TCI Product Feature

CUBIC Reagents

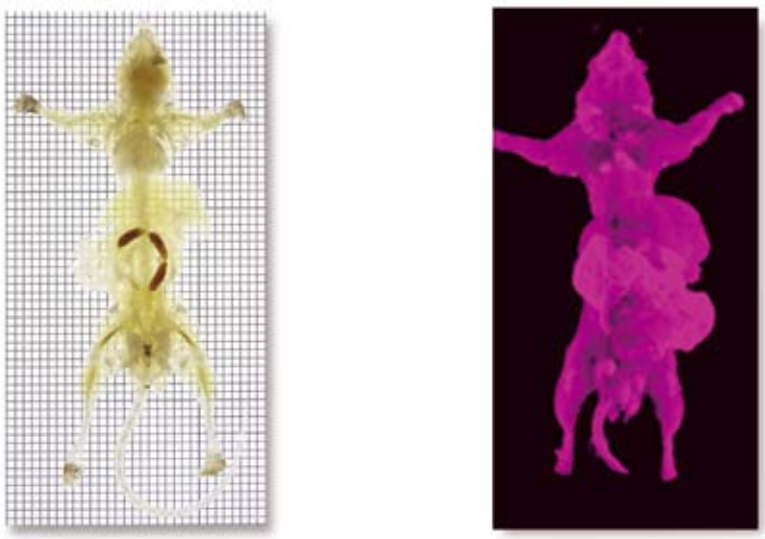
- for Animal Tissue, Whole-Organ and Whole-Body Clearing -

A tissue clearing method "CUBIC" has been developed by Prof. Hiroki R. Ueda and coworkers at The University of Tokyo / RIKEN. The CUBIC technique enables cyclopedic imaging at a single-cell resolution following whole-body and whole-organ clearing. CUBIC reagents (Product Number: T3740, T3741) that can be used in the tissue clearing method are provided by TCI.

● Advantages of clearing by CUBIC reagents

- Whole-body clearing is achieved using two reagents, T3740 CUBIC-L (for delipidation and decoloring) and T3741 CUBIC-R+ (for RI matching).
- The quenching of fluorescence signal is low.
- The period of sample treatment is shorter.
- The combination with light-sheet fluorescent microscopy (LSFM) or confocal laser-scanning microscopy (CLSM) enables the whole-organ / body imaging at a cellular resolution.

● Example : Mouse whole-body clearing



Fligure 1. Whole-body clearing (Left), Whole-body clearing with propidium iodide staining (Right)

Mouse whole-body clearing procedure

Pre-treatment	Delipidation	Wash x 3	Pre-treatment	RI match
50% CUBIC-L > 6 hr	CUBIC-L > 5 days	PBS > 2hr x 3	50% CUBIC-R+ 1 day	CUBIC-R+ > 1 day

Process	Reagent	Temp.	Time	Notes
Perfusion fixation	PBS 4% PFA in PBS			Finally, the mouse should be perfused with 50% CUBIC-L which is a 1:1 mixture of water and CUBIC-L.
Perfusion	PBS 50% CUBIC-L			
Pre-treatment	50% CUBIC-L	37℃	> 6 hr	Immerse the whole body of the mouse with gentle shaking (same in following steps). This step can be omitted.
Delipidation	CUBIC-L	37℃	> 5 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 1 day	

Work samples in a tube in which whole-body can be contained.
PFA : paraformaldehyde, RT : room temperature

Mouse whole-body clearing procedure for staining

Example : nuclear staining by propidium iodide (PI)

Pre-treatment	Delipidation / Staining	Wash x 3	Pre-treatment	RI match
50% CUBIC-L > 6 hr	PI in CUBIC-L > 7 days	PBS > 2hr x 3	50% CUBIC-R+ 1 day	CUBIC-R+ > 1 day

Process	Reagent	Temp.	Time	Notes
Perfusion fixation	PBS 4% PFA in PBS			Finally, the mouse should be perfused with 50% CUBIC-L which is a 1:1 mixture of water and CUBIC-L.
Perfusion	PBS 50% CUBIC-L			
Pre-treatment	50% CUBIC-L	37℃	> 6 hr	Immerse the whole body of the mouse with gentle shaking (same in following steps). This step can be omitted.
Delipidation / Staining	5 μg/mL PI in CUBIC-L	37℃	> 7 days	Refresh PI CUBIC-L on day 1, day 2, and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 1 day	

Example : nuclear staining by RedDot2

Pre-treatment	Delipidation	Wash	Staining	Wash	Pre-treatment	RI match
50% CUBIC-L > 6 hr	in CUBIC-L > 5 days	PBS > 2hr x 3	RedDot2 in PBS > 3 days	PBS > 2hr x 3	50% CUBIC-R+ 1 day	CUBIC-R+ > 1 day

Process	Reagent	Temp.	Time	Notes
Perfusion fixation	PBS 4% PFA in PBS			Finally, the mouse should be perfused with 50% CUBIC-L which is 1:1 mixture of water and CUBIC-L.
Perfusion	PBS 50% CUBIC-L			
Pre-treatment	50% CUBIC-L	37℃	> 6 hr	Immerse the whole body of the mouse with gentle shaking (same in following steps). This step can be omitted.
Delipidation	CUBIC-L	37℃	> 5 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash	PBS	RT	> 2hr x 3	Total 1 day
Staining	1 : 100 diluted RedDot2 in PBS*	RT	> 3 days	*Comprised of 0.5% Triton X-100 and 0.25% casein
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 1 day	

● Example : Mouse whole-organ clearing

Figure 2. Whole-brain clearing (Left), Whole-brain clearing with RedDot 2 staining and immunostaining (Right)

Mouse whole-organ clearing procedure

Fix	Wash x 3	Pre-treatment	Delipidation	Wash x 3	Pre-treatment	RI match
4% PFA 1 day	PBS > 2hr x 3	50% CUBIC-L 6 – 24 hr	CUBIC-L > 2 days	PBS > 2hr x 3	50% CUBIC-R+ 6 – 24 hr	CUBIC-R+ > 2 days

Process	Reagent	Temp.	Time	Notes
Tissue excision				After perfusion fixation
Tissue Fix	4% PFA in PBS	4℃	1 day	
Wash x 3	PBS	RT	> 2hr x 3	Shake gently (same in following steps). Total 1 day
Pre-treatment	50% CUBIC-L	37℃	6 – 24 hr	1:1 mixture of water and CUBIC-L This step can be omitted.
Delipidation	CUBIC-L	37℃	> 2 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	6 – 24 hr	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 2 days	

Work in a tube whose diameter is a little larger than that of organs. Appropriate liquid volume is essential as most of the organs are immersed in the liquid when the tube is in a horizontal position.

Mouse whole-organ clearing procedure for staining

Example : immunostaining

Fix	Wash x 3	Pre-treatment	Delipidation	Wash x 3	Staining	Wash x 3	Pre-treatment	RI match
4% PFA 1 day	PBS > 2hr x 3	50% CUBIC-L 6 – 24 hr	CUBIC-L > 2 days	PBS > 2hr x 3	antibodies > 3 days	PBS > 2hr x 3	50% CUBIC-R+ 6 – 24 hr	CUBIC-R+ > 2 days

Process	Reagent	Temp.	Time	Notes
Tissue excision				After perfusion fixation
Tissue Fix	4% PFA in PBS	4℃	1 day	
Wash x 3	PBS	RT	> 2hr x 3	Shake gently (same in following steps). Total 1 day
Pre-treatment	50% CUBIC-L	37℃	6 – 24 hr	1:1 mixture of water and CUBIC-L This step can be omitted.
Delipidation	CUBIC-L	37℃	> 2 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Staining	Antibody*1 in PBS*2	RT	> 3 days	*1 Use the fluorescent labeled antibody as a primary antibody. *2 Comprised of 0.5% Triton X-100, 0.25% casein and 0.01% sodium azide.
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	6 – 24 hr	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 2 days	

The staining protocol is not yet optimized completely. Please follow the latest publications.

References

1) 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 Reports* **2017**, *20*, 236.
2) 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.
3) 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.
4) RIKEN Quantitative Biology Center, CUBIC protocol and etc.
http://cubic.riken.jp/

The pictures are provided by Prof. Hiroki R. Ueda.

CUBIC Reagents

T3740	Tissue-Clearing Reagent CUBIC-L [for Animals]	25mL 100mL
T3741	Tissue-Clearing Reagent CUBIC-R+ [for Animals]	25mL 100mL

These products are under invention licenses by RIKEN, Japan.
Both of CUBIC-L and CUBIC-R+ are required for tissue-clearing.