

Rapid Transparency Reagent for Plants TOMEI

Plant internal structure can be observed clearly without dissecting.

Tissue-Clearing Reagent TOMEI [for Plants]

100mL [T3530]

*This item is unavailable except in Japan, U.S. and Europe.

Advantages

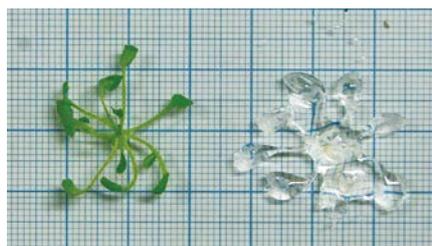
- Simple and rapid clearing of plant can be performed.
- Deeper part can be observed by using confocal microscope.

The transparency technique for plant analysis was developed by Prof. Matsunaga and coworkers. This method enables the clearing of *Oryza sativa*, *Arabidopsis thaliana* and many others in just a few hours. Using this technique, deeper internal structure can be clearly observed.



Non-treatment

"TOMEI-I"
treatment



The transparency technique "TOMEI-I" reduces the intrinsic fluorescence of chlorophyll, as well as reduced fluorescent protein, and thus observation benefits from the use of fluorescent dyes.

Alternatively, transparency technique "TOMEI-II" does not significantly reduce intrinsic fluorescence due to its mild conditions, but the transparency is adequate to detect fluorescent proteins.



Figure 2. Cleared *Arabidopsis thaliana* by "TOMEI-II"

Figure 1. Cleared *Oryza sativa* (upper) and *Arabidopsis thaliana* (lower) by "TOMEI-I"

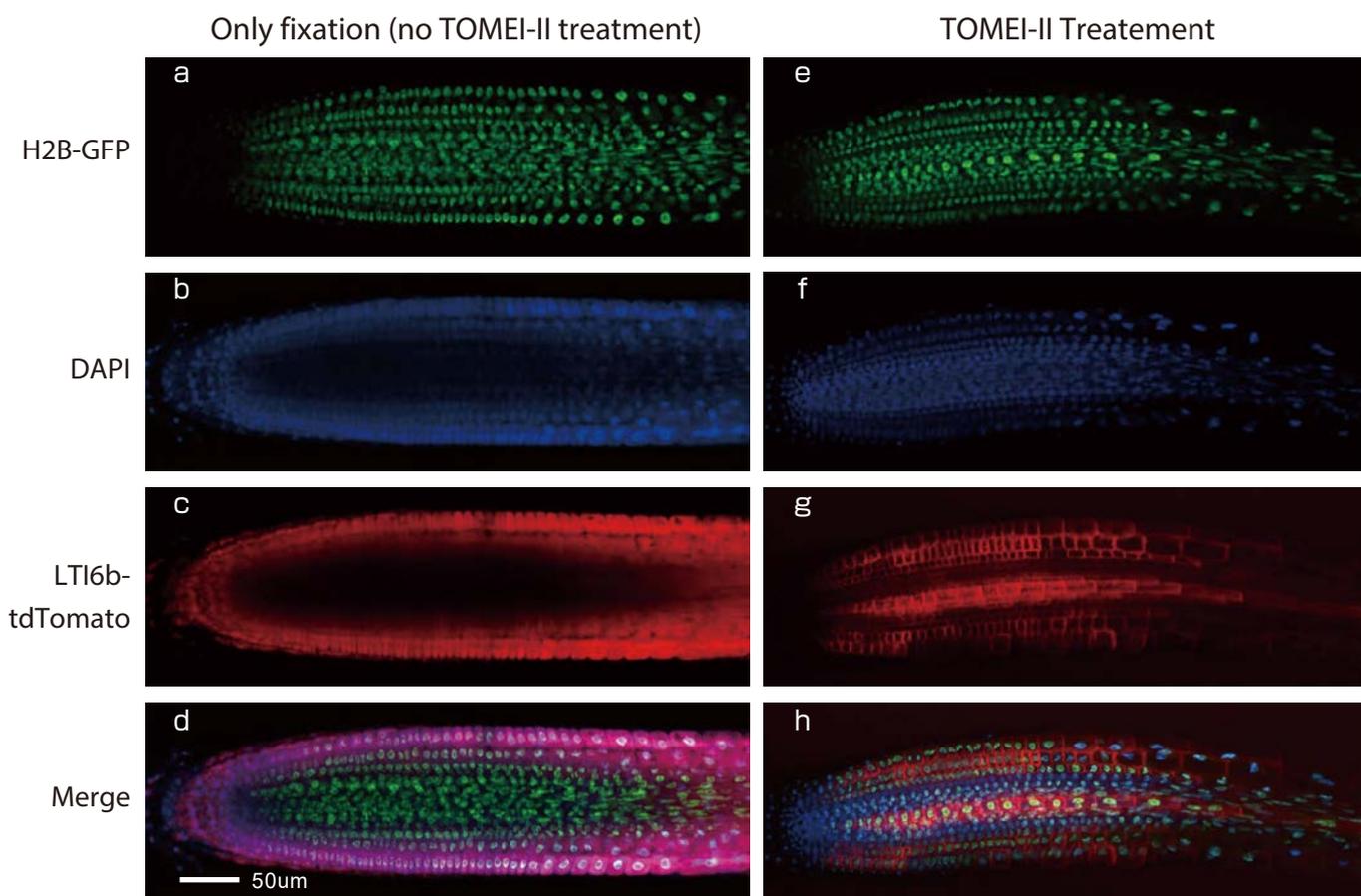


Fig 3. Optical sections images of root of *Arabidopsis thaliana* obtained using confocal microscope.

Nuclear is detected by DAPI (blue) and H2B-GFP (green), and cell membrane is detected by LTI6b–tdTomato (red). All of a-h are images of central part of root. Fluorescent of H2B-GFP (a, e) is detected strongly either in sample only fixation (a) and in TOMEI-II-treated sample (e). It's difficult to detect fluorescent of DAPI (b) and LTI6b-tdTomato (c) in central part of root only fixation. In contrast, fluorescent of DAPI (f) and LTI6b-tdTomato (g) is detected even in cells of central part of TOMEI-II-treated root.

*Image data and every kind of information have been provided by Dr. Yuki Sakamoto and Prof. Sachihiro Matsunaga.

Related Products

Paraformaldehyde

DAPI 2HCl [for Biochemical Research]

Acetic Acid

Evans Blue

25g / 500g [P0018]

5mg [A2412]

300mL [A2035]

25g [E0197]

Rapid Transparency Reagent for Plants: TOMEI

Application 1: Technique for detection of fluorescent protein, "TOMEI-II"

The transparency technique "TOMEI-II" is adequate for observation fluorescent protein such as GFP or YFP coexpressed as a reporter gene.

Reagents

- Fixative solution (4% paraformaldehyde in PBS)
*Preparation at time of use is recommended.
- PBS
- Staining solution
- Transparency reagent TOMEI (TCI Product No. **T3530**)
- 10%, 30%, 50% , 70% TOMEI (diluted with PBS)
*For the root specimens, 20% TOMEI diluted with PBS is necessary

Procedure

Fixing	<ol style="list-style-type: none"> 1. Fix the sample with adequate amount of fixative solution at room temperature. (Fixation time is determined by the sample size and plant type.) (Deaerate in fixative solution using vacuum pump or syringe when the sample is above ground part.) 2. Remove fixation solution and add PBS, then let it rest for 5 minutes at room temperature. 3. Remove PBS and add PBS again, then let it rest for 10 minutes at room temperature. 4. Remove PBS and add PBS again, then let it rest for 10 minutes at room temperature. 	
Staining	Remove PBS and add the staining solution, then let it rest with shading at room temperature. *1 (In case of double staining, re-stain after wasing.)	
Washing	<ol style="list-style-type: none"> 1. Remove the staining solution and add PBS, then let it rest for 10 minutes at room temperature. 2. Remove PBS and add PBS, then let it rest for 10 minutes at room temperature. 3. Remove PBS and add PBS, then let it rest for 10 minutes at room temperature. 	
Clearing	Above ground part	Underground part (root)
	<ol style="list-style-type: none"> 1. Remove PBS and add 10% TOMEI in PBS, then let it rest with shading at room temperature for 10 minutes. 2. Remove 10% PBS and add 30% TOMEI in PBS, then let it rest with shading at room temperature for 10 minutes. 3. Remove 30% TOMEI in PBS and add 50% TOMEI in PBS, then let it rest with shading at room temperature for 10 minutes. 4. Remove 50% TOMEI in PBS and add 70% TOMEI in PBS, then let it rest with shading at room temperature for 10 minutes. 5. Remove 70% TOMEI in PBS and add 100% TOMEI in PBS, then let it rest with shading at room temperature for 10 minutes. 6. Remove 100% TOMEI in PBS and add 100% TOMEI, then let it rest with shading at room temperature for 1 hour. (The time to treat should be considered depending on sample.) 7. Mount the sample on a microscope slide with TOMEI and observe it. *2 	<ol style="list-style-type: none"> 1. Remove PBS and add 20% TOMEI in PBS, then let it rest with shading at room temperature for 10 minutes. 2. Mount the sample on a microscope slide with 20% TOMEI in PBS and observe it.

*1 : The treatment time of DAPI staining is for 30 minutes with 5 µg/mL and the treatment time of Calcofluor White staining is for 10min with 1 g/L of Calcofluor White M3R and 0.5 g/L of Evans Blue, but their adjustments are needed for the purpose.

*2 : When the fluorescence of fluorescent protein reduces, after the treatment of 50% TOMEI in PBS, it may be possible to be solved by being mounted with 60% TOMEI in PBS after the treatment of 60% TOMEI in PBS for 1 hour.

Rapid Transparency Reagent for Plants: TOMEI

Application 2: Technique for analysis with only fluorescent dye, "TOMEI-I"

The transparency technique "TOMEI-I" is adequate for observation with only fluorescent dyes such as DAPI staining, Calcofluor White staining, etc.

Reagents

- Fixative solution (Acetic acid : Ethanol = 1 : 3)
*Preparation at time of use is recommended.
- PBS
- 70% Ethanol (diluted with PBS)
- 30% Ethanol (diluted with PBS)
- Staining solution
- Transparency reagent TOMEI (TCI Product No. **T3530**)
*For root specimens, 20% TOMEI diluted with PBS is necessary.

Procedure

Fixing	1. Fix the sample with adequate amount of fixative solution at room temperature. (Fixation time is determined by the sample size and plant type.) (In case of a seedling of <i>Arabidopsis thaliana</i> , it usually takes for about 1 to 2 hours.)	
	2. Remove fixation solution and add 70% ethanol, then let it rest for 5 minutes at room temperature. 3. Remove 70% ethanol and add 30% ethanol, then let it rest for 5 minutes at room temperature. 4. Remove 30% ethanol PBS and add PBS, then let it rest for 5 minutes at room temperature.	
Staining	Remove PBS and add the staining solution, then let it rest with shading at room temperature. *3 (In case of double staining, re-stain after washing.)	
Washing	1. Remove the staining solution and add PBS, then let it rest for 10 minutes at room temperature.	
	2. Remove PBS and add PBS, then let it rest for 10 minutes at room temperature. 3. Remove PBS and add PBS, then let it rest for 10 minutes at room temperature.	
Clearing	Above ground part	Underground part (root)
	1. Remove PBS and add TOMEI, then let it rest with shading at room temperature for 20 minutes. (The time to treat should be considered depending on sample.) 2. Mount the sample on a microscope slide with TOMEI and observe it.	1. Remove PBS and add 20% TOMEI in PBS, then let it rest with shading at room temperature for 10 minutes. 2. Mount the sample on a microscope slide with 20% TOMEI in PBS and observe it.

*3 : 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.

For further information please refer to our website at www.TCIchemicals.com.

TCI TOMEI



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