ScaleA2/U2/B4 Tissue Clearing

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PROCEDURE FOR PREPARATION

REFERENCE: *Hama H, Kurokawa H, Kawano H, Ando R, Shimogori T, Noda H, Fukami K, Sakaue-Sawano A, Miyawaki A. Scale: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain. Nature Neuroscience, 2011.*

http://www.nature.com.ezproxyhost.library.tmc.edu/neuro/journal/v14/n11/full/nn.2928.html#supplementary-information
http://www.labome.com/method/Optical-Clearing-of-Biological-Tissue-with-ScaleA2.html

- Use Sca*l*e at a ratio of 20 ml per 0.5 g tissue for effective clearing
- Sca*l*eA2 takes days or weeks to clear large specimens
- Sca*l*eU2 takes weeks or months.
- The clearing process could be accelerated by a transient tissue expansion induced by 8 M urea. As an added benefit, treating with ScaleB4 effectively depletes biological samples of background signals.

RAPID PROTOCOL

- Fixed biological samples are incubated in Sca*l*eA2 for 2 days
- Followed by 2 days in Sca*l*eB2 containing 8 M urea and 0.1% Triton X-100
- and finally incubation in Sca*l*eA2 or Sca*l*eU2 solution.

Stock solutions Required

10% (wt/vol) Triton X-100 solution
i) Dissolve 10 g of Triton X-100 (e.g., Nacalai Tesque, Code 355-01 or similar grade) in 80 ml of Milli-Q water by stirring.
ii) Add Milli-Q water to make 100 ml and stir until well mixed.
iii) Store at 4 ºC.

1. **Sca*l*eA2 solution** (1 liter)
4 M urea
0.1% (wt/vol) Triton X-100
10% (wt/wt) glycerol
i) Dissolve 240.24 g of urea crystals (e.g., Wako Chemical, Code 217-615 or similar grade) in 800 ml of Milli-Q water by stirring.
ii) Add 10 ml of 10% (wt/vol) Triton X-100 solution.
iii) Add 100 g of glycerol (e.g., Sigma, Code 191612 or similar grade).

iv) Mix well by stirring.
v) Add Milli-Q water to make 1,000 ml and stir until well mixed.
vi) Store at room temperature.

*ScaleA2 causes expansion of tissue
**ScaleA2 has a pH of 7.7 and refractive indices of 1.382, 1.387 and 1.380 at 589, 486 and 656 nm

2. **Sca*l*eB4 solution** (1 liter)
8 M urea
0.1% (wt/vol) Triton X-100
i) Dissolve 480.48 g of urea crystals in 800 ml of Milli-Q water by stirring.
ii) Add 10 ml of 10% (wt/vol) Triton X-100 solution.
iii) Add Milli-Q water to make 1,000 ml and stir until well mixed.
iv) Store at room temperature.

*pH of a ScaleB4 solution is 8.7, not sure of RI

3. **Sca*l*eU2 solution** (1 liter)v 4 M urea
0.1% (wt/vol) Triton X-100
30% (wt/wt) glycerol
i) Dissolve 480.48 g of urea crystals in 800 ml of Milli-Q water by stirring.
ii) Add 10 ml of 10% (wt/vol) Triton X-100 solution.
iii) Add Milli-Q water to make 1,000 ml and stir until well mixed.
iv) Store at room temperature.

*Sca*l*eU2 requires longer incubation times to achieve clearing, but it reduces tissue expansion and the fragili
brain samples and is therefore suitable for soft samples such as mouse embryos. Unsure of pH or RI.

Notes
a. Allow each solution to stand after preparation for at least 1 day prior to use.
b. Sterilization is not required.
c. Addition of preservative is not required

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title=ScaleA2/U2/B4_Tissue_Clearing&oldid=8167"

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