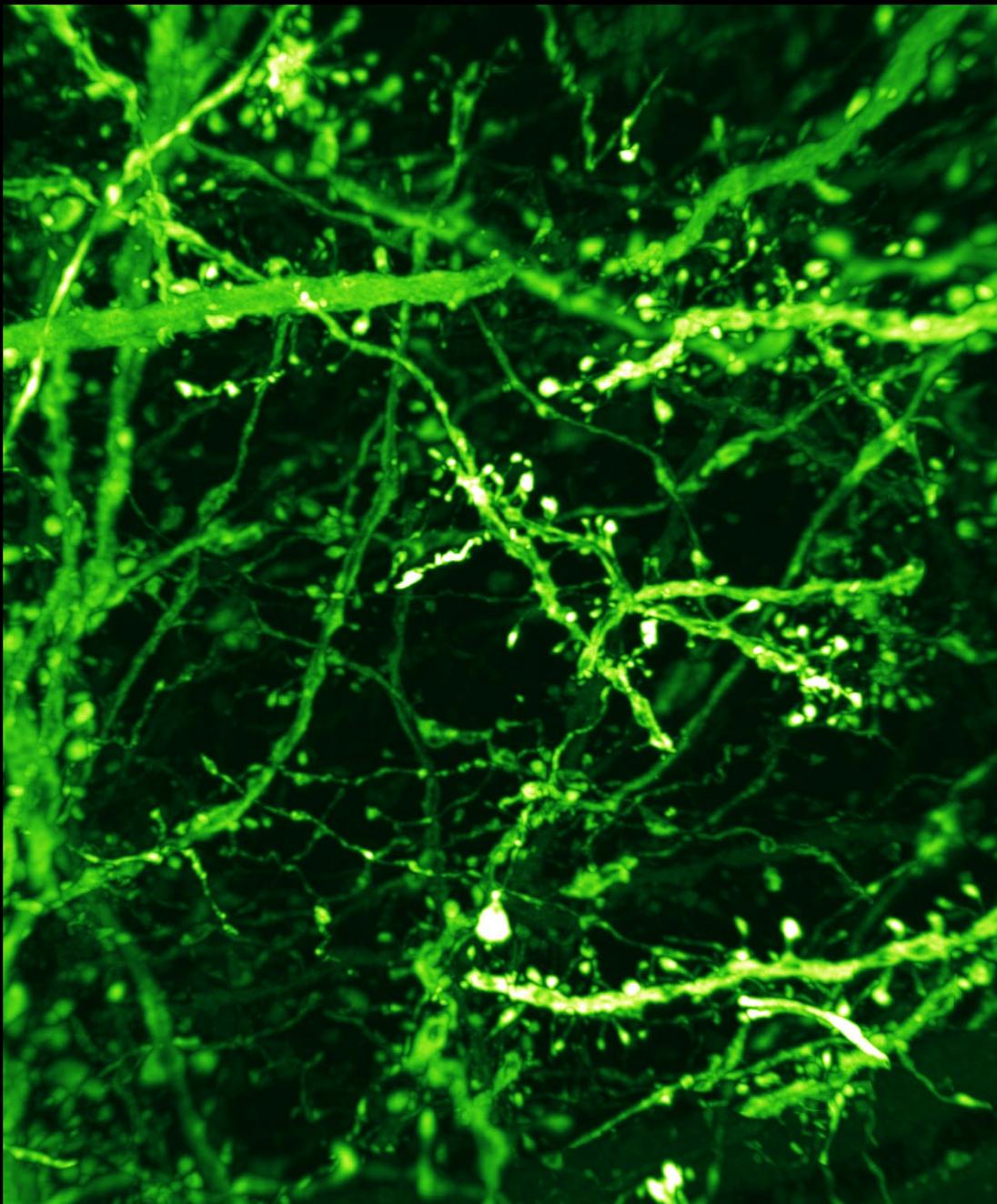


Tissue Optical Clearing Reagent

High-resolution imaging in deep tissue

SeeDB2



SeeDB2 Trial Kit Summary

Drs. Ke and Imai developed a new type of tissue clearing agent, SeeDB2. SeeDB2 is ideal for high-resolution three-dimensional imaging of fluorescent proteins. SeeDB2G and S are designed match the refractive indices of glycerol (1.46) and oil (1.52), minimizing spherical aberrations for high-NA glycerol- and oil- immersion objective lenses, respectively. SeeDB2 is particularly powerful in confocal and super-resolution microscopy using high-NA objective lenses. It is noteworthy that many of fluorescent proteins are highly preserved in SeeDB2, much better than in PBS or other commercialized mounting media optimized for fluorescent dyes. Thus, SeeDB2 is also an ideal mounting medium for samples labeled with fluorescent proteins. SeeDB2 is useful not only for thick brain tissues, but also for thin samples for cell biology or tissue sections.

Benefits of SeeDB2

- Easy-to-use
- High-resolution imaging
- No special equipment required
- Not quench fluorescent proteins
- Compatible with IF, FP and other fluorescent labels

SeeDB2 Applications

	Fixation	Clearing					Imaging
	Fixation 4%PFA/PBS(-)	Step 1. Permeabilization solution	Step 2. Clearing Solution 1	Step 3. Clearing Solution 2	Step 4. Clearing Solution 3 (SeeDB2G)	Step 5. Clearing Solution 4 (SeeDB2S)	Mounting: SeeDB2G or SeeDB2S
Processing temperature	4 °C	RT	RT	RT	RT	RT	RT
Processing time	1 day	12 - 16 hrs	6 - 24 hrs	6 - 10 hrs	> 12 hrs	> 12 hrs	



Figure 1. Mouse brain slice (adult, 1.5mm-thick) before and after clearing with SeeDB2.

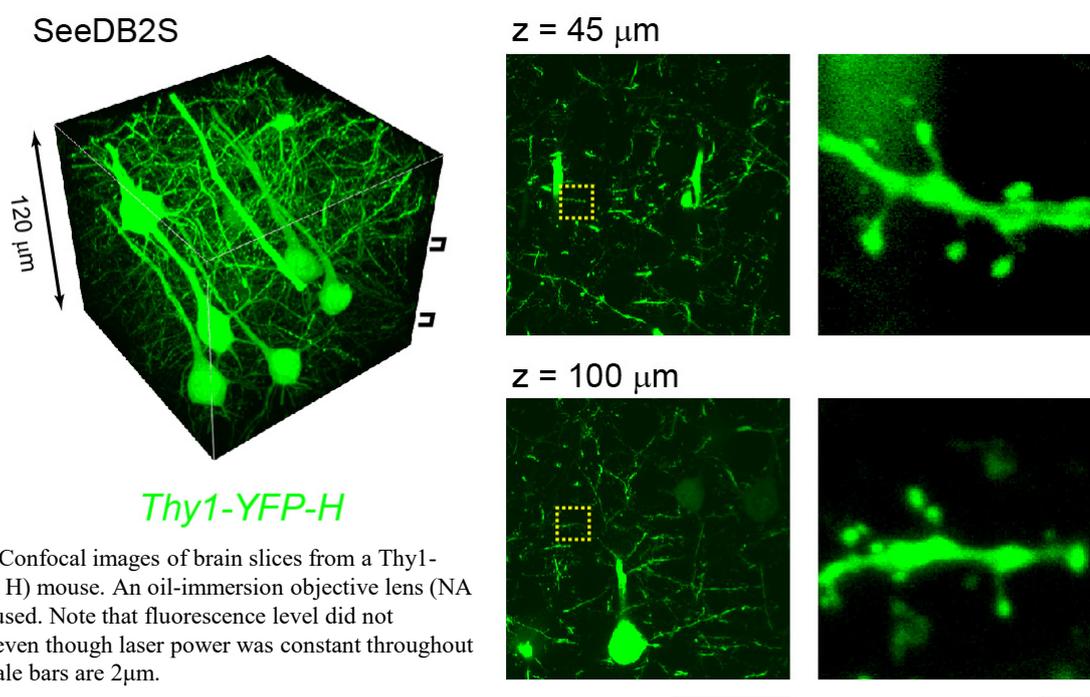


Figure 2. Confocal images of brain slices from a Thy1-YFP (line H) mouse. An oil-immersion objective lens (NA 1.4) was used. Note that fluorescence level did not decrease even though laser power was constant throughout depth. Scale bars are 2µm.

【Related product】

SeeDB Trial Kit Summary

Dr. Takeshi Imai, et al. developed a water-based optical clearing reagent, SeeDB (See Deep Brain), which clears fixed brain samples in a few days without quenching many types of fluorescent dyes, including fluorescent proteins and lipophilic neuronal tracers. SeeDB is a saturated solution of fructose in water with thioglycerol.

This method facilitates comprehensive and quantitative analyses for understanding neuronal circuitry, both in the adult and developing mouse brain.

Benefits of SeeDB

- **Easy-to-use**
- **No special equipment required**
- **Plasma membrane and ultrastructure remain intact**
- **Compatible with IF, FP and other fluorescent labels**

SeeDB applications

	Fixation	Clearing						Imaging
	Fixation 4%PFA/PBS(-)	Step 1. SeeDB:20w/v% Fructose Solution	Step 2. SeeDB:40w/v% Fructose Solution	Step 3. SeeDB:60w/v% Fructose Solution	Step 4. SeeDB:80w/v% Fructose Solution	Step 5. SeeDB:100w/v% Fructose Solution	Step 6. SeeDB	Mounting: SeeDB
Processing temperature	4 °C	RT	RT	RT	RT	RT	RT	RT
Processing time	1 day	4 - 8 hrs	4 - 8 hrs	4 - 8 hrs	> 12 hrs	> 12 hrs	24 hrs	

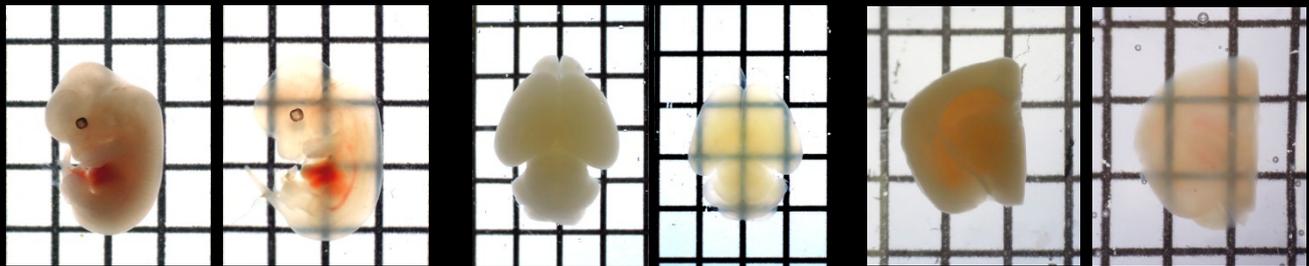


Figure.1 Mouse embryo and mouse brain before and after treatment with SeeDB

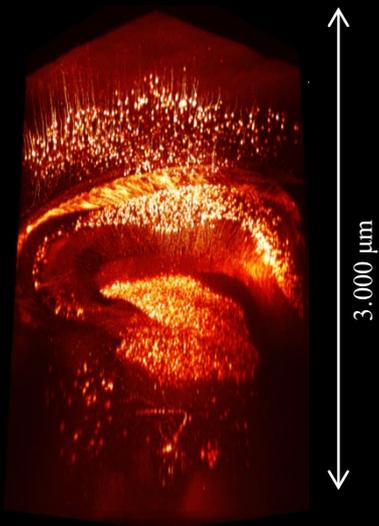


Figure 2. Imaging of Thy1-YFP(H Line) mouse brain after SeeDB process using Multiphoton microscope and Multiphoton dedicated objective :OLYMPUS, model:XLPLN10XSVMF.

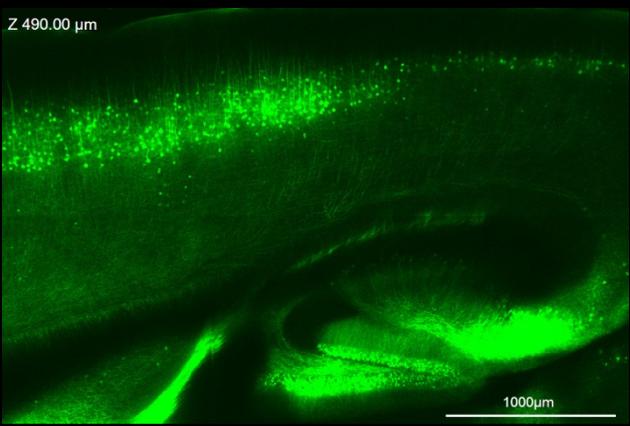


Figure 3. Imaging of Thy1-YFP(H Line) mouse brain after SeeDB process using Confocal microscope and objective :OLYMPUS, UPLSAPO 10X2.

Imaging chamber for tissue cleared samples

See Through Chamber

The See through chamber is an observation container used when observing tissue cleared samples with a microscope. The See through chamber is composed of 10 sets of silicon rubber sheets, cover glasses, slide glasses processed to make it easy to observe tissue cleared samples. There are five kinds of thickness of silicone rubber which becomes the spacer, 0.3 mm, 0.5 mm, 1.0 mm, 2.0 mm, and 3.0 mm, and it is possible to select the optimum size according to your samples. The silicone rubber sheet has a protective sheet affixed on both sides, it can be used in close contact with slide glass and cover glass by peeling off at the time of use.



【References】

- 1) Ke, M. T., Fujimoto, S. and Imai, T. : *Nat Neurosci* ,**6** (8),1154 (2013).
- 2) Ke, M. T., Fujimoto, S. and Imai, T.: *Bio-protocol* **4**(3), e1042 (2014).
- 3) Ke, M. T., and Imai, T. : *Curr Protoc Neurosci* ,**66**,2.22.1-2.22.19 (2014).
- 4) Ke et al.,: *Cell Reports* **14**, 2718(2016)
- 5) SeeDB Resources (<https://sites.google.com/site/seedbresources/>): updated information and technical TIPS from the authors.

Product name	Wako cat. No.	Package Size	Storage	Grade
SeeDB 2 Trial Kit	294-80701	1kit	Keep at RT	Tissue Optical Clearing Reagent
SeeDB Trial Kit	291-79601	1kit	Keep at RT	Tissue Optical Clearing Reagent
See Through Chamber, 0.3mm thick	294-35631	10set	Keep at RT	Tissue Optical Clearing Reagent
See Through Chamber, 0.5mm thick	291-35641	10set	Keep at RT	Tissue Optical Clearing Reagent
See Through Chamber, 1.0mm thick	295-35661	10set	Keep at RT	Tissue Optical Clearing Reagent
See Through Chamber, 2.0mm thick	292-35671	10set	Keep at RT	Tissue Optical Clearing Reagent
See Through Chamber, 3.0mm thick	299-35681	10set	Keep at RT	Tissue Optical Clearing Reagent