Extraction Of Proteins From Zebrafish Embryos For SDS-Gel Analysis

(Source: M. Westerfield from Zebrafish Book 5th Edition)

1. Remove embryos from their chorions by immersing them in 1 mg/ml pronase for 5 minutes, room temperature, in a Petri dish. Embryos younger than 24 hpf require shorter exposure to pronase (see REMOVING EMBRYOS FROM THEIR CHORIONS).

2. Transfer them to a clean beaker filled with 10% Hank's saline and rinse several times with clean 10% Hank's.

3. Triturate gently with a Pasteur pipette to expel the embryos from their chorions, if necessary.

4. Transfer embryos into microcentrifuge tubes (1.5 ml Eppendorf). Remove as much water as possible with a pipette.

5. Add cold SDS sample buffer (63 mM Tris-HCl pH 6.8, 10% glycerol, 5% ß-mercaptoethanol, 3.5% sodium dodecyl sulfate; see page 9.7) at 1 l per fish.

4. Homogenize with pestle. Add another 1 l of SDS sample buffer per fish to rinse pestle, homogenize a bit more.

5. Immerse tube in boiling water for 1 minute.

6. Spin in a microcentrifuge for 5 minutes. Collect supernatant and apply to gel. Alternatively, samples can be frozen at this point (-70°C) for later analysis.