LIFE SCIENCE

Plants Tissue-Clearing Reagent Suitable for Fluorescent Protein Observation

iTOMEI

For observing fluorescent protein clearly without complicated procedures

Decoloring solution

Tissue-Clearing Reagent iTOMEI-D [for Plants] 5mL / 25mL [T3940]

Mounting solution

Tissue-Clearing Reagent iTOMEI-M (RI 1.40) [for Plants] 5mL / 25mL [T4003]

Improved TOMEI (iTOMEI) developed by Prof. Sakamoto *et al*. is a method that specializes TOMEI[1] for fluorescent protein observation. This method enables simply transparency and clearly detecting fluorescent protein.[2,3,4] TCI offers suitable reagents for iTOMEI.

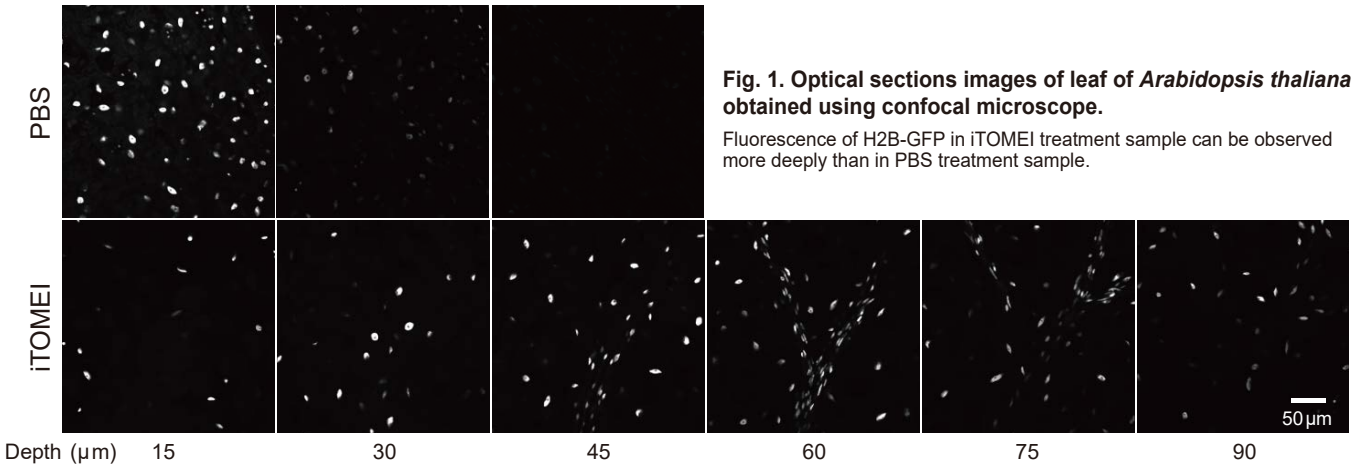


Fig. 1. Optical sections images of leaf of *Arabidopsis thaliana* obtained using confocal microscope.

Fluorescence of H2B-GFP in iTOMEI treatment sample can be observed more deeply than in PBS treatment sample.

Advantages

- **Enables transparency by simple osmosis in just a few days (more than 2 days).**
- **Keeps the fluorescence of a fluorescent protein such as GFP or tdTomato co-expressed as a reporter gene.**
- **Suppresses autofluorescence.**
- **Applicable to wide plant species including *Oryza sativa*, *Arabidopsis thaliana*, *Marchantia polymorpha*.**

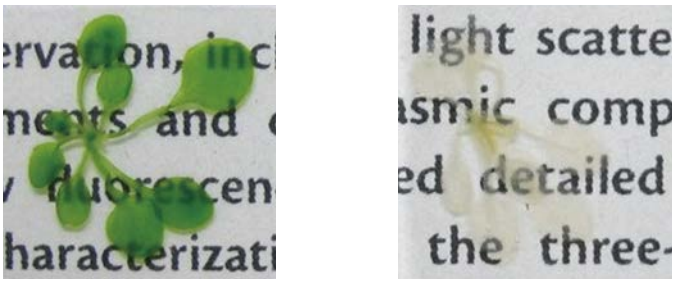


Fig. 2. Cleared *Arabidopsis thaliana* by "iTOMEI"

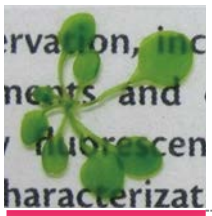
(left) Non-treatment (right) iTOMEI treatment

References

1) J. Hasegawa, Y. Sakamoto, S. Matsunaga, *et al., Plant Cell Physiol*. **2016**, *57*, 462. DOI: http://doi.org/10.1093/pcp/pcw027
2) Y. Sakamoto, S. Matsunaga, *et al., Commun. Biol*. **2022**, *5*, 12. DOI: https://doi.org/10.1038/s42003-021-02955-9
3) M. Sato, Y. Sakamoto, S. Matsunaga, H. Tsuji, *et al., Int. J. Mol. Sci*. **2022**, *23*, 40. DOI: https://doi.org/10.3390/ijms23010040
4) Y. Sakamoto, S. Matsunaga, Tokyo University of Science, Jpn. Kokai Tokkyo Koho 2020-026975, **2020**.

Plants Tissue-Clearing Reagent Suitable for Fluorescent Protein Observation: iTOMEI

Example of use



Reagents

- 1% PFA/PBS (Preparation at time of use is recommended)*1
- PBS
- Decoloring solution (Tissue-Clearing Reagent iTOMEI-D [for Plants])
- Mounting solution: Please use the suitable mounting solution for objective lens. Tissue-Clearing Reagent iTOMEI-M (RI 1.40) [for Plants] is optimized to silicone immersion objectives(RI=1.40). (Iohexol solution is used in the example below.)

Fixing	Fix the sample with 1% PFA/PBS for 1 hour at room temperature.*1 (Deaerate in fixative solution using vacuum pump or syringe when the sample is above ground part.)
Washing	Remove PBS and add the decoloring solution, then let it gently shake for 24 hours with shading at room temperature.*1
Decoloring	Remove the fixation solution and add PBS, then let it rest for 5 minutes at room temperature. Repeat the same work twice.*1
Washing	Remove the decoloring solution and add PBS, then let it rest for 5 minutes at room temperature. Repeat the same work twice more.
Staining	Remove PBS and add the staining solution, then let it rest with shading at room temperature.*2
Washing	Remove the staining solution and add PBS, then let it rest for 5 minutes at room temperature. Repeat the same work twice more.
Clearing	Remove PBS and add the mounting solution, then let it gently shake for 1 hour with shading at room temperature.*3
Mounting	Mount the sample on a microscope slide with the mounting solution, seal by manicure and observe it.

*1 : Operation time is determined by the sample size and plant type.
*2 : The treatment time of DAPI staining is for 30 minutes with 5 μg/mL and the treatment time of Calcofluor White staining is for 10 minutes with 1 g/L of Calcofluor White M3R and 0.5 g/L of Evans Blue, but their adjustments are needed for the purpose.
*3 : If you want to moderate the change in osmotic pressure, it is necessary to perform a gradual replacement with a low-concentration mounting solution.

For observing only fluorescent staining dyes

This reagent enables more rapid transparency (2-3 hours).

Tissue-Clearing Reagent TOMEI [for Plants] 100mL [T3530]

Related Products

Paraformaldehyde	25g / 500g [P0018]
DAPI 2HCl [for Biochemical Research]	5mg [A2412]
Acetic Acid	300mL [A2035]
Evans Blue	25g [E0197]
Acridine Orange	25g [A0132]

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