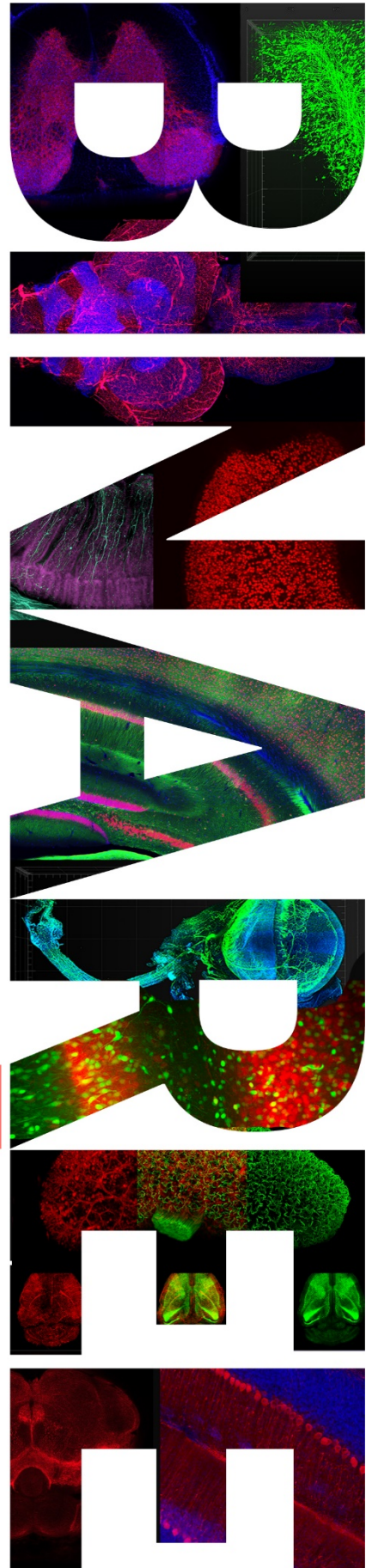


**BI****NAREE**  
make **visible**

TISSUE CLEARING

PROTOCOL

HRTC-001



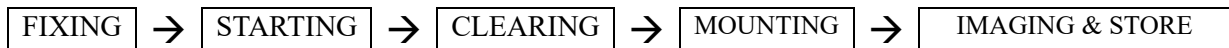
NAME OF PROTOCOL

Binaree Tissue Clearing™ kit (High Resolution)

Cat. No. HRTC-001

CODE OF PROTOCOL: C1001  
REVISION OF PROTOCOL: 1.1.8 (2020.08.28)

### [A] - Preparation | Planning you test



[Tissue Clearing & Imaging within 7 days]

When we designed the protocol, we considered not only the effectiveness of the clearing but also the working time of the researchers.

**Enjoy the tissue clearing!**

### [B] - Preparation | Taking the solutions

B-1. All the solutions should be stored at 4 °C.

B-2. Check Tissue Clearing Solution A & B and Mounting & Storage Solution for crystallization or precipitation before use.

B-3. Do not use the individual solutions from the other kit. Even if the names of solutions are the same, the component compositions are not the same. Each solution has a unique component composition depending on the purpose of the kit.

- ① **Starting Solution**
- ② **Tissue Clearing Solution A**
- ③ **Tissue Clearing Solution B**
- + Mounting & Storage Solution**

- The Mounting & Storage Solution (Cat. No. SHMS-060) is not included in Binaree Tissue Clearing Kit (HRTC-001). Only the Starter's kits (HRTC-101) contain the Mounting & Storage Solution.

- The solutions may become crystallized or precipitated. If this occurs, incubate it at 37 °C for 1-2 h before use.

### [C] - Preparation | Fixing the sample

C-1. The mouse is transcardial perfused with 4% PFA.

C-2. Incubate the sample with 4% PFA at 4 °C for overnight.

C-3. Wash the sample with 1X PBS while shaking at 4 °C for 20 min × 3 times.

C-4. Incubate the ②③ Tissue Clearing Solution A & B and + Mounting & Storage Solution at 37 °C for 1-2 h before use.

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## [D] - Protocol I Clearing the fixed sample

Please refer to APPENDIX and adjust the clearing step according to the tissue thickness.

D-1. Incubate the sample with ① **10 ml Starting Solution** at 4 °C until the sample sinks.

D-2. Incubate the sample with ② **10 ml Tissue Clearing Solution A** in a shaking at 50 rpm /37 °C for 48 h.

D-3. Wash the sample with distilled water while shaking at 50 rpm /4 °C for 30 min × 4 times.

The sample may become opaque and swell. This does not affect the clearing process; the sample will be cleared again in Mounting & Storage Solution.

D-4. Incubate the sample with ③ **10 ml Tissue Clearing Solution B** in a shaking at 50 rpm /37 °C for 48 h.

If the tissue not enough clear in step 4, washing (D-3) & tissue clearing (D-4) should be repeated until cleared.

D-5. Wash the sample with distilled water while shaking at 50 rpm /4 °C for 30 min × 4 times.

The sample may become opaque and swell. The sample may become opaque and swell. This does not affect the clearing process; the sample will be cleared again in Mounting & Storage Solution.

D-6. (optional) Add nuclear stain solution (e.g. DAPI, 20-40 µg/ml in distilled water) while shaking at 4 °C for overnight.

Wash the sample with distilled water while shaking at 50 rpm /4 °C for 30 min × 3 times.

D-7. Incubate the sample with + **20 ml Mounting & Storage Solution** in a shaking incubator at 50 rpm /37 °C for 12 - 24 h.

## [E] - Clearing Tips

E-1. If the sample contains air bubbles → Centrifuge the sample at 3,000 rpm /24 °C for 1 min.

E-2. If the sample is not entirely cleared → Repeat from step D-4 to step D-5.

E-3. If the rpm is not specified → Operate the shaking incubator gently.

E-4. Never wash the sample with PBS instead of distilled water at steps D-3 and D-5.

E-5. It is recommended to use the vial for tissue clearing rather than the chamber slide.

Drying causes crystallization of Tissue Clearing Solution A & B and Mounting & Storage Solution.

## [F] - Storage & Imaging Tips

F-1. After imaging, the samples are stored at room temperature in the Mounting & Storage Solution. Never refrigerate.

F-2. Take images within 7 days after the clearing for the best results.

F-3. Take images on the microscope. We recommend using a Light Sheet Fluorescence Microscope (LSFM) or

Confocal Laser Scanning Microscope (CLSM). Analyze and visualize the images with a microscopy image analysis software.

F-4. Mounting & Storage Solution is a solvent-free material that is safe to use in the Light Sheet Fluorescence Microscope (LSFM).

F-5. Refractive Index (RI) of the Mounting & Storage Solution is 1.45.

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Cat. No. HRTC-001

F-6. Be careful of making bubbles while filling the microscope chamber with the sample and the Mounting & Storage Solution. The bubbles may disturb the imaging.

F-7. To take images of tissue with less than 1 mm thickness via confocal microscope, use a slide chamber (2 wells or 4 wells) like the image below. Sealing the chamber with label tape reduces drying. Too much of the Mounting & Storage Solution can cause the sample in the chamber to shake. The optimal volume for 1 mm thick tissue is 200 µl.

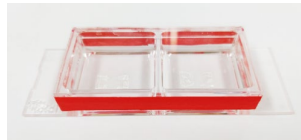


Figure 1. When taking images through confocal microscopy, the image chamber must be seal by label tape.

## [G] - Appendix

Read not only the appendix but also the protocol. The protocol describes the method in detail.

G-1. Mouse ≤ 8-weeks-old

step	Summary	Temp. (°C)	1 mm thickness	3 mm thickness	≤ 7 mm thickness (ex. half brain, spleen, spinal cord)	≥ 7 mm thickness (ex. whole brain, lung, kidney, heart)
B-2	4% PFA	4 °C	12 - 24 h	12 - 24 h	12 - 24 h	12 - 24 h
B-3	Wash with 1X PBS	4 °C	20 min × 3 times	20 min × 3 times	20 min × 3 times	20 min × 3 times
D-1	Starting Solution*	4 °C	2 - 12 h	12 - 24 h	24 h	24 - 48 h
D-2	Tissue Clearing Solution A	37 °C	4 - 6 h	24 - 48 h	48 h	48 - 72 h
D-3	Wash with distilled water	4 °C	10 min × 3 times	30 min × 4 times	30 min × 4 times	30 min × 4 times
D-4	Tissue Clearing Solution B	37 °C		24 - 48 h	24 - 48 h	48 - 72h
D-5	Wash with distilled water	4 °C		30 min × 4 times	30 min × 4 times	30 min × 4 times
D-6	(option) DAPI staining	4 °C	1 h	6 - 12 h	12 - 24 h	12 - 24 h
D-7	Mounting & Storage Solution	37 °C	< 1 day	> 1 day	> 1 day	> 2 days

**Note.** \*Samples were incubated in Starting Solution until the sample sank. However, even if the sample (ex. lung, spinal cord) does not sink in the Starting Solution after 2 days, proceed to the next steps.

**Note.** If the tissue not enough clear in step D-4, washing (step D-3) & tissue clearing (step D-4) should be repeated until cleared.

**Note.** When using tissue from mice older than 8 weeks, repeat the tissue clearing (step D-4) & washing (step D-5) until the tissue is clear.

## [H] - Contact Us | Technical support

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