3D Imaging Kit for Mouse Brain

Dream technology: optimized solution for clearing tissue without damage

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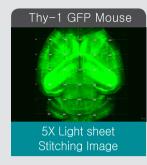


1	Fixing Solution	40 ml
2	Tissue Clearing Solution I	6 mL
3	Tissue Clearing Solution II	6 mL
4	Washing Solution	400 ml
5	Blotting Solution	20 ml
6	Mounting Solution I	10 ml
7	Mounting Solution II	10 ml
8	Image Chamber	1 ea

A WEEK FOR ALL CLEARING AND IMAGING









After washing step, the sample is exposed tolounting solution I

and incubated at 35°C/50rpm for 12 hours in the shaking incubator.

The sample is transferred to Mounting solution II and incubated

at 35℃/50rpm for 24hours in the shaking incubator.

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Fixing	Tiss	sue Clearing	Washin	g Immuno Staining Mour	nting		
1 day	2 day	3 day	4 day	5 day	6 day	imaging!!	7 day
ubated at4℃/50	ple fixed with4% PFA rpm in the shaking in ed sample is transfer	cubator until it is dep	Solution and incub	ated at35℃/50rpm			
		ssue Clearing Solutio	n lland react the sai	me way.			

After washing step

OPPOSITION

★ Preparation

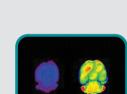
Pre-incubation of Tissue Clearing Solution (I, II) and Mounting Solution (I, II)

Fixation of transcardial perdusion sample at 4 °C over 12 hours using 4 %

- -Immerse the primary antibody in blotting solution (1/100-500 dilution) and incubated at 4°C/1000rpm for 72 hours in the centrifugal incubator. Do this a total of 2 times.
- -The primary antibody is transferred towashing solution
- and incubated for6 hours in the shaking incubator. Do this a total of 2 times.
- -Immerse the secondary antibody in blotting solution (1/100-500 dilution) and incubated at 4°C/1000rpm for 72 hours in the centrifugal incubator
- for 6 hours in the shaking incubator. Do this a total of 2 times.

at 35 ℃ for 1 hour to clear bottom of bottles.

Paraformaldehyde (PFA) solution









- 1. Put 4% PFA fixed sample in the Fixing solution and incubate at 4 °C, 50 rpm shaker to be deposited.
- 2. Put deposited sample in Tissue Clearing Solution I and incubate at 35 °C, 50 rpm shaker for 36 hours. After the first incubation repeat this step in Tissue Clearing Solution II.*
- 3. Add 50 ml of Washing Solution to sample and incubate at 4 °C, 50 rpm shaker for 4 hours.
- Repeat this step 3 times.* 4. Put washed sample in Mounting Solution I and incubate at 35 °C, 50 rpm shaker for 12 hours.
- 5. After mounting, centrifuge sample at 1200 rpm for 30 minutes and remove bubbles.
- 6. Put sample in Mounting Solution II and incubate at 35 °C, 50 rpm shaker for 24 hours.

8. After checking image, sample can be stored in image chamber or 1X PBS at 4 °C

7. Put transparent sample in Image Chamber and close a lid avoiding bubble.





* Cautions

Kit must be stored at 4 ℃ Opened bottle must be used within 1 month

- *Step 2, in case of whole brain Axon bundle can be found but proceed to washing step.
- *Step 3, if step 2 and 3 are repeated again you can achieve more transparent sample.



★ Immuno staining

After washing,

- 4-1 Centrifugal incubation at 4 ℃, 1000 rpm for 3 days in 1:100~1:500 primary antibody diluted with Blotting Solution. Repeat this step twice.
- 4-2 Add washing solution to sample and shake it for 6 hours. Repeat this step twice.
- 4-3 Centrifugal incubation at 4 °C, 1000 rpm for 3 days in 1:100~1:500 secondary antibody diluted with Blotting Solution.
- 4-4 Add washing solution and shake at 4 °C for 6 hours. Repeat this step twice.
- 4-5 Continue the beginning of step 4





