



Tissue-Clearing Reagent CUBIC-R+ [for Animals]

Product Information

Product No. : T3741

Description

This product is supplied as a ready-to-use solution for animal tissue-clearing. Tissue Clearing Reagent CUBIC-L (Product No. T3740) 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.