Antibody staining of Drosophila S2 cell

1. Coat glass chamber slides with 1ml of diluted (1:15 v/v in water) poly-L-lysine solution (SIGMA, P8902, 0.1% w/v in water) for 1h at RT or O/N at 4C.
2. Suck out coating solution.
3. Add 2 days fresh cultured cells in well and keep it 1~2h at 24C in incubator.
4. Wash cells once with 1*PBS (pH 7.4).
5. Fix cells with 4% formaldehyde in 1* PBS for 20min at RT. Quick wash cells 3 times with 1*PBS.
6. Add 100% methanol and incubate 10min at RT. Quick wash cells 3 times, then slow wash 3*15min with 1*PBS.
7. Blocking cells with 3% BSA for 1h at RT.
8. Add primary antibody with 3% BSA in PBST solution and incubate O/N at 4C.
9. Quick wash cells 3 times, then slow wash 4*15min with 1*PBST.
10. Blocking cells with 3% BSA for 1h at RT.
11. Add secondary antibody with 3% BSA in PBST solution and incubate 1h at RT.
12. Quick wash cells 3 times, then slow wash 4*15min with 1*PBST.
13. Take out the slide using the equipment. Dry it at RT by touching the edges with tissue paper.
14. Add one drop of mounting solution and put coverslip (#1) by touching only one side.
15. Seal all of four sides using nail polish.

RNA protein double staining in Drosophila S2 cells

1. Coat glass chamber slides with 1ml of diluted (1:15 v/v in DEPC-H_2O) poly-L-lysine solution (SIGMA, P8902, 0.1% w/v in water) for 1h at RT or O/N at 4C.
   *Note: Use DEPC-H_2O to make solution till post-hybridization wash.
2. Suck out coating solution.
3. Add 2 days fresh cultured cells in well and keep it 1~2h at 24C in incubator.
4. Wash cells once with 1*PBS (pH 7.4).
5. Fix cells with 4% formaldehyde in 1* PBS for 20min at RT. Quick wash cells 3 times with 1*PBS.
6. Add 100% methanol and incubate 10min at RT. Quick wash 3 times, then slow wash 3*15min with 1*PBS.
7. Pre-hybridize cells with 4x SSC, 50% formamide, 0.1% Tween-20 for 1h at RT.
8. Hybridize cells at 55C O/N in 1ml hybridization buffer containing 5% dextran sulfate, 4x SSC, 50% formamide, 0.1% Tween-20, and 1:100~200 diluted probe.
9. Wash cells 3*20min with 2x SSC, 50% formamide, 0.1% Tween-20, 3*20min 0.1x SSC, 0.1% Tween-20 at 55C.
10. Rinse cells 3 times with 1*PBS. Block cells with 1*blocking solution (Roche) for 1h at RT.
11. Incubate cells with Anti-dig-HRP and primary antibody against protein of interest in 1*blocking solution at 4C O/N. Rinse cells once and wash 5*15min with PBT.
12. Blocking cells with 3% BSA for 1h at RT.
13. Incubate cells with Fluorescence-conjugated secondary antibody in 1*blocking solution at RT for 1 hr.
14. Quick wash cells 3 times, then slow wash 4*15min with PBT.
15. Rinse cells once with 1*PBS. Add TSA working solution and incubate 15 min at RT.
16. Quick wash twice and slow wash 3*5min with PBT.
17. For nuclear staining, incubate cells with 1:10,000 diluted DAPI in PBS for 15min at RT. Rinse 3 times, then wash 3 *10min with PBS.
18. Take out the slide using the equipment. Dry it at RT by touching the edges with tissue paper.
19. Add one drop of mounting solution. Put coverslip (#1) by touching only one side.
20. Seal all of four sides by using nail polish.