RapiClear reagents

To achieve the best transparent result after applying the RapiClear (RC) reagents, we suggest that

1. Mouse fixed by 4% paraformaldehyde solution via cardiac perfusion.
2. Tissues can be sectioned manually, using a vibratome, cryo-section, paraffin section, or a tissue matrix with razor blades.
3. Fix the tissue slices in a 24-well plate with 4% paraformaldehyde solution on an orbital shaker or rocker for 2~4 h at RT.
4. Wash with PBS 3 times, 5 min/time.
5. Transfer samples into 2% Triton X-100 solution overnight at 4℃ for permeabilization.
6. Keep the specimen in blocking buffer on an orbital shaker or rocker at 4℃ overnight

   *__Blocking buffer__ (10% normal goat serum, 1% Triton-X 100, and 0.2% sodium azide in PBS): Store this solution for only a short period of time (overnight at most) at 4℃.
7. Incubate the specimen with primary antibody in a 24-well plate (350 μl/well) on an orbital shaker or rocker at 4℃ for 2 days.

   ***Ab dilution buffer** (1% normal goat serum, 0.2% Triton-X 100, and 0.2% sodium azide in PBS) : Store this solution for only a short period of time (overnight at most) at 4℃.
8. Wash the specimen with washing buffer for >1 hrs at room temperature for 2 times. Then, keep the specimen in washing buffer on an orbital shaker or rocker at 4℃ for overnight. (**Note:** washing step is quite important for immunostaining!)

   *__Washing buffer__ (3% NaCl and 0.2% Triton-X 100 in PBS): Store this nonhazardous buffer at 4℃.

9. Incubate the specimen with secondary antibody on an orbital shaker or rocker at 4℃ for 1 day.
10. Wash the specimen with washing buffer for >1 hrs at room temperature for 2 times. Then, keep the specimen in washing buffer on an orbital shaker or rocker at 4℃ for overnight. (**Note:** washing step is quite important for immunostaining!)
11. Post-fix the specimen in a 24-well plate with 4% paraformaldehyde solution on an orbital shaker or rocker for 2 h at RT.
12. Wash with PBS 3 times, 5 min/time.
13. DAPI, Hoescht or Propidium iodide for nuclear staining if needed.
14. Transfer the specimen to a 24-well plate with the RC reagent with 5~10 times of the sample volume.
15. Place the plate on an orbital shaker or rocker to gently mix and immerse the specimen with the RC reagent for hours at RT.
16. Mount the specimen in fresh RC reagent between two coverslips separated by iSpacer® sticker(s). Press gently around the sticker to seal the coverslips.
17. Remove the extra solution at the edges with Kimwipes.
18. Fill the space outside the iSpacer® with Neo-Mount™ medium (Merck Co., LTD.) or clear nail polish to seal the edges between the two coverslips.
19. The images are acquired by a confocal microscopy system.