

New

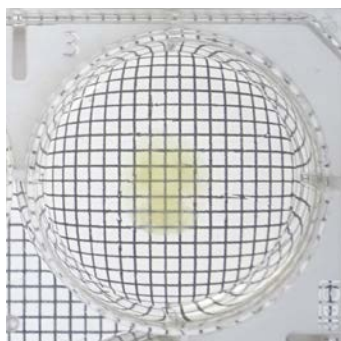
# Animal Tissue-Clearing Reagent CUBIC

## Products

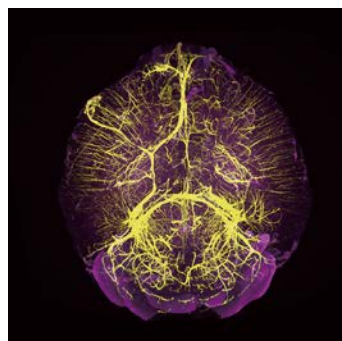
<b>CUBIC-L</b> (for delipidation and decoloring)	25mL / 100mL [T3740]
<b>CUBIC-R+(N)</b> (for RI matching)	25mL / 100mL / 500mL [T3983]
<b>CUBIC-R+(M)</b> (for RI matching)	25mL / 100mL [T3741]
<b>CUBIC-B</b> (for decalcification)	25mL / 100mL [T3780]
<b>CUBIC-HL</b> (for delipidation strongly and quenching autofluorescence)	25mL / 100mL [T3781]
<b>CUBIC-P</b> (with perfusion before tissue excision)	25mL / 100mL [T3782]
<b>CUBIC-X1</b> (for expansion)	25mL / 100mL [T3866]
<b>CUBIC-X2</b> (for RI matching with expansion)	25mL / 100mL [T3867]
<b>CUBIC-HV™1 3D immunostaining kit</b> (Casein separately)	1kit [C3717]
<b>CUBIC-HV™1 3D nuclear staining kit</b>	1kit [C3709]

## Related Products

<b>Mounting Solution (RI 1.520)</b> [for CUBIC-R+]	50mL [M3294]
<b>Mounting Solution (RI 1.467)</b> [for CUBIC-X2]	50mL [M3292]



Whole-brain clearing



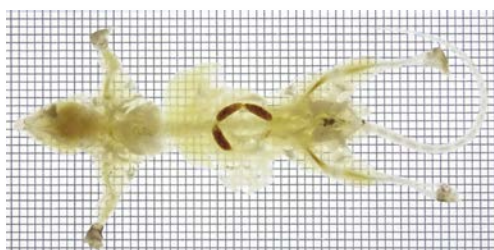
Whole-body clearing  
with nuclei staining and immunostaining

These products were developed by Prof. Hiroki R. Ueda (The University of Tokyo / RIKEN) and are under invention licenses by RIKEN, Japan.

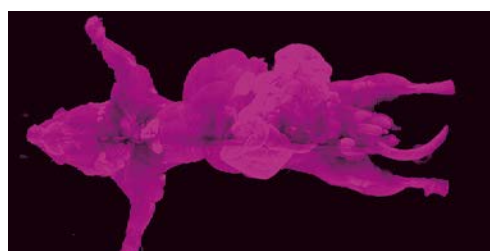
\*CUBIC-HV™ is a registered trademark of CUBICStars Co.

## Advantages

- **Basic protocol ;**  
Clearing of whole mouse bodies as well as animal organs can be achieved by using two reagents in sequence: CUBIC-L [T3740] for delipidation and either CUBIC-R+(N) [T3983] or CUBIC-R+(M) [T3741] for RI matching.
- **Optional protocol ;**  
The following products can easily clear tissues, such as bones or highly fatty tissues which were previously difficult to clear.  
CUBIC-B [T3780] for bone  
CUBIC-HL [T3781] for highly fatty tissues
- **For efficiently aiding with perfusion fixation for mouse perfusion ;**  
CUBIC-P [T3782]
- **Expansion protocol ;**  
The following products can clear tissues with expansion.  
CUBIC-X1 [T3866] for expansion tissues  
CUBIC-X2 [T3867] for RI matching with keeping the expanded size of tissues
- **For staining thick and large specimens uniformly ;**  
CUBIC-HV™1 3D immunostaining kit [C3717] for 3D immunostaining  
CUBIC-HV™1 3D nuclear staining kit [C3709] for 3D nuclear staining
- **Tissue expansion enables acquisition of images easy.**
- **Preserve the fluorescent protein signals except CUBIC-HL [T3781].**
- **Using light-sheet fluorescent microscopy (LSFM) or confocal laser-scanning microscopy (CLSM) enables the whole-organ / body imaging at a cellular resolution.**



Whole-body clearing



Whole-body clearing  
with propidium iodide staining

## Direction for Use : Mouse whole-organ clearing protocol

Fix 4% PFA 1 day	Wash x 3 PBS > 2 hr x 3	Delipidation 50% CUBIC-L 6 - 24 hr	Delipidation CUBIC-L > 2 days	Wash x 3 PBS > 2 hr x 3	(Staining) Stains > 3 days	(Wash x 3) PBS > 2 hr x 3	(Fixation) 1% FA 1 day	(Fixation) 1% FA 1 hr	(Wash x 3) PBS > 2 hr x 3	RI match 50% CUBIC-R+ 1 day	RI match CUBIC-R+ > 1 day
------------------------	-------------------------------	--	-------------------------------------	-------------------------------	----------------------------------	---------------------------------	------------------------------	-----------------------------	---------------------------------	-----------------------------------	---------------------------------

Process	Reagent	Temp.	Time	Notes
Tissue excision				After perfusion fixation
Tissue Fix	4% PFA in PBS	4°C	1 day	
Wash x 3	PBS	RT	> 2 hr x 3	Shake gently (Same in following steps). Total 1 day
(Delipidation)	50% CUBIC-L	37°C or RT	6 - 24 hr	1:1 mixture of water and CUBIC-L. Optional
Delipidation	CUBIC-L	37°C	> 2 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4.
Wash x 3	PBS	RT	> 2 hr x 3	Total 1 day
(Staining)	Stains	RT	> 3 days	Optional
(Wash x 3)	PBS	RT	> 2 hr x 3	Total 1 day, When stained
(Fixation)	1% FA	4°C	1 day	Diluted of 37% FA by PBS. When stained.
(Fixation)	1% FA	37°C	1 hr	When stained.
(Wash x 3)	PBS	RT	> 2 hr x 3	When stained.
RI match	*50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+
RI match	*CUBIC-R+	RT	> 1 day	

\*Both CUBIC-R+(N) [T3983] and CUBIC-R+(M) [T3741] can be used.

### Application

#### ● An adult mouse brain after excision



#### ● After pre-treatment step of 4 mL 50% CUBIC-L at room temperature overnight



#### ● After delipidation step of 4 mL CUBIC-L at 37°C for 5 days (Refresh CUBIC-L on day 1, day 2 and day 4)



#### ● After pre-treatment step of 4 mL CUBIC-R+(M) at room temperature overnight



#### ● Observation the sample in Mounting Solution (RI = 1.520) [M3294] after RI matching of 4 mL CUBIC-R+(M) at room temperature overnight



Each sample of these images is immersed in each reagent.

- Light penetrates the organ.
  - CUBIC-L does not get colored after treatment.
- Above points are the signs of end of delipidation.

Total

- CUBIC-L : 14 mL
- CUBIC-R+(M) : 6 mL

The reagent volumes of the left example is in the case of usage in a 5 mL-tube. Work in a tube whose diameter is a little larger than that of organs and the volume of reagents is half of that of tubes.

PFA : paraformaldehyde, RT : room temperature

# Animal Tissue-Clearing Reagent CUBIC

## Direction for Use : Mouse whole-brain clearing with expansion protocol

Fix 4% PFA 1 day	Wash x 3 PBS > 2 hr x 3	Delipidation 50% CUBIC-L 3 hr	Delipidation CUBIC-L 5 - 14 days	Wash PBS 1 day	Staining Stains 3 days	Wash PBS 1 day	Fixation 1% FA 1 day	Fixation 1% FA 1 hr	Wash x 3 PBS > 2 hr x 3	Swelling CUBIC-X1 2.5 days	RI match CUBIC-X2 1.5 days
------------------------	-------------------------------	-------------------------------------	--	----------------------	------------------------------	----------------------	----------------------------	---------------------------	-------------------------------	----------------------------------	----------------------------------

Process	Reagent	Temp.	Time	Notes
Tissue excision				After perfusion fixation
Tissue Fix	4% PFA in PBS	4°C	1 day	
Wash x 3	PBS	RT	> 2 hr x 3	Shake gently (Same in following steps). Total 1 day
Delipidation	50% CUBIC-L	37°C	3 hr	1:1 mixture of water and CUBIC-L.
Delipidation	CUBIC-L	37°C	5 - 14 days	Refresh CUBIC-L every 4 days. 5 days for 1-week-old mice 7 days for 3-week-old mice 14 days for 8-week-old and 6-month-old mice
Wash	PBS	RT	1 day	
Staining	Stains	RT	3 days	
Wash	PBS	RT	1 day	
Fixation	1% FA	4°C	1 day	Diluted of 37% FA by PBS.
Fixation	1% FA	37°C	1 hr	
Wash x 3	PBS	RT	> 2 hr x 3	
Swelling	CUBIC-X1	4°C	2.5 days	
RI match	CUBIC-X2	RT	1.5 days	Refresh CUBIC-X2 every 12 hours.

## Application

- Example of usage of mouse brain clearing and expansion
- Pre-treatment step of 3 mL 50% CUBIC-L at 37°C for 3 hours after PBS wash
- Delipidation step of 3 mL CUBIC-L at 37°C for 14 days  
(Refresh CUBIC-L on day 4, day 8 and day 12)
- Wash by PBS, staining by staining reagents and wash by PBS
- Expansion step of 30 mL CUBIC-X1 at 4°C for 2.5 days
- Observation the sample in Mounting Solution (RI = 1.467) [M3292] after RI matching of 40 mL CUBIC-X2 at room temperature for 1.5 days  
(Refresh CUBIC-X2 every 12 hours)

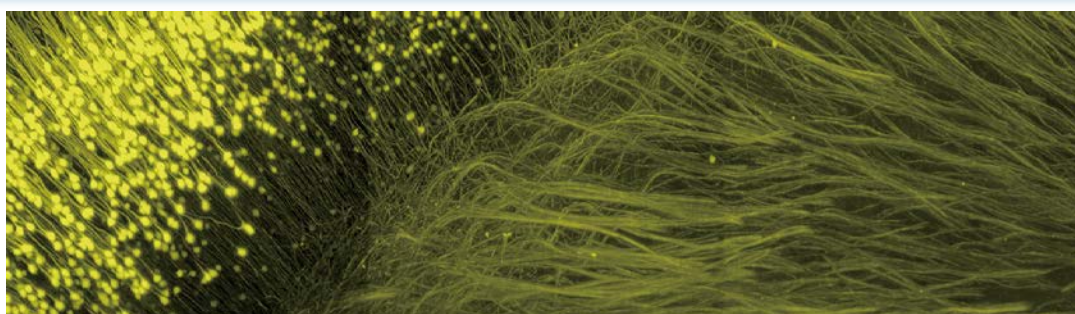
### Total

- CUBIC-L : 10.5 mL
- CUBIC-X1 : 30 mL
- CUBIC-X2 : 120 mL

\*For nuclear staining, use 30 µg/mL Propidium iodide (PI) and 1.5 M NaCl in PBS.

Since the expanded brains are fragile, careful handling is required after the swelling step.

PFA : paraformaldehyde, RT : room temperature



Magnified view of a transgenic mouse brain after clearing-expansion protocol

## 3D Tissue Staining

# 3D Tissue Staining Kits CUBIC-HV™

### Introduction

- Stain bulky specimens uniformly.  
(Includes two nuclear stains and an antibody control)
- CUBIC-L [T3740] and either CUBIC-R+(N) [T3983] or CUBIC-R+(M) [T3741] (sold separately) required for upstream / downstream sample processing.



### Kit Components

#### CUBIC-HV™1 3D immunostaining kit (Casein separately)

1 kit [C3717]

- 2 x Immunostaining Buffer (Casein separately) (for 10 tests)
- 1 x Immunostaining Washing Buffer (for 10 tests)
- 10 x Immunostaining Additive (for 10 tests)
- Anti NeuN Mouse IgG1 antibody (1mg/mL) (for 2 tests)
- Subdivided Casein (1 vial)
- 10 packs of 15mL tube

#### CUBIC-HV™1 3D nuclear staining kit

1 kit [C3709]

- 1 x 3D Nuclear Staining Buffer (for 10 tests)
- 100 x 3D nuclear staining washing buffer (for 10 tests)
- 200 x DAPI 2HCl (1mg/mL in Water) [for Cell Staining] (for 10 tests)
- 100 x Propidium Iodide (1mg/mL in Water) [for Cell Staining] (for 10 tests)
- 10 packs of 5mL tube

These volumes are for mouse adult brains. Components of the kits are subject to change without notice.

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

\*CUBIC-HV™ is a registered trademark of CUBICStars Co.

## References

### Using CUBIC-X1 and CUBIC-X2, Mouse Brain Expansion

A three-dimensional single-cell-resolution whole-brain atlas using CUBIC-X expansion microscopy and tissue clearing

T. C. Murakami, T. Mano, S. Saikawa, S. A. Horiguchi, D. Shigetani, K. Baba, H. Sekiya, Y. Shimizu, K. F. Tanaka, H. Kiyonari, M. Iino, H. Mochizuki, K. Tainaka, H. R. Ueda, *Nat. Neurosci.* **2018**, *21*, 625.

DOI: <https://doi.org/10.1038/s41593-018-0109-1>

### Using CUBIC-L, CUBIC-R+, CUBIC-B, CUBIC-HL, CUBIC-P, Mouse Whole Body, Brain, Lung, Liver, Leg, Kidney, Marmoset Brain, Human Brain, Kidney, Liver, Lung Clearing [Immunohistochemistry after CUBIC protocol]

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.

DOI: <https://doi.org/10.1016/j.celrep.2018.07.056>

### Mouse Whole Body, Brain, Lung Clearing

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.

DOI: <http://dx.doi.org/10.1016/j.celrep.2017.06.010>

### Mouse Brain, Marmoset Brain Clearing

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.

DOI: <http://dx.doi.org/10.1016/j.cell.2014.03.042>

### With CUBIC Perfusion, Mouse Whole Body, Heart, Lung, Kidney, Liver Clearing

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.

DOI: <http://dx.doi.org/10.1016/j.cell.2014.10.034>

### Application to Pathological Tissue 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.

DOI: <https://doi.org/10.1038/s41598-017-09117-0>

### 3D Tissue-staining and observation technique by CUBIC-HV™ kit

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. Saïdo, M. Fukayama, H. Onoe, K. Touhara, T. Isa, A. Kakita, M. Shibayama, H. R. Ueda, *Nat. Commun.* **2020**, *11*, 1982.

DOI: <https://doi.org/10.1038/s41467-020-15906-5>

Scan the QR code



or Access the URL <https://bit.ly/3wY11N3> for Technical Guidebook

Scan the QR code



or Access the URL <https://bit.ly/3wXw63t> for Q&A

For further information please refer to our website at [www.TCIchemicals.com](http://www.TCIchemicals.com). ▶▶▶

TCI CUBIC



### Ordering and Customer Service

#### TCI AMERICA

Tel : 800-423-8616 / 503-283-1681  
Fax : 888-520-1075 / 503-283-1987  
E-mail : [Sales-US@TCIchemicals.com](mailto:Sales-US@TCIchemicals.com)

#### TCI EUROPE N.V.

Tel : +32 (0)3 735 07 00  
Fax : +32 (0)3 735 07 01  
E-mail : [Sales-EU@TCIchemicals.com](mailto:Sales-EU@TCIchemicals.com)

#### TCI Deutschland GmbH

Tel : +49 (0)6196 64053-00  
Fax : +49 (0)6196 64053-01  
E-mail : [Sales-DE@TCIchemicals.com](mailto:Sales-DE@TCIchemicals.com)

#### Tokyo Chemical Industry UK Ltd.

Tel : +44 (0)1865 78 45 60  
E-mail : [Sales-UK@TCIchemicals.com](mailto:Sales-UK@TCIchemicals.com)

#### TCI Chemicals (India) Pvt. Ltd.

Tel : 1800 425 7889 / 044-2262 0909  
Fax : 044-2262 8902  
E-mail : [Sales-IN@TCIchemicals.com](mailto:Sales-IN@TCIchemicals.com)

#### 梯希爱(上海)化成工业发展有限公司

Tel : 800-988-0390 / 021-67121386  
Fax : 021-6712-1385  
E-mail : [Sales-CN@TCIchemicals.com](mailto:Sales-CN@TCIchemicals.com)

#### TOKYO CHEMICAL INDUSTRY CO., LTD.

Tel : +81 (0)3-5640-8878  
E-mail : [globalbusiness@TCIchemicals.com](mailto:globalbusiness@TCIchemicals.com)

Availability, price or specification of the listed products are subject to change without prior notice. Reproduction forbidden without the prior written consent of Tokyo Chemical Industry Co., Ltd.