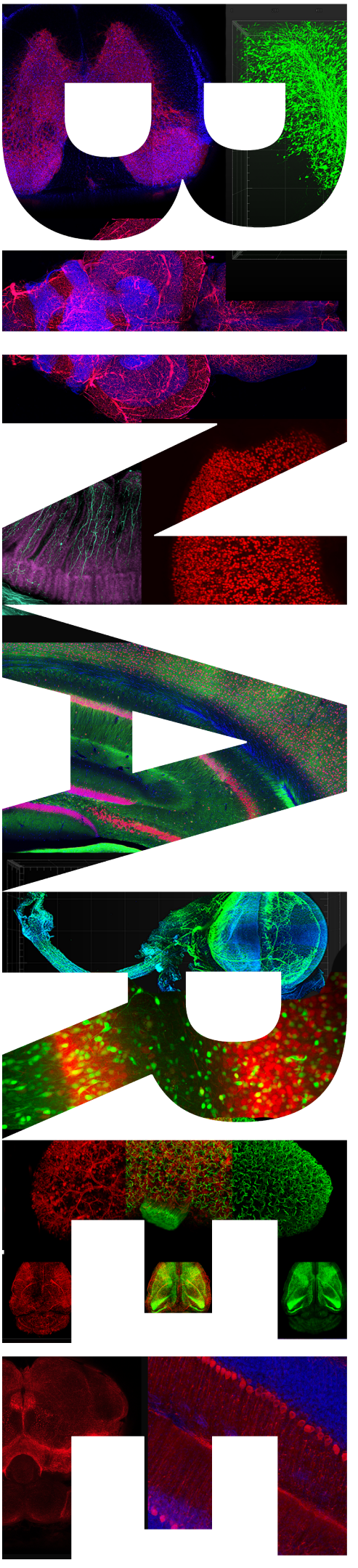


BINAREE

make visible

TISSUE CLEARING

PROTOCOL

NAME OF PROTOCOL

The optical clearing protocol for Spheroid

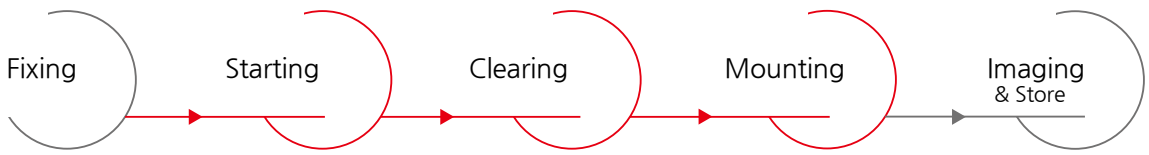
Cat.No. HRSC-101

CONDITION OF SAMPLE : Spheroid
≤ Length 1 ㎜ x Width 1 ㎜ x Height 1 ㎜

CODE OF PROTOCOL : C1001

REVISION OF PROTOCOL : 1.1.9 (2021.01.02)

[A] - Preparation I Planning you test



[Spheroid Clearing & Imaging within 2 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 of the solutions should be stored at 4˚C.

B-2. Check Spheroid 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.

① Starting Solution

② Spheroid Clearing Solution

+ Mounting & Storage Solution

- The Mounting & Storage Solution (Cat. No. SHMS-060) is not included in Binaree Tissue Clearing Kit for Spheroid (HRSC-001). Only the Starter's kits (HRSC-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 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 ②Spheroid 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 Spheroid Clearing Solution in a shaking at 50 rpm /37°C for 12-24 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, spheroid 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 1-2 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 12 h or more.

[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 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.

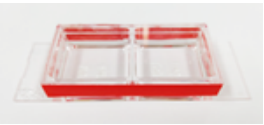


Figure 1. When taking images through confocal microscopy, the image chamber must be seal by label tape.

[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 3 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 1.46.

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

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