Protein extraction with deyolk for Western blot protocol

1. Zebrafish embryos (~ 100 embryos from different stages) were dechorionated in eppendorf tube either with pronase (50 μg/ml) and swirl for 10~15 min or use forceps to remove manually in egg water.
2. Wash embryos with ice-cold 1xPBS.
3. Manually deyolk using a P200 pipet tip by pipetting **three to five** times in ice-cold 1xPBS.
4. Wash embryos with ice-cold 1xPBS.
5. Spin down embryos and discard suspensions in a small microcentrifuges.
6. Zebrafish embryos were homogenized in cholate extraction buffer on ice. (2 μl cholate extraction buffer per embryo)
8. Store at -80℃; thaw for western blot.

Cholate extraction buffer.

Final : 10mM Tris pH~8.0

2% Sodium Deoxycholate (Sigma D-6750)

protease inhibitor cocktail tablets from Roche

Store at 4℃