Immunofluorescence for Apg7 and COXIV in C2C12 Myotubes

Procedure:

1. Prepare ice-cold 4% paraformaldehyde/PBS.
2. Take cultured C2C12 myotubes on coverslip in 6-well plate. Wash with ice-cold 1X PBS (~2 ml for each well) once.
3. Fix the cells in ice-cold 4% paraformaldehyde/PBS (~1.5 ml for each well) at RT for 5 min.
4. Wash cells with ice-cold PBS for 5 min x 2. Alternatively, cells can be kept in 4^{o}C before proceeding to further steps.
5. Permeabilize cells in 0.3% Triton/PBS on ice for 5 min x 2.
6. Wash in PBS for 5 min x 2, WIPE.
7. Block non-specific binding with 5% Normal Goat Serum (NGS)/PBS (150 µl per coverslip) and incubate for 30 min at RT.
8. Wash in PBS for 5 min x 3.
9. Apply mouse anti-COXIV antibody diluted 1:500 in 5% NGS/PBS and rabbit anti-Apg7 antibody diluted 1:50 in 5% NGS/PBS and incubate overnight at 4^{o}C in a container covered with seranwrap.
10. Wash in PBS for 5 min x 3, WIPE.
11. Apply goat anti-mouse-IgG-FITC diluted 1:25 and goat-anti-Rabbit-IgG-Rhodamine diluted 1:25 in 5% NGS/PBS and incubate at RT for 30 min.
12. Wash in PBS for 5 min x 3.
13. Incubate coverslip with 1 µg/ml DAPI at RT for 20 min.
14. Wash in PBS for 5 min x 4, WIPE.
15. Mount the coverslip upside down on slide using VECTASHIELD mounting media.

Reagent preparation:
10% Triton X-100: Triton X-100 50 ml, ddH_2O 450ml.
0.3% Triton X-100/PBS: 10X PBS 50 ml, 10% Triton X-100 15ml, ddH_2O 435ml
5% NGS/PBS: NGS 50 µl, 1X PBS 950 µl.