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ProductInformation

Plant Cell Viability Assay Kit

Product Code **PA0100**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Plant Cell Viability Assay Kit is designed for the differential viability staining of plant cells. The kit employs a dual color fluorescent staining system to highlight viable and non-viable cells.[1,2] This procedure has been used to stain intact plant tissue,[3,4] callus tissue,[5] cell suspension culture,[6] and protoplasts.[2]

Viable cells are living cells with intact plasma membranes. These cells can be distinguished by the presence of intracellular esterase activity. This activity is assayed through the enzymatic hydrolysis of fluorescein diacetate or related compounds, such as carboxyfluorescein or calcein AM. These lipophilic compounds are membrane-permeable and non-fluorescent. In the plant cell, they are hydrolyzed to highly polar fluorescent compounds. Because of their polar nature, these compounds are unable to diffuse across the plasma membrane and are retained within viable cells, producing an intense green fluorescence within the cytoplasm. Fluorescein diacetate was found to be the optimal dye for staining viable plant cells. It does not photobleach as quickly as calcein AM, and produces much less background fluorescence than carboxyfluorescein diacetate in plant cells.

Non-viable cells could be either living or dead. They can be distinguished from viable cells by their non-intact or damaged plasma membranes. Propidium iodide and related compounds, such as ethidium homodimers-1 and –III, can only enter cells with damaged membranes, whereupon they intercalate into double-stranded nucleic acids. This results in a bright red fluorescence in non-viable cells, particularly in the nucleus where the concentration of nucleic acids is highest. Propidium iodide is the optimal dye for staining non-viable plant cells. In plant cells it specifically labels nucleic acids, whereas ethidium homodimers-1 and –III both bind non-specifically to the plant cell wall.

Spectral Properties

Fluorescein: Ex_{max}: 494 nm; Em_{max}: 518 nm
Propidium iodide: Ex_{max}: 536 nm; Em_{max}: 617 nm

Reagents

- Fluorescein Diacetate Solution
  Product Code F 4804 — 0.1 mL
- Propidium Iodide Solution
  Product Code P 4373 — 0.1 mL

If used as recommended, this kit contains sufficient material for at least 100 dual staining assays.

Materials Required But Not Provided

- Plant tissue
- 1.5 mL microcentrifuge tubes
- Water, Molecular Biology Reagent (Product Code W 4502)
- Microscope slides and coverslips
- Fluorescence microscope

Precautions and Disclaimer

This product is for R&D use only; not for drug, household, or other uses. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

The components of this kit must be protected from light and stored at –20 °C with desiccant. Propidium iodide is stable in aqueous solution. However, fluorescein diacetate will hydrolyze in aqueous solution. Dilutions of the dyes should be used within one day.

Procedure

NOTE: This protocol was optimized using a *Nicotiana tabacum* cv. NT-1 cell suspension culture. The recommended dilutions of fluorescein diacetate and propidium iodide are suitable for staining a number of plant species and tissue types. However, dye concentrations, volumes, and staining times may need to be optimized depending on the species and tissues being studied.

1. Thaw kit components at room temperature or in a 30-37 °C water bath. After thawing, a precipitate may be seen in the propidium iodide solution. This can be redissolved by vortexing vigorously.

2. Using a wide-mouth pipette, collect 500 µl of cells and transfer to a 1.5 mL vial.

3. Add an equal amount of water or culture medium, and invert to mix.

4. Transfer 90 µL of the diluted cells to a new vial.

5. Dilute the stains 1:100 in water to make a 10X stain solution. Add 1 µL each of fluorescein diacetate and propidium bromide to a tube containing 98 µL of water or PBS, pH 7.4, and vortex the solution to mix.

6. Add 10 µL of the 10X stain solution to 90 µL of cells, and mix by gently tapping the tube. To stain intact plant tissues, prepare an amount of 1X stain solution to completely submerge the sample.

7. Incubate the samples at room temperature for 1 to 2 minutes. Thin tissues, such as *Arabidopsis* seedling roots, stain completely over this time period. Thicker tissues may need to be sectioned or stained for a longer period.

8. Place approximately 10 µL of stained cells on a microscope slide and cover with a glass coverslip. Thin tissues, such as *Arabidopsis* seedling roots, can be placed directly on a slide and covered with a coverslip. Thicker tissues must be sectioned before placing the coverslip. A small amount of the 1X stain solution can be placed on the slide to completely fill the space between the slide and the coverslip.

9. View the stained samples with a fluorescence microscope. Fluorescein and propidium iodide can be viewed simultaneously with a fluorescence microscope equipped with the proper dual band fluorescence filter set. These dyes can also be viewed separately. Fluorescein can be viewed with an FITC band pass filter, and propidium iodide can be viewed with a TRITC band pass filter.

10. Count the number of green and red cells in several different fields of view to estimate the ratio of viable to non-viable cells.

References

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3. Koyama, H., et al., Brief exposure to low-pH stress causes irreversible damage to the growing root in *Arabidopsis thaliana*: pectin-Ca interaction may play an important role in proton rhizotoxicity. J. Exp. Bot., **52,** 361-368 (2001).
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