Simultaneous Measurement of Cell Color and Vacuolar pH

< Measurement of Absorption Spectrum >

1. Pour the protoplast suspension onto a x20 poly-L-lysine pre-coated glass-bottom petri dish (35 mm ϕ) and allow to stand for 1 minute at room temperature.
2. After removing the extra suspension buffer, very carefully pour 2 ml of suspension buffer into the dish.
3. Set the petri dish on a inverted microscope and measure the absorption spectrum with a micro-spectrophotometer (MCPD-7000; Photal).

< Measurement of Vacuolar pH: Preparation of Microelectrodes >

1. Make double-barreled pipettes (1B100F-4; WPI, washed with acetone and water) with vertical pullers. Cut one of the pipettes 1.5 cm from the tip.
2. Heat the pipettes at 200℃. After 1 hour, dip the blunt end of the longer pipette in 0.1% (v/v) trimethylchlorosilane/chloroform for 1 second, then heat again for one more hour.
3. Cool down the pipettes and apply melted wax to bind them together.
4. Add 0.5 μl of proton-ionophore solution (5% v/v Hydrogen Ionophore II-Cocktail A, 0.05% w/v nitrocellulose in THF) to the longer pipette.
5. Store the pipettes in a dry box over night.
6. Add 0.2 μl of Hydrogen Ionophore II-Cocktail A to the longer pipette, and allow to stand at vacuum condition for 5 minutes.
7. Add 0.5 M KCl to the shorter pipette.
8. Add 0.1 M MES-Tris, 0.5 M KCl, pH 6.0 to the longer pipette.

Tips・The diameter of the tip must be optimized according to the plant cells.
・All the liquid must be back-filled to the pipettes.
・The pipettes must be free of air bubbles.

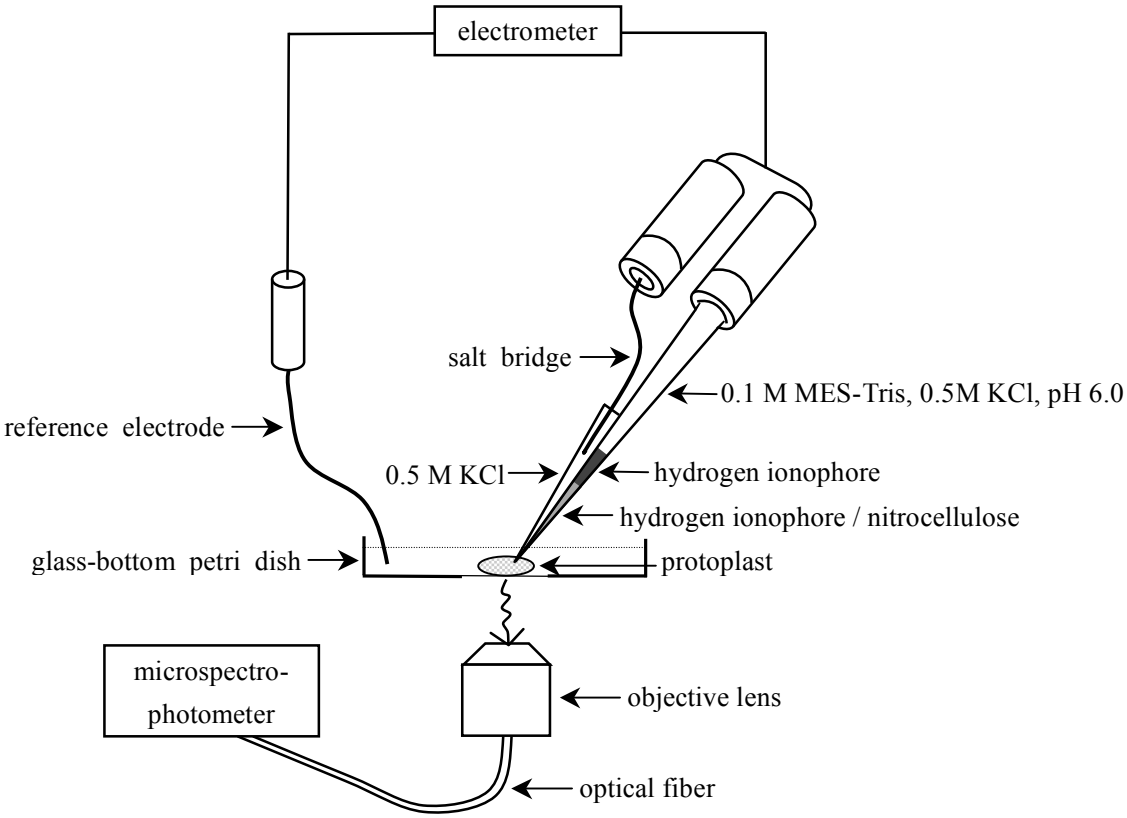
< Measurement of Vacuolar pH: Calibration and pH Measurement >

1. Insert the longer pipette into the holder, and connect the shorter pipette via salt bridge.
2. Calibrate the pH electrode using standard buffers. Measure the potential difference with a electrometer (FD223; WPI), and collect data with Chart for windows v. 5.0.1 with an A/D converter (PowerLab/4sp; ADInstruments).
3. Insert the microelectrode into the cell-color recorded protoplast using a micromanipulator and record its electric potential.
4. Recalibrate the microelectrode.
5. Calculate pHv using the latter calibration data.

Tips・Use only electrodes that have responded well'.
・Adopt data which drift was less than constant numerical value.

< Reference >

Yoshida K, Toyama-Kato Y, Kameda K, and Kondo T (2003) *Plant Cell Physiol* 44:262-268.
Yoshida K, Osanai M, Kondo T (2003) *Phytochemistry* 63:721-726.


electrometer
salt bridge
0.1 M MES-Tris, 0.5M KCl, pH 6.0
reference electrode
0.5 M KCl
hydrogen ionophore
hydrogen ionophore / nitrocellulose
glass-bottom petri dish
protoplast
microspectro-
photometer
objective lens
optical fiber