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RapiClear® 1.49 Solution

Ready-to-use
Making biological sample transparent rapidly

#RC149001, 10mL
#RC149002, 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® 1.49

1. Samples can be directly transferred from water, buffer solutions, and glycerin into RapiClear® 1.49 medium.
2. The transparent effect is reversible if samples re-immerse in water or buffer solutions.
3. RapiClear® 1.49 is ready-to-use.
4. RapiClear® 1.49 allows visualization of internal targets up to 1 mm below tissue surface.
5. Application of RapiClear® 1.49 will not introduce sample deformation.

Pretreatment (before immunostaining/ clearing)

1. For organoid

   4% paraformaldehyde fixed organoids 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 2~3 days at 35˚C~37˚C incubator.

2. For hard tissue (bones, teeth, etc.)

   4% paraformaldehyde fixed bones and teeth should be treated with 50mL 10% EDTA, pH 7.5~8.5, on an orbital shaker for 7~10 days, at RT

3. For biopsy samples

   The dissected biopsies were washed with saline to remove the residual blood. Afterward, samples were fixed in 4% formaldehyde for 1 day and then washed in 1% PBST (1% Triton X-100 in PBS solution containing 0.05% sodium azide) on an orbital shaker for 4~6 days at 4˚C.

Immunolabeling

For the immunostaining protocol, kindly find the procedures on the next page.

Clearing

The volume of RapiClear® used and the time required for clearing should be adjusted according to the tissue size. Basically, 5 times the sample volume of RapiClear® is recommend. Tissue slices may become transparent in 1~2hr.

Note: Pre-warm the RapiClear® to 37℃ before mounting can facilitate solution penetration.

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

REFERENCE

1. Ohtsuki G. Modification of synaptic-input clustering by intrinsic excitability plasticity on cerebellar Purkinje cell dendrites. *J Neurosci* (2019). https://doi.org/10.1523/JNEUROSCI.3211-18.2019
2. Liau ES et al. Visualization of Motor Axon Navigation and Quantification of Axon Arborization In Mouse Embryos Using Light Sheet Fluorescence Microscopy. *J Vis Exp* (2018). http://dx.doi.org/10.3791/57546
3. Hulsmans M et al. Macrophages Facilitate Electrical Conduction in the Heart. *Cell* (2017). http://dx.doi.org/10.1016/j.cell.2017.03.050

STABILITY AND STORAGE

RapiClear® 1.49 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 should be cardiac perfused with cold freshly prepared 4% PFA and normal saline solution.
2. Fix the dissected tissues again in a 50mL tube with 50mL 4% paraformaldehyde solution on an orbital shaker O/N at 4℃.
3. Wash out paraformaldehyde with ~50mL PBS 3 times, 15 min/time.
4. Tissue is sectioned manually, using either vibratome, cryo-section or a tissue matrix with razor blades.

***Note:** if there is endogenous fluorescent proteins expression in the sample, please skip the steps from #5~#9

5. Dehydrate the sample with 5mL Methanol/PBS series: 25%, 50%, 75%, 100% on a shaker at RT; 20min each.
6. Place the sample in 5mL 100% Methanol, overnight on a shaker, at 4℃
7. Bleach in 5mL 5% H2O2/Methanol (1 volume of 30% H2O2 to 5 volumes of Methanol), overnight on a shaker, at 4℃
8. Rehydrate with 5mL Methanol/PBS series: 100%, 75%, 50%, 25%, PBS; on a shaker at RT; 20min each.
9. Wash in 5mL PBS 3 times on a shaker at RT; 30min each.
10. Transfer the sample 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 2~3 days at 35˚C~37˚C incubator for permeabilization.
11. Wash with PBS 3 times, 15 min/time on an orbital shaker or rocker at RT.
12. Keep the specimen in a 2mL tube filled with freshly prepared blocking buffer on an orbital shaker at 4˚C, 1~2 days. *Blocking buffer (10% normal goat serum, 1% Triton-X 100, 2.5% DMSO, and 0.05% sodium azide in PBS)
13. Incubate the specimen with primary antibody in a 2mL tube filled with the Ab dilution buffer on an orbital shaker at 4˚C or RT for 3~5 days. Incubation at higher temp. will promote antibody penetration into the tissue sample. But one of the tradeoffs will be the potential non-specific binding of Ab. *Ab dilution buffer (1% normal goat serum, 0.2% Triton-X 100, 2.5% DMSO, and 0.05% sodium azide in PBS).
14. Wash the specimen with washing buffer for 1hr at RT, 3 times. Then keep the specimen in washing buffer on an orbital shaker at 4˚C overnight. *Washing buffer (3% NaCl and 0.2% Triton-X 100 in PBS)
15. Incubate the specimen with secondary antibody in a 2mL tube filled with the Ab dilution buffer on an orbital shaker at 4˚C or RT for 2~3 days.
16. 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.
17. Wash with PBS 3 times, 20 min/time.
18. Stain with DAPI or SYTOX for nuclear staining if needed. For example, incubate the 0.5mm thick specimen in a 2mL tube filled with SYTOX working solution (1:2500 dilution) on an orbital shaker at 4˚C overnight
19. Wash with PBS 3 times, 1hr/time on an orbital shaker at RT.
20. Clear sample with RapiClear at RT, overnight~1 day. (2mL tube fill with RapiClear; This RapiClear can be reused several times). Pre-warm the RC to 37˚C before mounting can facilitate solution penetration.
21. Mount the cleared specimen with fresh RapiClear reagent in iSpacer microchamber. Press gently around the iSpacer to seal the coverslips.
22. Remove the excess solution at the edges with Kimwipes.
23. (Optional) Fill the space outside the iSpacer with clear nail polish to seal the edges between the two coverslips.
24. Image sample with a high-speed confocal microscope and the oil/glycerin/silicone/water lens with high NA and long WD for high-quality imaging.

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

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