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LIFE SCIENCE

Animal Tissue-Clearing Reagent CUBIC

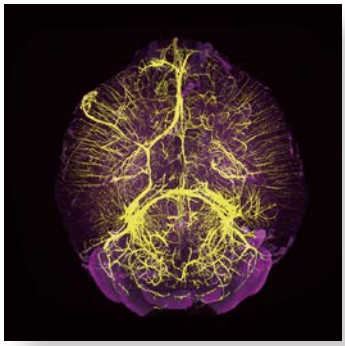
Products

CUBIC trial kit (including mounting solution) ; All-in-One basic tissue clearing kit containing CUBIC-L (50mL), CUBIC-R+(M) (50mL), and RI-Matched Mounting Solution (RI = 1.520, 50mL)	1kit	[C3942]
CUBIC-L (for delipidation and decoloring)	25mL / 100mL / 500mL	[T3740]
CUBIC-R+(N) (for RI matching)	25mL / 100mL / 500mL	[T3983]
CUBIC-R+(M) (for RI matching)	25mL / 100mL	[T3741]
CUBIC-B (for decalcification)	25mL / 100mL	[T3780]
CUBIC-HL (for delipidation strongly and quenching autofluorescence)	25mL / 100mL	[T3781]
CUBIC-P (with perfusion before tissue excision)	25mL / 100mL	[T3782]
CUBIC-X1 (for expansion)	25mL / 100mL	[T3866]
CUBIC-X2 (for RI matching with expansion)	25mL / 100mL	[T3867]
CUBIC-HV™1 3D immunostaining kit (Casein separately)	1kit	[C3717]
CUBIC-HV™1 3D nuclear staining kit	1kit	[C3709]

Related Products

Mounting Solution (RI 1.520) [for CUBIC-R+]	50mL	[M3294]
Mounting Solution (RI 1.467) [for CUBIC-X2]	50mL	[M3292]
Mouse Anti-NeuN Monoclonal Antibody	0.1mg/vial	[M3586]
Goat Anti-Mouse IgG$_1$ Fab Fragment Cyanine 3 Conjugate	0.05mg/vial	[G0598]

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.
  The difference between CUBIC-R+(N) [T3983] and CUBIC-R+(M) [T3741]:
  CUBIC-R+(N) is inexpensive and easier to handle because it raises less precipitation.
  The fluorescence signal may decay, but the fluorescence signals of samples in CUBIC-R+(N) can be observed for several days after immersion. CUBIC-R+(M) is superior in retaining the fluorescence signal. However, at low temperatures such as in winter, it may precipitate. In that case, it can be resolved by placing the sample at 37°C for a few days.
  For these reasons, it is recommended to try CUBIC-R+(N) first and then use CUBIC-R+(M) if fluorescence signal cannot be found.

- **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]**

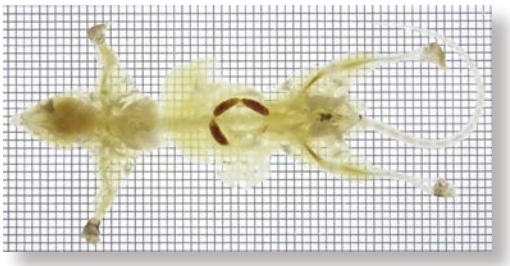
- **Expansion protocol ;**
  The following products can clear tissues with expansion.
  CUBIC-X1 **[T3866]** for expansion tissues
  CUBIC-X2 **[T3867]** for RI matching with keeping the expanded size of tissues

- **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

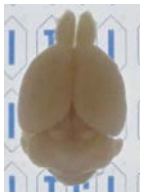
Fix	Wash x 3	Delipidation	Delipidation	Wash x 3	(Staining)	(Wash x 3)	(Fixation)	(Fixation)	(Wash x 3)	RI match	RI match
4% PFA 1 day	PBS > 2 hr x 3	50% CUBIC-L 6 – 24 hr	CUBIC-L > 2 days	PBS > 2 hr x 3	Stains > 3 days	PBS > 2 hr x 3	1% FA 1 day	1% FA 1 hr	PBS > 2 hr x 3	50% CUBIC-R+ 1 day	CUBIC-R+ > 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	Wash x 3	Delipidation	Delipidation	Wash	Staining	Wash	Fixation	Fixation	Wash x 3	Swelling	RI match
4% PFA 1 day	PBS > 2 hr x 3	50% CUBIC-L 3 hr	CUBIC-L 5 - 14 days	PBS 1 day	Stains 3 days	PBS 1 day	1% FA 1 day	1% FA 1 hr	PBS > 2 hr x 3	CUBIC-X1 2.5 days	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.

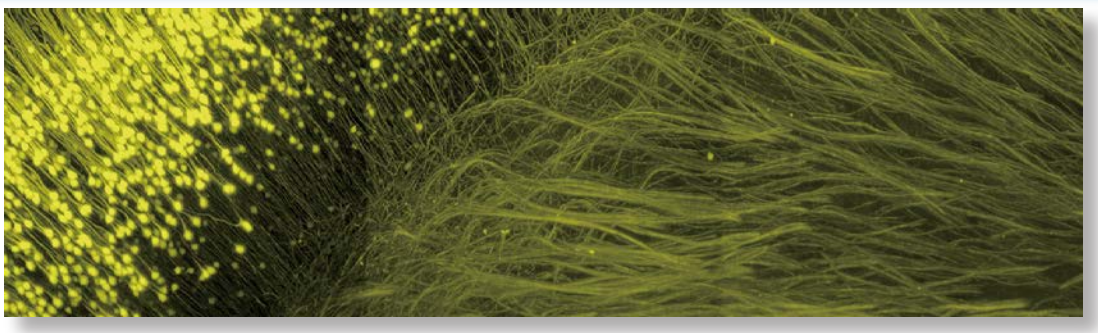
Application

- **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



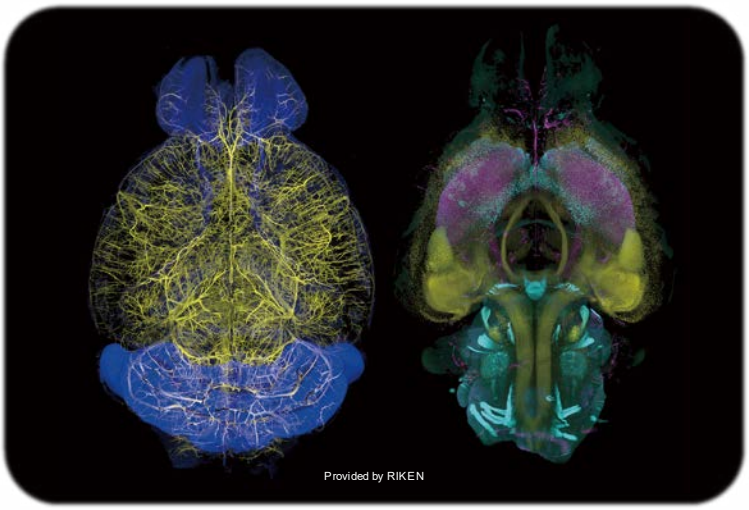
Magnified view of a transgenic mouse brain after clearing-expansion protocol

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) 1kit [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 1kit [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.

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