New

LIFE SCIENCE

TCI

Rapid Transparency Reagent for Plants TOMEI

Plant internal structure can be observed clearly without dissecting.

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 *et al.* at Tokyo University of Science. 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

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

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" doesn't significantly reduce intrinsic fluorescence due to its mild conditions, but the transparency is adequate to detect fluorescent proteins.

Only fixation   "TOMEI-II" treatment

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

1) J. Hasegawa, Y. Sakamoto, S. Nakagami, M. Aida, S. Sawa, S. Matsunaga, *Plant Cell Physiol.* **2016**, *57*, 462.

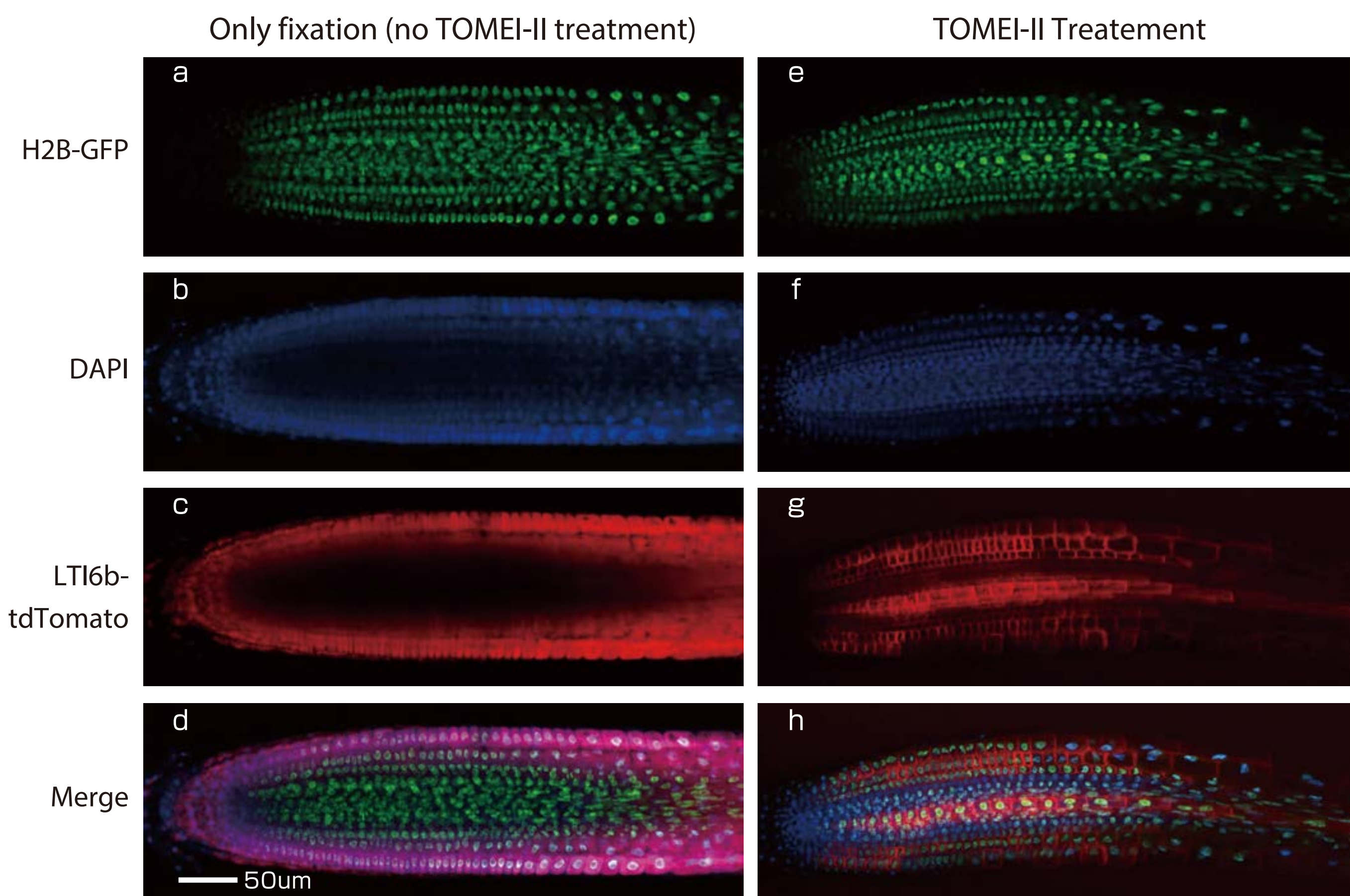

Fig 3. Optical sections images of root of *Arabidopsis thaliana* obtained using confocal microscope.

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 at Tokyo University of Science.

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

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

Related Products

Product	Size	Code
Paraformaldehyde	25g / 500g	[P0018]
DAPI・2HCl [for Biochemical Research]	5mg	[A2412]
Acetic Acid	300mL	[A2035]
Evans Blue	25g	[E0197]

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 code **T3530**)
- 10%, 30%, 50% , 70% TOMEI (diluted with PBS)
  *For the root specimens, 20% TOMEI diluted with PBS is necessary

Procedure

<table>
<tr><td>Fixing</td><td colspan="2">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 5min at room temperature.
3) Remove PBS and add PBS again, then let it rest for 10min at room temperature.
4) Remove PBS and add PBS again, then let it rest for 10min at room temperature.</td></tr>
<tr><td>Staining</td><td colspan="2">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.)</td></tr>
<tr><td>Washing</td><td colspan="2">1) Remove the staining solution and add PBS, then let it rest for 10min at room temperature.
2) Remove PBS and add PBS, then let it rest for 10min at room temperature.
3) Remove PBS and add PBS, then let it rest for 10min at room temperature.</td></tr>
<tr><td rowspan="2">Clearing</td><td>Above ground part</td><td>Underground part (root)</td></tr>
<tr><td>1) Remove PBS and add 10% TOMEI in PBS, then let it rest with shading at room temperature for 10min.
2) Remove 10% PBS and add 30% TOMEI in PBS, then let it rest with shading at room temperature for 10min.
3) Remove 30% TOMEI in PBS and add 50% TOMEI in PBS, then let it rest with shading at room temperature for 10min.
4) Remove 50% TOMEI in PBS and add 70% TOMEI in PBS, then let it rest with shading at room temperature for 10min.
5) Remove 70% TOMEI in PBS and add 100% TOMEI in PBS, then let it rest with shading at room temperature for 10min.
6) Remove 100% TOMEI in PBS and add 100% TOMEI, then let it rest with shading at room temperature for 1hour.
(The time to treat should be considered depending on sample.)
7) Mount the sample on a microscope slide with TOMEI and observe it. *2</td><td>1) Remove PBS and add 20% TOMEI in PBS, then let it rest with shading at room temperature for 10min.
2) Mount the sample on a microscope slide with 20% TOMEI in PBS and observe it.</td></tr>
</table>

*1 : The treatment time of DAPI staining is for 30min 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 1hour.

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 code **T3530**)
  *For root specimens, 20% TOMEI diluted with PBS is necessary.

Procedure

<table>
<tr><td>Fixing</td><td colspan="2">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 Arabidopsis thaliana, it usually takes for about 1 to 2 hours.)
2) Remove fixation solution and add 70% ethanol, then let it rest for 5min at room temperature.
3) Remove 70% ethanol and add 30% ethanol, then let it rest for 5min at room temperature.
4) Remove 30% ethanol PBS and add PBS, then let it rest for 5min at room temperature.</td></tr>
<tr><td>Staining</td><td colspan="2">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 wasing.)</td></tr>
<tr><td>Washing</td><td colspan="2">1) Remove the staining solution and add PBS, then let it rest for 10min at room temperature.
2) Remove PBS and add PBS, then let it rest for 10min at room temperature.
3) Remove PBS and add PBS, then let it rest for 10min at room temperature.</td></tr>
<tr><td rowspan="2">Clearing</td><td>Above ground part</td><td>Underground part (root)</td></tr>
<tr><td>1) Remove PBS and add TOMEI, then let it rest with shading at room temperature for 20min.
(The time to treat should be considered depending on sample.)
2) Mount the sample on a microscope slide with TOMEI and observe it.</td><td>1) Remove PBS and add 20% TOMEI in PBS, then let it rest with shading at room temperature for 10min.
2) Mount the sample on a microscope slide with 20% TOMEI in PBS and observe it.</td></tr>
</table>

*3 : The treatment time of DAPI staining is for 30min 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.

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

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