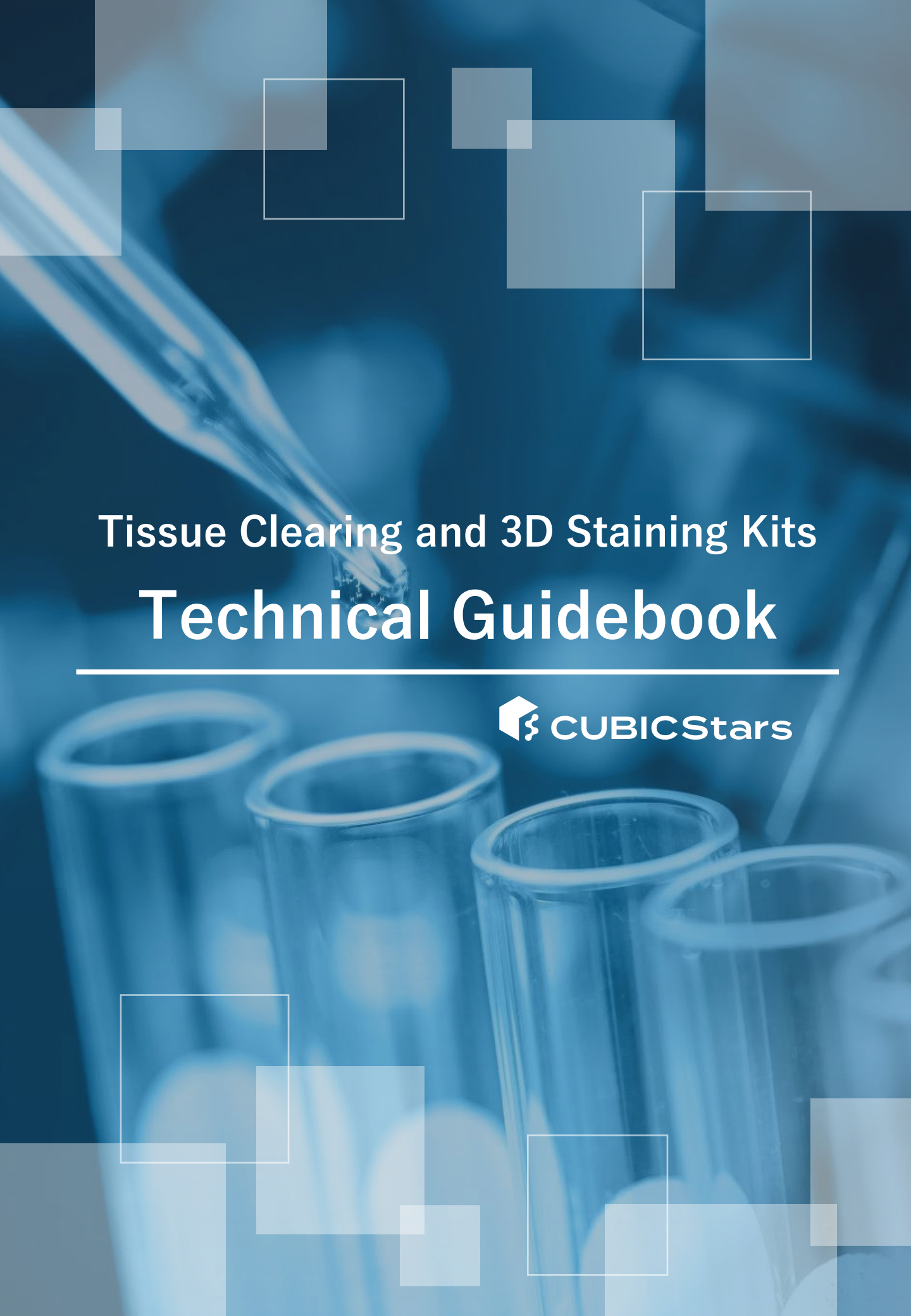

# Tissue Clearing and 3D Staining Kits

# Technical Guidebook

CUBICStars

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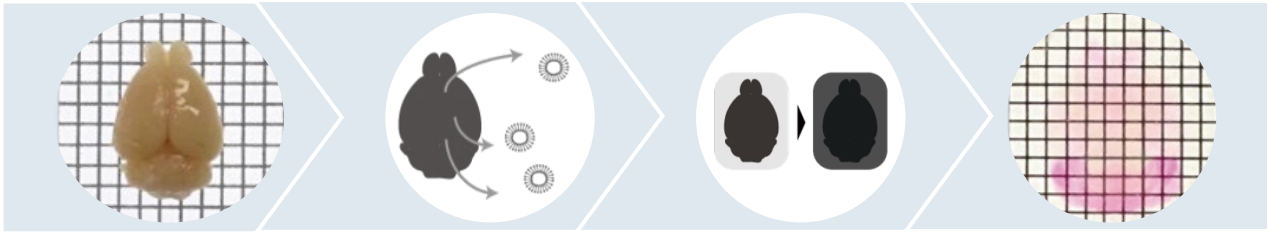
# 01 Technical Overview

Our CUBIC Technology was developed by RIKEN and the University of Tokyo over about 10 years to allow observation of all cells in organs and the body. Today, our technology is used by researchers around the world and gained high trust as a versatile tissue clearing and 3D tissue staining method. Our kits are designed for safe use and easy introduction.

## CUBIC Tissue Clearing Reagent Kits

- The world's most powerful and highly versatile water-soluble clearing reagents that allow transparency of organs and the whole body.
- Can be performed with only general lab equipment and does not require special dedicated equipment such as an electrophoresis apparatus.
- Uses light-sheet microscopes (LSFM) and confocal laser scanning microscopes to allow imaging of the whole 3D organ with a resolution to the cellular level.

### Tissue Transparency Workflow



PFA fixed sample

Remove lipids and pigments with CUBIC-L

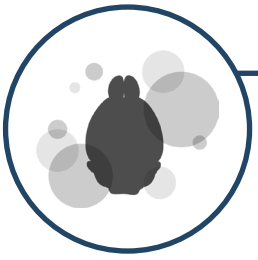
RI matching with CUBIC-R

Transparency sample

## CUBIC-HV 3D Staining Kit

- The world's highest-performance 3D staining technology (patented by CUBICStars, Inc.)
- Practical application of a special tissue staining process that uniformly stains 3D blocks of tissue
- Highly reproducible protocol compatible for various nuclear staining and antibody staining

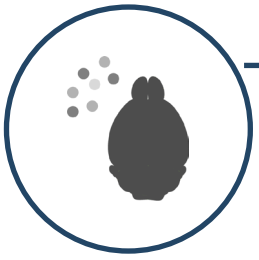
### Main Technology



**Patented Technology 1**

**High concentration stain probe method**

A new technology that allows concentration levels to increase 10 to 100 times than the regular method, penetrating reagents and antibodies to the depths quickly.



**Patented Technology 2**

**Penetration enhancer stain probe**

An additive that penetrates reagents and antibodies to the depths quickly made from ideal compounds selected among over 500 types.

# 02 Tissue Clearing Reagent Kit CUBIC

## Features

- Highly versatile water-soluble CUBIC tissue clearing kits that have a powerful transparency level
- Compatible with all organs including the bone. Fluorescent protein signals can be observed after clearing the sample.
- Our high simplicity and reproducibility protocol solves issues pertaining to safety, waste processing, microscope compatibility, fluorescent protein signal retainability, etc.


## Products

### CUBIC Basic Clearing Reagents

**Allows animal organs to become transparent by immersing in two types of reagents**

| | | |
|---|---|---|
| **CUBIC-L [CSCR001]** | For delipidation and decolorization | USD580/kit<br>500 mL/kit |
| **CUBIC-R [CSCR002]** | For clearing (RI matching). High versatility and easy handling. | USD 400 /kit<br>500 mL/kit |

*Can experiment approx. 20 mouse brains with 500 mL of reagent.
*CUBIC-R causes each axis to swell 1.5 times to increase transparency and performance resolution during microscope imaging.
*The product structure of kits is subject to change without prior notice.

### CUBIC Bone Tissue Clearing Reagent

**Reagent optimized for bone tissue transparency. Use with CUBIC-L/R.**

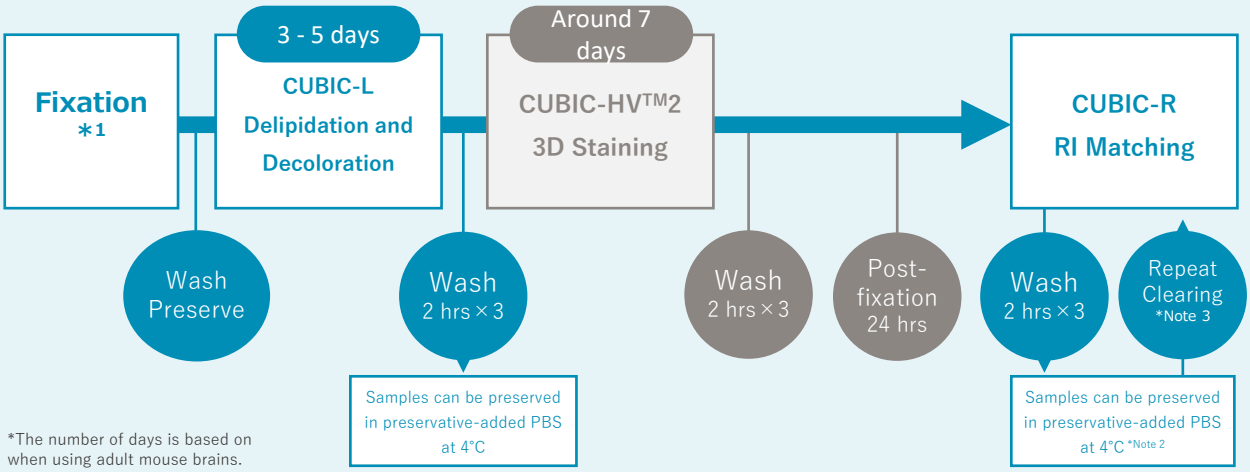
| | | |
|---|---|---|
| **CUBIC-B [CSCR003]** | Bone tissue clearing reagent optimized for bone tissue decalcification | USD 165 /kit<br>100 mL/kit |

*Can experiment approx. 5 to 10 mouse lower limbs with 100 mL of reagent.
*The product structure of kits is subject to change without prior notice.

Reference 1 K. Matsumoto, H. R. Ueda, et al., Nat. Protoc. 2019, 14, 3506-3537 https://doi.org/10.1038/s41596-019-0240-9
Reference 2 K. Tainaka, H. R. Ueda, et al., Cell Rep. 2018, 24, 2196-2210 https://doi.org/10.1016/j.celrep.2018.07.056

# CUBIC Tissue Clearing Reagent Kit : Application Example 1

## Basic Process of Clearing and Staining Mouse Organs




*Note 1 We recommend using 4% paraformaldehyde (pH 7~7.5) for the fixative. Transparency is greatly affected by fixation conditions. It is important to minimize the difference between controlled conditions, such as creating large amounts of paraformaldehyde and storing them in the freezer. Overfixation and yellowing may occur if the pH is alkaline.

*Note 2 Transparency samples can be embedded with agarose gel. This allows stable preservation for a long time.

*Note 3 Frequently repeating clearing results in tissue damage.

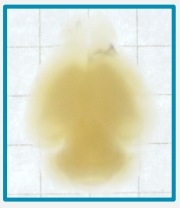
## Mouse Organ Clearing Procedure (Basic Protocol)

| Step | Reagent | Temperature | Time | Notes |
|---|---|---|---|---|
| **Extract organ** | | | | After perfusion fixation. |
| **Post-fixation** | 4% PFA in PBS | 4°C | 1 day | |
| **Wash x 3** | PBS | RT | Up to 2 hrs x 3 | Shake gently (the same applies to the steps below). |
| Before replacement | 50% CUBIC-L | RT Or 37°C | 6 – 24 hrs | Optional step. Mix an equal amount of CUBIC-L and water. |
| **Delipidation and decoloration** | CUBIC-L | 37°C | > 2 days | Change CUBIC-L to a new solution every two days. |
| **Wash x 3** | PBS | RT | Up to 2 hrs x 3 | Exchange or wash the tube every time to prevent carry-over of the solution. |
| Staining | CUBIC-HV™2, stain probe (antibody, staining) | RT | Up to 7 days | Optional step. |
| Wash x 3 | CUBIC-HV™2 | 4°C | Up to 1.5 hrs x 3 | Conduct if staining. |
| Post-fixation | 1% formaldehyde | 4°C | 1 day | Conduct if staining. Use 37% formaldehyde diluted with PBS. |
| Post-fixation | 1% formaldehyde | 37°C | 1 hr | Conduct if staining. Transfer the solution treated overnight at 4°C to the solution treated at 37°C. |
| Wash x 3 | PBS | RT | Up to 2 hrs x 3 | Conduct if staining. |
| **Before replacement** | 50% CUBIC-R | RT Or 37°C | 1 day | Mix an equal amount of CUBIC-R and water. |
| **Clearing** | CUBIC-R | RT Or 37°C | > 1 day | |

*Details on the 3D staining procedure on pages 8 – 10.

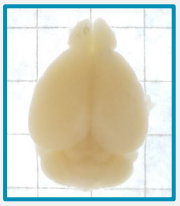
## Practical Example of Mouse Organ Clearing

Adult mouse brain after extraction and PFA fixation

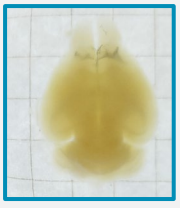


After replacement with 50% CUBIC-L
(Treated overnight at 37°C)

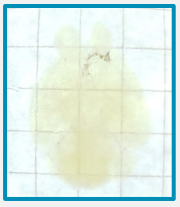
After delipidation and decoloration with 100% CUBIC-L
(Treated for four days at 37°C)



After washing with PBS



After shaking in 4 mL 50% CUBIC-R
(Treated overnight at room temperature)

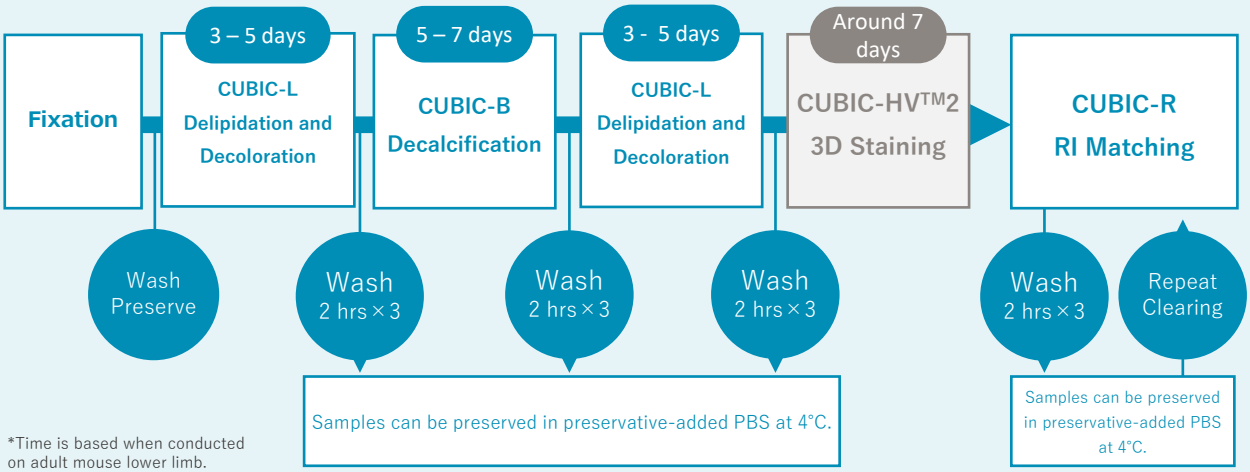


After shaking in 4 mL CUBIC-R (overnight at room temperature),
Immersed in mounting agent for observation (RI = 1.522)

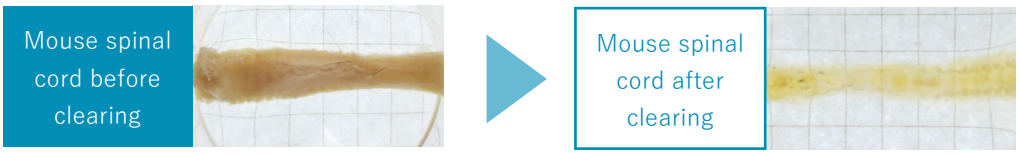
* Amount of reagent and treatment time varies depending on the organ.
* Please use a slightly larger tube wider than the organ's diameter. Most of the organ should be immersed in the reagent when the tube is laid on its side.
* Shake the organ immersed in the reagent at a speed that shakes the whole organ.
* We do not recommend rotation as it will create bubbles.

# CUBIC Tissue Clearing Reagent Kit : Application Example 2

## Mouse Bone Tissue Clearing Basic Process




*Time is based when conducted on adult mouse lower limb.




## Mouse Bone Clearing Procedure (Basic Protocol)

| Step | Reagent | Temperature | Time | Notes |
|---|---|---|---|---|
| **Fixation** | 4% PFA in PBS | 4°C | 1 day | |
| Prior replacement | 50% CUBIC-L | RT Or 37°C | 6 – 24 hrs | Optional step. Mix an equal amount of CUBIC-L and water. |
| **Delipidation** | CUBIC-L | 37°C | 3 - 7 days* | Shake gently (the same applies to the steps below). Change once every two days. |
| **Wash** | PBS | RT | Up to 2 hrs x3 | Exchange or wash the tube every time to prevent carry-over of the solution. |
| **Decalcification** | CUBIC-B | 37°C | 5 - 7 days | Change CUBIC-L to a new solution at least once. |
| **Wash** | PBS | RT | Up to 2 hrs x3 | Exchange or wash the tube every time to prevent carry-over of the solution. |
| **Delipidation** | CUBIC-L | 37°C | 2 - 4 days | |
| **Wash** | PBS | RT | Up to 2 hrs x3 | Exchange or wash the tube every time to prevent carry-over of the solution. |
| Stain | CUBIC-HV™2, staining probe | RT | 5 - 7 days | Optional step. |
| Wash x 3 | CUBIC-HV™2 | 4°C | Up to 1.5 hrs x 3 | Conduct if staining. |
| Post-fixation | 1% FA | 4°C | 1 day | Conduct if staining. Use 37% formaldehyde diluted with PBS. |
| Post-fixation | 1% FA | 37°C | 1 hr | Conduct if staining. Transfer the solution treated overnight at 4°C to the solution treated at 37°C. |
| Wash x 3 | PBS | RT | Up to 2 hrs x 3 | Conduct if staining. |
| **Before replacement** | 50% CUBIC-R | RT Or 37°C | 1 day | Mix an equal amount of CUBIC-R and water. |
| **Clearing** | CUBIC-R | RT Or 37°C | > 1 day | |

Reference Tainaka et al. Cell Reports 2018

# 03 CUBIC-HV™2 3D Tissue Staining Kit

## Features

- Productized based on CUBIC-HistoVIsion, the world's highest-performance 3D tissue staining technology.
- The kit includes reagents that allow equal staining of the inside and outside of the 3D samples.
- To be used together with CUBIC clearing reagents (sold separately).
- Available in a starter kit that includes a staining pot optimized for experiments and a general kit (the staining pot can be used repeatedly).

## Products

**Buffers and tools optimized for 3D tissue staining. Applicable for antibody and nuclear staining. Equally stains a whole mouse brain within a week at the shortest.**

| Product Name | Kit Contents | Price |
|---|---|---|
| **CUBIC-HV™2 3D tissue staining kit [CSSR003]** | • Pre-wash buffer<br>• Staining buffer<br>• Additive A - F<br>• Wrapping reagent<br>• Wash buffer<br>• Supplement for staining buffer (supplement) | USD 990/kit<br>10 tests/kit |
| **CUBIC-HV™2 3D tissue staining kit (Starter) [CSSR004]** | • The above reagents + Staining pot | USD1,580/kit<br>10 tests/kit |
| **3D tissue staining pot for CUBIC-HV™2 [CSSR005]** | • Staining pot | USD 590/kit<br>10 tests/kit |

*The amount used in the above structure is applicable in the application example of adult mouse brains. The amount required will vary depending on the size and type of organ.

*The product structure of kits is subject to change without prior notice.

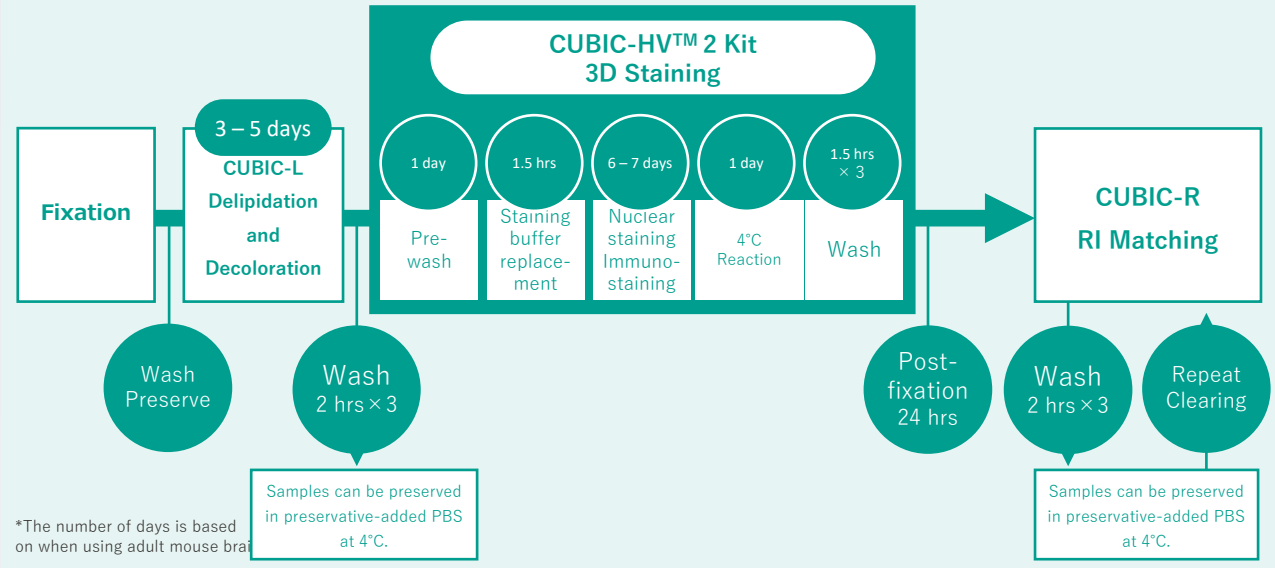
*Dissolve the supplement in the staining buffer right before use. Dissolving takes approx. 2 hours.

*Purchase formaldehyde available in the market separately.

Reference E. A. Susaki, H. R. Ueda, et al., Nat. Commun. 2020, 11, 1982. https://doi.org/10.1038/s41467-020-15906-5

# CUBIC-HV™2 3D Staining Kit : Application Example 3

## Basic Process of 3D Staining Mouse Brains Using CUBIC-HV™2




## Steps to 3D Staining Mouse Brains (Basic Protocol)

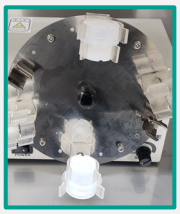
| Step | Reagent | Temperature | Time | Notes |
|---|---|---|---|---|
| **Extract organ** | | | | After perfusion fixation. |
| **Post-fixation** | 4% PFA in PBS | 4°C | 1 day | |
| **Wash x 3** | PBS | RT | Up to 2 hrs x 3 | Shake gently (the same applies to the steps below). |
| Before replacement | 50% CUBIC-L | RT Or 37°C | 6 – 24 hrs | Optional step. Mix an equal amount of CUBIC-L and water. |
| **Delipidation and Decoloration** | CUBIC-L | 37°C | > 2 days | Change CUBIC-L to a new solution every two days. |
| **Wash x 3** | PBS | RT | Up to 2 hrs x 3 | Exchange or wash the tube every time to prevent carry-over of the solution. |
| **Staining pre-wash** | 1× CUBIC-HV™2 Pre wash buffer | 37°C | Overnight | |
| **Staining before replacement** | 2× CUBIC-HV™2 Staining buffer | 37°C | 1.5 hrs | Add the additive.<br>Conduct reactions of the primary antibody and secondary antibody (Fab fragment) separately during replacement. |
| **Staining** | 2× CUBIC-HV™2 Staining buffer | RT~37°C | 6 - 7 days | Add the sample, additive, antibody solution, and nuclear staining reagent to the staining pot. Conduct staining in the wrapping reagent. |
| **Reaction after staining** | 2× CUBIC-HV™2 Staining buffer | 4°C | Overnight | Set the reaction chamber in the previous step to 4°C as it is. |
| **Wash x 3** | 2× CUBIC-HV™2 Wash buffer | 4°C | Up to 1.5 hrs x 3 | Store the buffer in ice beforehand. |
| **Post-fixation** | 1% Formaldehyde | 4°C | 1 day | Use 37% formaldehyde diluted with PBS. |
| **Post-fixation** | 1% Formaldehyde | 37°C | 1 hr | Transfer the solution treated overnight at 4°C to the solution treated at 37°C. |
| **Wash x 3** | PBS | RT | Up to 2 hrs x 3 | |
| **Before replacement** | 50% CUBIC-R | RT Or 37°C | 1 day | Mix an equal amount of CUBIC-L and water. |
| **Clearing** | CUBIC-R | RT Or 37°C | > 1 day | |

## 3D Staining Mouse Organ Practical Example

Adult mouse brain after treating with CUBIC-L

Pour the solution containing the preservative, staining probe, and staining buffer into the sample pot.

Add the sample treated with CUBIC-L and sample wrapping reagent for staining.



Set the sample in a rotator. Rotate at a speed that maintains a relative top and bottom position inside the pot without the sample flipping.

*If the sample flips inside the pot, the top and bottom of the sample will always be upward/downward in the gravity direction, resulting in unequal staining.

After staining, remove the sample from the pot and wash with the wash buffer. Shake vigorously in the first wash to remove the sample wrapping reagent.

Conduct clearing with CUBIC-R after washing and post-fixation.

# 04 Frequently Asked Questions

## Questions on Staining Reagents, Antibodies, and Fluorescent Proteins

**Q** **What fluorescence reagents and stains can be used in CUBIC-HV™2 ?**

**A** When using antibodies, we recommend using antibodies that are directly fluorescently labeled with Alexa Fluor® dyes or FabuLight™ Fab fragment secondary antibodies from Jackson Immunolab. Regarding Alexa Fluor® dyes, AF488 is not compatible with CUBIC-R. Please use dyes such as FITC instead. In the CUBIC-HV protocol, we do not recommend using general IgG secondary antibodies, as staining the depths is not guaranteed. For nuclear staining, we recommend propidium iodide (PI), SYTOX™-Green, or RedDot™2.

**Q** **Can primary antibodies available in the lab be used with CUBIC-HV™2 ?**

**A** In the references, some proteins that have maintained their antigenicity before and after clearing. However, not all proteins have been confirmed, so consider the antibodies at hand before use.
To check if the antibodies are compatible, compare the stains of slices before and after processing with CUBIC-L.

**Q** **Can fluorescently labeled secondary antibodies be used after primary antibodies, similar to normal immunohistostaining?**

**A** For CUBIC-HV™2, the FabuLight™ Fab fragment secondary antibody reacts with the primary antibody to create a complex before 3D staining. We do not recommend staining 2D slices in the general two steps (primary antibody to secondary antibody) because staining of the depths is not guaranteed.

**Q** **What fluorescent proteins can be used?**

**A** Fluorescent signals are maintained for EGFP, EYFP, Venus, tdTomato, mCherry, and mKate2. For other fluorescent proteins, please check before use on your own.

## Questions on Clearing

**Q** **What types of containers should be used during clearing?**

**A** CUBIC tissue clearing is designed to improve transparency and microscope resolution by slightly expanding the tissue. Thus, we recommend using tubes, containers, etc. with a diameter wider than the tissue. For example, when a tube is filled halfway with the reagent, the sample should not come out of the liquid surface when the tube is laid on its side. CUBIC reagents are aqueous, so polypropylene and polyethylene containers can be used safely.

**Q** **Will the expansion of tissues affect the experiment?**

**A** Various cells may expand, but the physical relationship of the cells is maintained. The rigidness of the structure may vary depending on the tissue (nerves, veins, etc.) and may cause artifacts, so confirmation is required according to the situation.

**Q** **Is fixation still required even when clearing is done right after the organ is extracted from the animal?**

**A** CUBIC tissue clearing agents are optimized for PFA fixation. Lysis will occur on samples that are not fixed, so please fix the samples.

**Q** **Can samples be cleared even if some time has passed after dissection and fixation?**

**A** Samples that have been immersed in fixative for a long time (over several weeks) can still be cleared.
However, since the fixation condition is a parameter that greatly affects the transparency condition, we recommend maintaining the preparation condition and fixation period of the fixative as much as possible. In our protocol, we recommend washing with PBS within 24 hours after perfusion fixation of the animal, and promptly starting the CUBIC-L treatment.

## Questions on Clearing

### Q Can paraffin-embedded samples become transparent?

**A** Paraffin-embedded samples can become transparent after deparaffinization treatment. Please refer to the following treatment method for more details.

Reference CUBIC pathology: three-dimensional imaging for pathological diagnosis
S. Nojima et al., Sci. Rep. 2017, 7, 9269.
https://doi.org/10.1038/s41598-017-09117-0

### Q How much reagent is required for clearing?

**A** When making the whole mouse body transparent, the amount of reagent should be enough to immerse the whole body. For organs, choose a tube so that when the tube is filled halfway with the reagent, the whole organ is immersed when the tube is laid on its side. For example, for mouse brains, pour 10 to 15 mL of CUBIC-L into a 20 to 30 mL tube. The actual amount will vary according to the sample size and container used. As an example, 200 to 400 mL of CUBIC-L and 100 to 200 mL of CUBIC-R is required to clear the whole mouse body, and a total of 20 to 40 mL of CUBIC-L and 10 to 20 mL of CUBIC-R is required to clear each mouse organ (approx. $1cm^3$).

### Q What is the reason if clearing does not go well?

**A** It may be due to the following reasons. Please consider the measures below.

**a) The pH level of the PFA solution used in fixation is high**
When the pH level is over 8, overfixation occurs, and the organ becomes yellow, causing clearing to slow down. Please set the pH level around 7 to 7.5.
In addition, the transparency level differs depending on the PFA pH level and processing time. We recommend maintaining the fixation time and fixative preparation procedure as much as possible.

**b) Delipidation is not complete.**
Either extend the delipidation step or consider the exchange frequency of CUBIC-L. We recommend exchanging to a new CUBIC-L solution every two days and shaking at 37°C.

**c) Clearing is not complete.**
When moisture is added during CUBIC-R, the RI drops, and transparency becomes insufficient. Please exchange CUBIC-R.

## Questions on Clearing

**Q** How long does delipidation take?

**A** For adult mouse tissue, delipidation takes three to seven days. The length of delipidation depends on the organ.
Set the delipidation time according to the type and size of the organ, and experiment purpose. For example, complete transparency is required for light-sheet microscope observation, but less time and incomplete transparency are acceptable for partial observation using two-photon microscopes.

## Questions on After Clearing

**Q** What should we do with the liquid waste after the experiment?

**A** Dispose of the liquid waste according to the specified disposal method after consulting with the person in charge of waste in your institution. Furthermore, in general, samples and reagents used in the experiment should be disposed of as medical waste and infectious waste, and unused CUBIC disposed of as organic waste and fire-resistant waste because it has a high water content.

**Q** How should samples be stored after clearing?

**A** Samples can be stored at room temperature with the used CUBIC-R. Furthermore, the sample may harden if the water in the CUBIC-R solvent evaporates. Seal the container with parafilm, etc.
Samples can also be stored at room temperature in agarose gel. This method is more stable.
To archive, samples can be stored at 4°C inside the preservative-added PBS, such as sodium azide after washing CUBIC-R with PBS.

**【Agarose Gel Storing Procedure】**
In an appropriate tube, add 2% agarose gel to the CUBIC-R reagent used in the clearing. Heat and dissolve the solution. Insert the transparent sample into the agarose solution before it hardens. Cool enough to harden.

For more details, refer to the article or website listed below.

Website for reference: http://cubic.riken.jp/

Reference: Advanced CUBIC tissue clearing for whole-organ cell profiling
K. Matsumoto et al., Nat. Protoc. 2019, 14, 3506. https://doi.org/10.1038/s41596-019-0240-9

## Questions on After Clearing

**Q** **The transparent sample cannot be observed well.**

**A** We recommend observing with light-sheet microscopes for tissue clearing observation (LSFM), confocal laser scanning microscopes (CLSM) with objective lenses compatible with high RI, and two-photon microscopes. When observing, immerse the sample in the mounting agent for observation, and use objective lenses compatible with the RIs. In recent years, affordable light-sheet microscopy for tissue clearing is developed.

Reference: descSPIM: Affordable and Easy-to-Build Light-Sheet Microscopy for Tissue Clearing Technique Users
K. Otomo et al., bioRxiv. 2023 https://doi.org/10.1101/2023.05.02.539136

**Q** **What is the refractive index of the reagents?**

**A** The refractive index (RI) of CUBIC-R is 1.522. Avoid mixing solvents such as water with CUBIC-R to change the refractive index.

**Q** **Are CUBIC-L and CUBIC-R the same as CUBIC-1 and CUBIC-2 mentioned in the articles?**

**A** CUBIC-1 (Sca/eCUBIC-1, Reagent-1) and CUBIC-2 (Sca/eCUBIC-2, Reagent-2) are different from CUBIC-L and CUBIC-R. CUBIC-1 and CUBIC-2 are the first-generation CUBICs, and CUBIC-L and CUBIC-R are the improved second-generation CUBICs with much higher transparency.

For functions, CUBIC-1 shows the same delipidation and decoloration as CUBIC-L, and CUBIC-2 shows the same refractive index matching as CUBIC-R.

*Regarding the information above, clearing and staining results vary depending on the transparency sample, staining reagents, and devices used.
Please consider the appropriate processing time and reagent concentration level.

# 04 References

## Transparency Examples of the Clearing of the Mouse Body and Organs (Brain, Lungs, Liver, Limb, and Kidney), Marmoset Brain, and Human Organs (Brain, Kidney, Liver, and Lungs) (Immunostaining Protocol After Clearing with CUBIC)


Chemical Landscape for Tissue Clearing based on Hydrophilic Reagents
K. Tainaka, T. C. Murakami, E. A. Susaki, C. Shimizu, R. Saito, K. Takahashi, A. Hayashi-Takagi, H. Sekiya, Y. Arima, S. Nojima, M. Ikemura, T. Ushiku, Y. Shimizu, M. Murakami, K. F. Tanaka, M. Iino, H. Kasai, T. Sasaoka, K. Kobayashi, K. Miyazono, E. Morii, T. Isa, M. Fukayama, A. Kakita, H. R. Ueda, Cell Rep. 2018, 24, 2196. https://doi.org/10.1016/j.celrep.2018.07.056

Whole-Body Profiling of Cancer Metastasis with Single-Cell Resolution
S. I. Kubota, K. Takahashi, J. Mishida, Y. Morishita, S. Ehata, K. Tainaka, K. Miyazono, H. R. Ueda, Cell Rep. 2017, 20, 236. http://doi.org/10.1016/j.celrep.2017.06.010

Whole-Brain Imaging with Single-Cell Resolution Using Chemical Cocktails and Computational Analysis
E. A. Susaki, K. Tainaka, D. Perrin, F. Kishino, T. Tawara, T. M. Watanabe, C. Yokoyama, H. Onoe, M. Eguchi, S. Yamaguchi, T. Abe, H. Kiyonari, Y. Shimizu, A. Miyawaki, H. Yokota, H. R. Ueda, Cell 2014, 157, 726. http://doi.org/10.1016/j.cell.2014.03.042

Whole-Body Imaging with Single-Cell Resolution by Tissue Decolorization
K. Tainaka, S. I. Kubota, T. Q. Suyama, E. A. Susaki, D. Perrin, M. Ukai-Tadenuma, H. Ukai, H. R. Ueda, Cell 2014, 159, 911. http://doi.org/10.1016/j.cell.2014.10.034


## Application Example of CUBIC for Human Pathological Diagnosis


CUBIC pathology: three-dimensional imaging for pathological diagnosis
S. Nojima, E. A. Susaki, K. Yoshida, H. Takemoto, N. Tsujimura, S. Iijima, K. Takachi, Y. Nakahara, S. Tahara, K. Ohshima, M. Kurashige, Y. Hori, N. Wada, J. Ikeda, A. Kumanogoh, E. Morii, H. R. Ueda, Sci. Rep. 2017, 7, 9269. https://doi.org/10.1038/s41598-017-09117-0


## Whole Organ/Body 3D Staining and Observation with CUBIC-HistoVIsion (CUBIC-HV™ Kit Basic Protocol)


Versatile whole-organ/body staining and imaging based on electrolyte-gel properties of biological tissues
E. A. Susaki, C. Shimizu, A. Kuno, K. Tainaka, X. Li, K. Nishi, K. Morishima, H. Ono, K. L. Ode, Y. Saeki, K. Miyamichi, K. Isa, C. Yokoyama, H. Kitaura, M. Ikemura, T. Ushiku, Y. Shimizu, T. Saito, T. C. Saido, M. Fukayama, H. Onoe, K. Touhara, T. Isa, A. Kakita, M. Shibayama, H. R. Ueda, Nat. Commun. 2020, 11, 1982. https://doi.org/10.1038/s41467-020-15906-5


## Affordable Light-Sheet Microscopy for Tissue Clearing Observation


descSPIM: Affordable and Easy-to-Build Light-Sheet Microscopy for Tissue Clearing Technique Users
K. Otomo, T. Omura, Y. Nozawa, Y. Saito, E. A. Susaki
bioRxiv 2023. https://doi.org/10.1101/2023.05.02.539136


## Protocol Article on Tissue Clearing and Nuclear Staining


Advanced CUBIC tissue clearing for whole-organ cell profiling
Katsuhiko Matsumoto, Tomoki T. Mitani, Shuhei A. Horiguchi, Junichi Kaneshiro, Tatsuya C. Murakami, Tomoyuki Mano, Hiroshi Fujishima, Ayumu Konno, Tomonobu M. Watanabe, Hirokazu Hirai & Hiroki R. Ueda
Nature Protoc. 2019 https://doi.org/10.1038/s41596-019-0240-9

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