Flow cytometry protocol

1. Dechorinate all embryos (~20 embryos for 24, 36, and 48 hpf) in eppendorf tube either with pronase (50 μ g/ml) and swirl for 10~15 min or use forceps to remove manually in egg water.
2. Wash embryos with ice-cold 1xPBS.
3. Gently pipette up and down three to five times with P200 to deyolk in ice-cold 1xPBS.
4. Wash embryos with ice-cold 1xPBS.
5. Spin down embryos and discard suspensions using a small microcentrifuges.
6. Embryos were digested with a trypsin (0.25%)/EDTA (1 mM) solution for 10 min at room temperature and pipette gently to create a single cell suspension using P200.
7. Inactivation of trypsin with CaCl2 (1 mM) /lamb serum (5%).
8. Filter whole suspension through sterile mesh (40 μm) (BD Falcon, cat. # 352235, Franklin Lakes, NJ, USA) and transferred to a collection tube.
9. The cell suspensions were centrifuged at 2000 rpm at 4 °C for 10 min.
10. Discard suspensions and add 1ml of ice-cold 1xPBS and centrifuge again for 10 min.
11. Discard suspensions and the cells were fixed in 70% ethanol overnight at -20 °C.
12. The cells were centrifuged at 2000 rpm at 4 °C for 10 min and resuspended in ice-cold 1xPBS.
13. The suspended cells were passed through a 40-μm filter.
14. The suspended cells were stained with RNase A (200 μg/ml) and propidium iodide (PI) (50 ng/ml) for at least 15 min at 4 °C in the dark.
15. PI-positive cells were quantified by flow cytometry.