



## Tissue-Clearing Reagent CUBIC-L [for Animals]

### Product Information

Product No. : T3740

### Description

This product is supplied as a ready-to-use solution for animal tissue-clearing. Tissue Clearing Reagent CUBIC-R+ (Product No. T3741) is also required for a series of procedures.

### Directions for Use

#### Mouse whole-organ clearing protocol (after perfusion fixation, tissue excision)

Process	Reagent	Temp.	Time	Notes
Tissue fixation	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
(Pre-treatment)	50% CUBIC-L	37 °C	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. Optional.
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+.
RI matching	CUBIC-R+	RT	> 1 day	

\*In case of antibody, use the fluorescent labeled antibody as a primary antibody. For nucleus staining, use 10 µg/mL Propidium iodide (PI) in 0.1 M Phosphate buffer (PB) pH 7.4 containing 0.5 M NaCl.

## Mouse whole-body clearing protocol

Process	Reagent	Temp.	Time	Notes
Perfusion fixation	PBS			Finally, the mouse needs to be perfused with 50% CUBIC-L which is a 1:1 mixture of water and CUBIC-L.
	4% PFA in PBS			
Perfusion	PBS			
	50% CUBIC-L			
(Pre-treatment)	50% CUBIC-L	RT	> 6 hr	Immerse the whole body of the mouse with gentle shaking (same in following steps). Optional.
Delipidation	CUBIC-L	37 °C	> 5 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. Optional.
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+.
RI matching	CUBIC-R+	RT	> 2 days	

\*In case of antibody, use the fluorescent labeled antibody as a primary antibody.  
For nucleus staining, use 10 µg/mL Propidium iodide (PI) in 0.1 M Phosphate buffer (PB) pH 7.4 containing 0.5 M NaCl.

The above protocol is mentioned as examples. Please adjust the experimental conditions according to samples and purposes of the experiment.

### Precautions

Precipitations sometimes arise, but they do not affect the quality of clearing.  
Samples may swell but detailed structures are preserved.

### Storage

The product can be stored at room temperature.

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