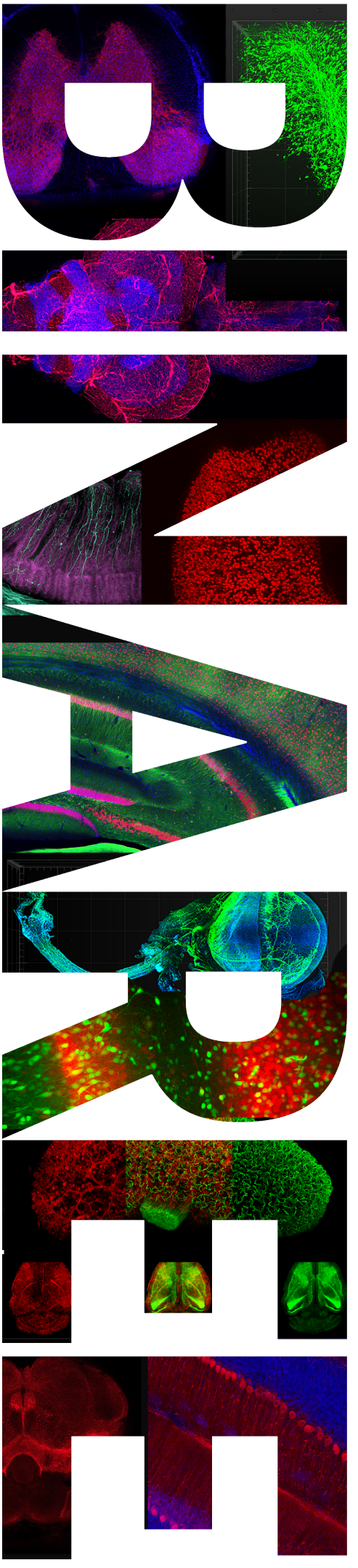


BINAREE

make visible

TISSUE CLEARING

PROTOCOL

NAME OF PROTOCOL

The Tissue clearing protocol for Organoid

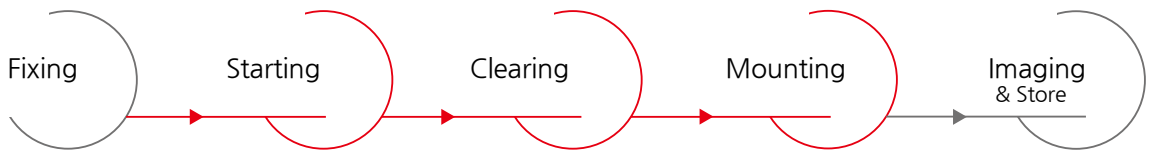
Cat.No. HROC-001

CONDITION OF SAMPLE : Organoid
〈Length 1 ㎜ x Width 1 ㎜ x Height 1 ㎜

CODE OF PROTOCOL : C1001

REVISION OF PROTOCOL : 1.1.8 (2020.08.25)

[A] - Preparation I Planning you test



[Organoid Clearing & Imaging within 4 days]

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

Enjoy the tissue clearing!

[B] - Preparation I Taking the solutions

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

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

①Starting Solution

②Organoid Clearing Solution

- The Mounting & Storage Solution (Cat. No. SHMS-060) is not included in Binaree Tissue Clearing Kit for Organoid (HROC-001).
- The solutions may become crystallized or precipitated. If this occurs, incubate it at 37 °C for 1-2 h before use.

[C] - Preparation I Fixing the sample

C-1. Incubate the sample with 4% PFA at 4 °C for 15 min.

C-2. Wash the sample with 1 x PBS while shaking at 4°C for 10 min X 3 times.

C-3. Incubate the ② Organoid Clearing Solution and + Mounting & Storage Solution at 37 °C for 1-2 h before use.

[D] - Protocol | Clearing the fixed sample

D-1. Incubate the sample with ① 0.5 ml Starting Solution at 4 °C until the sample sinks.

D-2. Incubate the sample with ② 0.5 ml Organoid Clearing Solution in a shaking at 50 rpm / 37 °C for 24 h.

D-3. Wash the sample with distilled water while shaking at 50 rpm/ 4 °C for 10 min X 3 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.
If the sample not enough clear in D-2, organoid clearing (D-2) & washing (D-3) should be repeated until cleared.

D-4. (optional) Add nuclear stain solution (e.g. DAPI, 20-40 µg/ml in distilled water) while shaking at 4 °C for 3 h.

D-5. Incubate the sample with + 0.3 ml Mounting & Storage Solution in a shaking incubator at 50 rpm/ 37 °C for at least 1-2 day.

[E] - Clearing Tips

E-1. If the sample contains air bubbles ➡ Centrifuge the sample at 3,000 rpm/24 °C for 1min. E-2. If the sample is not entirely cleared ➡ Repeat from step D-2 to step D-3.

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 step D-3.

E-5. It is recommended to use the vial for organoid clearing rather than the chamber slide. Taking image via confocal microscope, use a slide chamber (2 wells or 4 wells) like the image below. Sealing the chamber with label tape reduces drying.

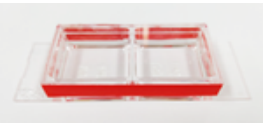


Figure 1. When taking images through confocal microscopy, the image chamber must be seal by label tape.

[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 Confocal Laser Scanning Microscope (CLSM).

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.

[G] - 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)53-939-5012 ○ Fax : +82-(0)53-382-5012