CUBIC Tissue Clearing Protocol

Materials and Reagents

Urea: Fisher Bioreagents, Cat# BP169-500
N, N, N', N'-tetrakis (2-hydroxypropyl) ethylenediamine: Sigma-Aldrich, Cat# 122262-1L
polyethylene glycol mono-p-isooctylphenyl ether/Triton X-100: MP, Cat# 194854
Sucrose: Sigma-Aldrich, Cat# S7903-1KG
Triethanolamine (2,2`,2``-nitrilotriethanol): Sigma, Cat# 90279-500ML

CUBIC-Reagent I (Up to 1 month shelf live)

25 wt%	Urea
25 wt%	N,N,N',N'-tetrakis (2-hydroxypropyl) ethylenediamine
15 wt%	Triton X-100

Note: N, N, N', N'-tetrakis(2-hydroxypropyl) ethylenediamine is dense, warm up to 40-50 degrees before use to make it more liquid.

CUBIC-Reagent II (Up to 1 month shelf live)

50 wt%	Sucrose
25wt%	Urea
10 wt%	Triethanolamine
0.1% (v/v)	Triton X-100

Carrier Solution

1 X	PBS
0.3 %	Triton X-100
0.5 %	BSA
1 %	Goat Serum
0.05 %	Sodium Azide (when necessary)

Whole Brain Clearing

1. Perfuse mouse transcardially with 4% PFA/PBS, post-fix dissected mouse brain 16-24 hrs at 4 degree
2. Wash post-fixed mouse brain with PBS 3 times with gentle shaking, 1 hr each time
3. Immerse mouse brain in 10 ml CUBIC-Reagent I at 37degree with gentle shaking for 28 days, change CUBIC-Reagent I every 3 days
4. Wash mouse brain with PBS 3 times at room temperature, >2 hrs each time while gently shaking
5. Immerse mouse brain in CUBIC-Reagent II for 7 days, change CUBIC-Reagent II every 2 days
6. Cleared brain can be kept in CUBIC-Reagent II at room temperature

300 μm Brain Sections CUBIC Clearing with Staining

1. Fix 300 μm Brain Sections in 4%PFA/PBS at 4 degree 16-18 hrs
2. Wash sections with PBS 3 times with gentle shaking, 1 hr each time
3. Immerse sections in CUBIC-Reagent I at room temperature with gentle shaking for 3 days, change CUBIC-Reagent I every day
4. Wash sections with PBS 3 times at room temperature, 2 hrs each time while gently shaking
5. Immerse sections in carrier solution >3h at room temperature while gently shaking
6. Immerse sections in primary antibodies diluted in carrier solution for 2-3 days at room temperature or 6-7 days at 4 degree with gentle shaking
7. Wash sections with PBS 3 times at room temperature, 2 hrs each time while gently shaking
8. Immerse sections in secondary antibodies + DAPI (600 nM) diluted in carrier solution for 2-3 days at room temperature or 6-7 days at 4 degree with gentle shaking
9. Wash sections with PBS 3 times at room temperature, >2 hrs or overnight each time while gently shaking (note: the longer the better)
10. Immerse sections in CUBIC-Reagent II at room temperature with gentle shaking for 1 day before imaging
11. Mount in 300μm imaging gaskets, load coverslips, imaging

Note: Replace PBS with PB (PH 7.6) when necessary
Incubating time in each step could be adjustable

Reference:

Susaki EA, Tainaka K, Perrin D, Yukinaga H, Kuno A, Ueda HR.
Advanced CUBIC protocols for whole-brain and whole-body clearing and imaging.
Nat Protoc. 2015 Nov;10(11):1709-27.

Matsumoto K, Mitani TT, Horiguchi SA, Kaneshiro J, Murakami TC, Mano T, Fujishima H, Konno A, Watanabe TM, Hirai H, Ueda HR. Advanced CUBIC tissue clearing for whole-organ cell profiling. Nat Protoc. 2019 Dec;14(12):3506-3537.