NAME OF PROTOCOL
The tissue clearing protocol for organoid (Cat. No.SHOC-001)

CONDITION OF SAMPLE	CODE OF PROTOCOL	REVISION OF PROTOCOL
Length 1 ㎜ x Width 1 ㎜ x Height 1 ㎜	C1003	1.1.2 (2019.08.21)

Preparation | Planning your test.

When we designed the protocol, we considered not only the effect of the clearing but also the working time of the researchers. *Enjoy the tissue clearing!*

Preparation | Takes the solutions.

A-1. All of the solutions might be **stored at 4℃**.

A-2. **Crystallization or precipitation while solutions are stored at 4℃**; It is no effect for the clearing process. It will be liquid again after incubating the solutions at 37℃ (Refer to preparation step B-3).

A-3. **Do not use the individual solution 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 depend on the purpose of the kit.

1	Starting Solution	30 ㎖
2	Organoid Clearing Solution	30 ㎖
+	Mounting & Storage Solution	60 ㎖

- The Mounting & Storage Solution (Cat. No. SHMS-060) is **not included in the kit. Only the Starter's kits contain** the Mounting & Storage Solution.
- The solutions may be **crystallized or precipitated**, incubate it at 37℃ for 1~2 h before use.

Preparation | Fixing the sample (organoid tissue).

B-1. Incubate the sample with 4% PFA at 4℃ for 15 min.

B-2. Wash the sample with 1 X PBS in shaking incubator at room temperature for 10 min X 3 times.

B-3. Incubate the (2) Organoid Clearing Solution and (+) Mounting & Storage Solution at 37℃ for 1~2 h before use.

Protocol | Clearing the fixed sample.

1. Incubate the sample with (1) 500 ㎕ Starting Solution at 4℃ for until the sample sinks (at least overnight or more) .
2. Incubate the sample with (2) 500 ㎕ Organoid Clearing Solution in shaking incubator at 50 rpm/37℃ for 6 h.
3. Wash the sample with **distilled water** in shaking incubator at 50 rpm/4℃ for 10 min X 3 times.

   ⚠ The sample may be **back to be opaque reversely and swell**.
   It is no effect for the clearing process, the sample will be cleared again at the end of protocol.
4. Incubate the sample with (+) 500 ㎕ Mounting & Storage Solution in shaking incubator at 50 rpm/37℃

for at least 4 h or more.

Clearing Tips | Troubleshootings

C-1. If the sample contains air bubbles ➡ Centrifuge the sample at 3,500 rpm/24˚C for 30 min.

C-2. If the sample is not entirely clearing ➡ Repeat step #3 and then from #2 to step #4.

C-3. If the rpm is not specified ➡ Usually operate the shaking incubator gently.

C-4. Never wash the sample with PBS instead of distilled water at step #3.

C-5. Use the vial or tube for tissue clearing better than the slide chamber.

Storage & Imaging Tips

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

D-2. Take images within 7 days after the clearing for the best result.

D-3. Take images on the microscope. We recommend using the Light Sheet Fluorescence Microscope (LSFM) or Confocal Laser Scanning Microscope (CLSM). And analyze and visualize the images with the microscopy image analysis software.

D-4. + Mounting & Storage Solution the solvent-free material that is safe for using the Light Sheet Fluorescence Microscope (LSFM).

D-5. Refractive Index (RI) of + Mounting & Storage Solution is 1.45.

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

End of Protocol

Contact Us | Technical support

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