May 2020

RapiClear® 1.52 Solution

Ready-to-use
Making biological sample transparent rapidly

#RC152001, 10mL
#RC152002, 100mL

INTRODUCTION

RapiClear® is a water-soluble clearing reagent for enhanced visualization of both fluorescence and non-fluorescence labeled biological specimens. It can be applied in viewing cell morphology in tissues of mammals, plants, insects, and even the biomaterial scaffold such as collage, chitosan, and cellulose. Targets that are usually indistinguishable or blurry due to specimen opacity can now be clearly visualized simply by applying RapiClear® in the mounting procedure.

Advantages of RapiClear® 1.52

1. Samples can be directly transferred from water, buffer solutions, and glycerin into RapiClear® 1.52 medium.
2. The transparent effect is reversible if samples re-immerse in water or buffer solutions.
3. RapiClear® 1.52 is ready-to-use, no need to be centrifuged.
4. RapiClear® 1.52 allows visualization of internal targets up to 1~5 mm below tissue surface.
5. Application of RapiClear® 1.52 introduces just limited sample deformation.
6. RapiClear® 1.52 has a refractive index of nD = 1.52, which is close to the refractive index of coverslip and immersion oil.

OPERATION

1. For effective clearing, 4% paraformaldehyde fixed samples should be treated with 2% PBST (2% Triton X-100 in PBS solution containing 0.05% sodium azide) on an orbital shaker or rocker for 1~2 days at RT or 25℃ incubator for permeabilization before mounted in RapiClear® 1.52.
2. The volume of RapiClear® 1.52 used and the time required for clearing should be adjusted according to the tissue size. Basically, 5 times the sample volume of RapiClear® 1.52 is recommend. Tissue slices (e.g., 1mm thickness) may become transparent in 1~2hr.

   Note: Pre-warm the RapiClear® to 37℃ before mounting can facilitate solution penetration.
3. Mounting cleared sample with fresh RapiClear® 1.52 in iSpacer microchamber (SunJin Lab Co.) is recommend to prevent flattening.
4. Press gently around the edges of the coverslip to ensure a safety seal.
5. Remove carefully the exceeding solution with Kimwipes.
6. Fill the space outside the iSpacer with clear nail polish if necessary.

For more information, please check the "**Instruction**" in our website: www.sunjinlab.com/instruction/

REFERENCE

1. Tan SH et al. AQP5 enriches for stem cells and cancer origins in the distal stomach. *Nature* (2020). https://doi.org/10.1038/s41586-020-1973-x
2. Ayala-Nunez NV et al. Zika virus enhances monocyte adhesion and transmigration favoring viral dissemination to neural cells. *Nat Commun* (2019). https://doi.org/10.1038/s41467-019-12408-x
3. Gomariz A et al. Quantitative spatial analysis of haematopoiesis-regulating stromal cells in the bone marrow microenvironment by 3D microscopy. *Nat Commun* (2018). https://doi.org/10.1038/s41467-018-04770-z

STABILITY AND STORAGE

RapiClear® 1.52 can be stored at -20℃~RT. When stored at 4℃ or -20℃, the product is stable for at least 1 year.

WARNING AND PRECAUTIONS

Repeated exposure may cause skin dryness or cracking. Prevent skin contact is suggested.

MANUFACTURER

SunJin Lab Co., Taiwan, R.O.C.

Immunostaining Protocol

1. Animal fixed with 4% paraformaldehyde solution via cardiac perfusion.
2. Tissues is sectioned manually, using either vibratome, cryo-section or a tissue matrix with razor blades.
3. Fix the tissue slices in a 6-well plate with 4% paraformaldehyde solution on an orbital shaker or rocker for 1~2hr at RT.
4. Wash with PBS 3 times, 15 min/time on an orbital shaker or rocker at RT.
5. Transfer samples into 2% PBST (e.g., 50ml tube filled with 2% Triton X-100 in PBS solution containing 0.05% sodium azide) on an orbital shaker or rocker for 1~2 days at RT or 25˚C incubator for permeabilization.
6. Wash with PBS 3 times, 15 min/time on an orbital shaker or rocker at RT.
7. Keep the specimen in a 1.5ml tube filled with freshly prepared blocking buffer on an orbital shaker or rocker at 4˚C, 1~2 days. *Blocking buffer (10% normal goat serum, 1% Triton-X 100, 2.5% DMSO, and 0.2% sodium azide in PBS)
8. Incubate the specimen with primary antibody in a 1.5ml or 2ml tube filled with the Ab dilution buffer on an orbital shaker or rocker at 4˚C or 15˚C for 3~4 days. Incubation at RT or higher temp. will promote antibody penetration into the tissue sample. But one of the tradeoffs will be the possible non-specific binding of Ab. *Ab dilution buffer (1% normal goat serum, 0.2% Triton-X 100, 2.5% DMSO, and 0.2% sodium azide in PBS).
9. Wash the specimen with washing buffer for 1hr at RT 3 times. Then, keep the specimen in washing buffer on an orbital shaker or rocker at 4˚C overnight. *Washing buffer (3% NaCl and 0.2% Triton-X 100 in PBS)
10. Incubate the specimen with secondary antibody in a 1.5ml or 2ml tube filled with the Ab dilution buffer on an orbital shaker or rocker at 4˚C or 15˚C for 2 days.
11. Wash the specimen with washing buffer for 1hr at RT 3 times. Then, keep the specimen in washing buffer on an orbital shaker or rocker at 4˚C overnight.
12. Wash with PBS 3 times, 15 min/time.
13. Stain with DAPI or SYTOX for nuclear staining if needed. For example, incubate the 0.5mm thick specimen in a 1.5ml tube filled with SYTOX working solution (1:1500 dilution) on an orbital shaker at RT for 1.5hrs.
14. Wash with PBS 3 times, 1hr/time on an orbital shaker at RT.
15. Clear sample with RapiClear O/N at RT (1.5ml tube fill with RapiClear; This RapiClear can be reused several times). Pre-warm the RC to 37˚C before mounting can facilitate solution penetration.
16. Mount the cleared specimen with fresh RapiClear reagent in iSpacer microchamber. Press gently around the iSpacer to seal the coverslips.
17. Remove the extra solution at the edges with Kimwipes.
18. Fill the space outside the iSpacer with clear nail polish to seal the edges between the two coverslips.
19. The images are acquired using a confocal microscopy system.

For more information, please check the "**Instruction**" in our website: www.sunjinlab.com/instruction/ or email to sunjinlab@gmail.com