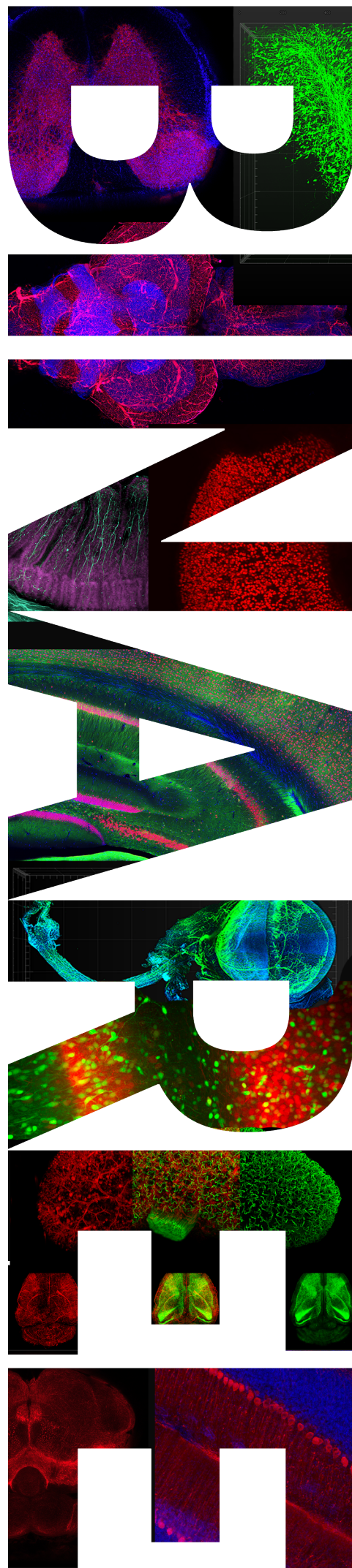


**BI****NAREE**  
make **visible**

TISSUE CLEARING

PROTOCOL



NAME OF PROTOCOL

**The Tissue clearing protocol for whole organs**

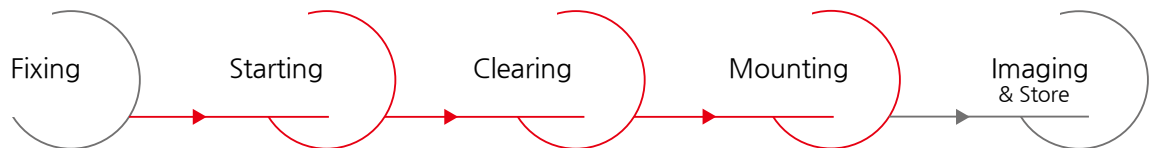
(Cat.No. HRTC-001)

CONDITION OF SAMPLE : Adult whole mouse brain

CODE OF PROTOCOL : C1001

REVISION OF PROTOCOL : 1.1.8 (2019.11.25)

[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 of the solutions should be stored at 4°C.

B-2. Check Tissue Clearing Solution and Mounting & Storage Solution for crystallization or precipitation before each use. Redissolve any precipitation by warming the solution at 37°C for 1-2h and then 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

+ 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 1 x PBS while shaking at 4°C for 20 min X 3 times.

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

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

- D-1. Incubate the sample with ① 10 ml Starting Solution at 4° until the sample sinks (at least 24 h or more).
- D-2. Incubate the sample with ② 10 ml Tissue Clearing Solution 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 1 h X 4 times.  
The sample may become opaque and swell. This does not affect the clearing process; the sample will be cleared again at the end of protocol.
- D-4. Incubate the sample with ③ 10 ml Tissue Clearing Solution in a shaking at 50 rpm/37°C for 48 h.  
If the tissue not enough clear in step 4, tissue clearing (step 4) & washing (step 5) should be repeated until cleared.
- D-5. Wash the sample with **distilled water** while shaking at 50 rpm/4°C for 1 h X 4 times.  
The sample may become opaque and swell.
- D-6. (optional) Add nuclear stain solution (e.g. DAPI, 20-40 µg/ml in 0.1 x PBS) while shaking at 4°C for overnight.
- D-7. Incubate the sample with + 20 ml Mounting & Storage Solution in a shaking incubator at 50 rpm/37°C for at 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. **If the mouse is older than 5 weeks-old** read Appendix 1 at the end of the document.
- E-6. It is recommended to use the vial for tissue clearing rather than the chamber slide.  
Drying causes crystallization of Tissue Clearing Solution and Mounting & Storage Solution.

## [F] - Storage & Imaging Tips

- F-1. Store the cleared sample in +Mounting & Storage Solution at the room temperature (20~25°C).
- 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.
- 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.

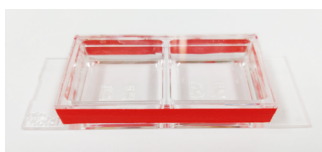


Figure 1. When taking images through confocal microscopy, the image chamber must be seal by label tape.

## [G] - Appendix 1 | Adjustment of processing time to size depending on mouse age

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

### G-1 Mouse ≤ 5-weeks-old

step	Summary	temp	1 mm thickness	≤ 7 mm thickness (ex. Half brain)	≥ 7 mm thickness (ex. Whole brain)
B-2	4% PFA	4°C	overnight	12 -24 h	12-24 h
B-3	Wash with 1 x PBS	4°C	20 min X 3 times	20 min X 3 times	20 min X 3 times
D-1	Starting Solution1*	4°C	24 h	24 h	24 h
D-2	Tissue Clearing Solution	37°C	24 h	48 h	48 h
D-3	Wash with distilled water	4°C		1 h X 4 times	1 h X 4 times
D-4	Tissue Clearing Solution	37°C		48 h	48 h
D-5	Wash with distilled water	4°C		1 h X 4 times	1 h X 4 times
D-7	Mounting & Storage Solution	37°C	< 1day	>1 day	>1 day

Note. 1\* 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 3 days, proceed to the next steps.

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

Note. The spinal cord should be incubated for a long time (3-4 days) in Tissue Clearing Solution and then washed.

Tissue clearing (step D-4) & washing (step D-5) should be repeated until cleared.

## [H] - Contact Us | Technical support

Binaree, Inc. (Headquaters)

○47 Gyeongdaero17-gil Buk-gu, STE#608 IT Convergence Bldg(115)., Daegu, 41566, Republic of Korea.

○Website: binaree.com ○ Email : lab@binaree.com

○Tel : +82-(0)53-291-5012 ○ Fax : +82-(0)53-382-5012