfer to microfuge tubes and wash 2x with cold PBS.

Remove liquid as much as possible and freeze in liquid nitrogen, If need be, store at – 70 °C.

Thaw the embryos at RT, spin for 2 min to pellet and remove any residual liquid.

Add 200 μl of SDS sample buffer and homogenize with microfuge pestle until uniform in consistency.

Boil for 5 min and spin for 2 min at top speed.

Transfer S/N to a new microfuge tube. If significant pellet remains, repeat the homogenization step.