

# Electro Cell Manipulator™ ECM® 600

## Methods for Rapid Verification of Electroporation in Various Cell Types

### 1) *Dictyostelium discoideum* (slime mold)

Cells, in particular growth and developmental phases, were washed twice in ice-cold Bonner's standard salt solution (BSS), and once in 50 mM sucrose. An aliquot (250 - 300  $\mu$ l) of cell suspension in the presence of 50  $\mu$ g/ml FITC dissolved in 50 mM sucrose was transferred into an ice-cold chamber equipped with two flat stainless steel electrodes that were separated at a distance of 2 mm. Electroporation and subsequent washings by centrifugation were performed cold, and the samples observed under a fluorescence microscope to determine the ratio of cells with FITC to total cells. Incidentally, FITC was barely incorporated into the cells without electroporation.

**excerpted from *Differentiation*, Springer-Verlag, 1988, pg. 91.**

### 2) *Saccharomyces* spp. (yeasts)

Yeast were grown in YPD medium, harvested while in exponential phase and resuspended in a minimal medium (50 mM Na + 50 mM K phosphate buffer, pH 6.8, 1 mM glucose, 5 mM  $\text{NH}_4\text{Cl}$ ) and 0.1 mM FITC dextran (green fluorescence, 0.01 mol FITC per mol glucose). Control aliquots were removed before and after the electrical pulse experiments, corresponding to exposure of 5 and 40 minutes to the FITC-dextran. These initial and final controls are used to determine the spontaneous uptake and adsorption, and also loss of viability which is unrelated to electroporation. For each electric field strength, E, a single Aliquot (14-25  $\mu$ l;  $6 \times 10^7$  cells/ml) of cell suspension was used to fill a parallel plane (stainless-steel electrodes) electroporation chamber at room temperature ( $23 \pm 3^\circ\text{C}$ ). Cells were then placed in a micro-centrifuge tube, held for a 5 min uptake/recovery period, centrifuged at 5000-7500 x g for 3 min, and washed twice with a minimal medium.

**excerpted from *FEBS Letters*, Vol. 229 (1), 1988, pg. 31.**

### 3) *Fusarium oxysporum* (fungi macroconidia)

Macroconidia were suspended in water containing various concentrations (50 - 150 mg/ml) of FITC-albumin incubated at  $28^\circ\text{C}$  for 1 - 5 hr, and subjected to electroporation. Macroconidia were collected by centrifugation, washed 3 times with water, and observed with an Olympus fluorescence microscope.

For spore germination, electroporated spores were washed with water, suspended in Czapek-Dox medium, and incubated statically at  $28^\circ\text{C}$  for two weeks. Sample solutions were sterilized by membrane filtration and the reaction chamber was autoclaved before experiment. All of the experiments were carried out under a stream of sterile air.

**excerpted from *J. Phytopathology*, Matsuda, Vol. 125, 1989, pg. 91.**

### 4) P3X63Ag8, CV-1, HeLa, Mouse Kidney Cells (MKC) mammalian)

The P3X63Ag8 cells and trypsinized CGV-1, HeLa or secondary MKCs were centrifuged at 250 X g, resuspended and washed in the appropriate electroporation buffer by recentrifugation. FITC-dextran of SFV-C protein were added to the cell suspension before electroporation. The electroporation chamber was cooled to  $0^\circ\text{C}$ , and filled with cell suspension ( $10^5$  cells). Thereafter, the cells were kept for 10 min at  $0^\circ\text{C}$  (diffusion loading time). They were then subjected to a short centrifugation (5s, reaching 1500 rpm at the end of acceleration) before they were suspended in the appropriate resealing buffer or growth medium. Fluorescence microscopy was performed with a Nikon fluorescence microscope.

**Excerpt from *Experimentia*, Birkhauser-Verlay, Vol. 44, 1988, pg. 199.**