RapiClear® 1.47

Ready-to-use
Making biological sample transparent rapidly

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 collagen, 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® 1.47
1. Samples can be directly transferred from water, buffer solutions, and glycerin into RapiClear® 1.47 medium.
2. The transparent effect is reversible if samples re-immers into water or buffer solutions.
3. RapiClear® 1.47 is ready-to-use, no need to be centrifuged.
4. RapiClear® 1.47 allows visualization of internal targets up to 0.5 mm below tissue surface.
5. Application of RapiClear® 1.47 will not introduce sample deformation.

OPERATION
1. For effective clearing, 4% paraformaldehyde fixed samples should be treated with 2% PBS overnight at RT before mounted in RapiClear® 1.47.
2. The volume of RapiClear® 1.47 used and the time required for clearing should be adjusted according to the tissue size. Basically, 5 times the sample volume of RapiClear® 1.47 is recommend. Tiny tissue (e.g., fly brain) treated with RapiClear® 1.47 may become completely transparent within 1 minute. Tissue slices (e.g., ~0.5mm thickness) may become transparent in 30–60 minutes.
   Note: Pre-warm the RapiClear® to 37°C before mounting can facilitate solution penetration.
3. Cleared sample mounting in fresh RapiClear® 1.47 between two coverslips separated by spacer stickers (iSpacer, 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 to seal the edges between the two coverslips.

For more information, please check the “Demonstration” in our website: www.sunjinlab.com

STABILITY AND STORAGE
RapiClear® 1.47 can be stored at 15°C–RT. Invert the bottles several times to mix the contents before use.

WARNING AND PRECAUTIONS
Repeated exposure may cause skin dryness or cracking. Preventing skin contact is suggested.

TECHNICAL ASSISTANCE
E-mail: sunjinlab@gmail.com

Manufacturer:
SunJin Lab Co.
www.sunjinlab.com
4F., No.11, Ln. 22, Bo’ai St., East Dist., Hsinchu City 30068, Taiwan
Phone: 886-3-516-5085
Fax: 886-3-516-5087
**Immunostaining Protocol**

1. Animal fixed by 4% paraformaldehyde solution via cardiac perfusion.

2. Tissues can be sectioned manually (e.g., 500μm), using a vibratome, cryo-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 h at RT.

4. Wash with PBS 3 times, 10 min/time.

5. Transfer samples into 2% PBST (2% Triton X-100 in PBS solution) overnight at RT for permeabilization.

6. Keep the specimen in blocking buffer on an orbital shaker or rocker at 4°C 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°C.

7. Incubate the specimen with primary antibody in a 24-well plate (500 μl/well) on an orbital shaker or rocker at 4°C 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°C.

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°C 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°C.

9. Incubate the specimen with secondary antibody on an orbital shaker or rocker at 4°C 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°C for overnight. (Note: washing step is quite important for immunostaining!)

11. DAPI or SYTOX for nuclear staining if needed.

12. Wash with PBS 3 times, 30 min/time.

13. Transfer the specimen to a 24-well plate with the RapiClear (RC) reagent with ~5 times of the sample volume. Pre-warm the RC to 37°C before mounting can facilitate solution penetration.

14. Place the plate on an orbital shaker or rocker to gently mix and immerse the specimen with the RC reagent for minutes ~ hours at RT.

15. Mount the cleared specimen in fresh RC reagent between two coverslips separated by iSpacer® sticker(s). Press gently around the sticker to seal the coverslips.

16. Remove the extra solution at the edges with Kimwipes.

17. Fill the space outside the iSpacer with clear nail polish to seal the edges between the two coverslips.

18. The images are acquired by a confocal microscopy system.