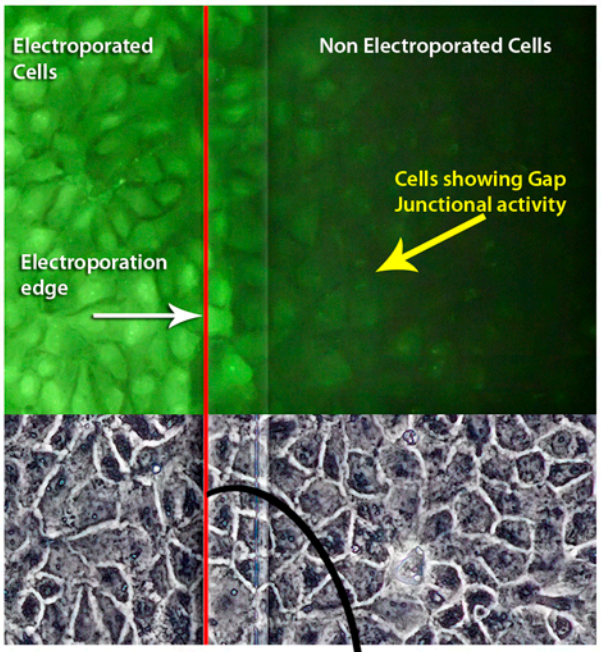
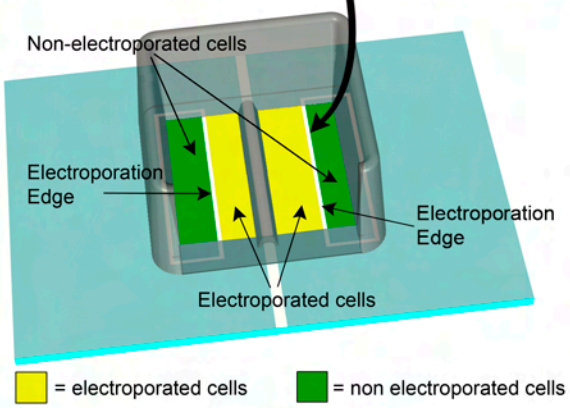
InSitu Porator for Gap Junction and Signal Transduction Studies

Uniquely Designed for Adherent Cells.

The disposable InSitu Chamber uses a glass slide with a conductive and transparent coating for cell growth and electroporation.
The InSitu Porator discharges optimised electrical pulses to different areas of the slide such that there are electroporated and non-electroporated cells immediately adjacent to each other.
This offers the opportunity to study gap junctional, intercellular communication by introducing fluorescent dyes, whose migration through gap junctions can be observed.

Epithelial cells showing gap junctional activity





= electroporated cells = non electroporated cells

InSitu Chamber showing Electroporated and Non Electroporated Areas

Adherent Cell Electroporation

Application	Molecules Introduced
Gap Junctions	Fluorescent Dyes
Signal Transduction	Peptides
	siRNA
	Nucleotides
	Others

- No damage to cells
- Unique design offers highest efficiences
- Extensively tested on a wide variety of adherent cells
- Easy programming for improved optimisation
- Sterile environment to grow and monitor the cells
- Disposable chambers eliminate cross contamination

Non Traumatic conditions for Signal Transduction studies.

The ability to compare directly, electroporated and control cells is especially valuable for **Signal Transduction** studies where various molecules such as **peptides, pro-drugs, siRNA** or **decoy oligonucleotides** can be introduced into cells ***without cell trauma.***
For cells with gap junctions, the material (e.g. peptide) introduced can migrate into the non-electroporated cells via their gap junctions and affect the signal.

This offers a powerful demonstration that the inhibition of the signal must be due to the peptide and cannot be an artifact of electroporation since the cells were not electroporated!

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InSitu Porator

Easy to use with improved safety operation

The InSitu Porator has advanced yet flexible output functions, with a simple to operate menu to easily optimise the conditions for each cell line or type of molecule being introduced.

High efficiencies with exclusive features

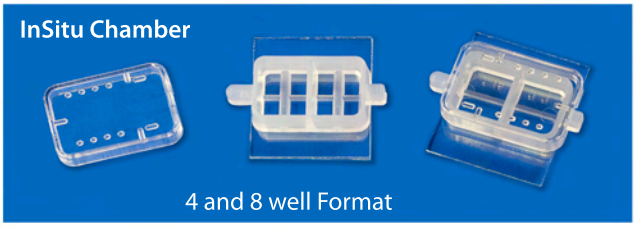
The InSitu Porator maximises electroporation efficiences in a number of ways:

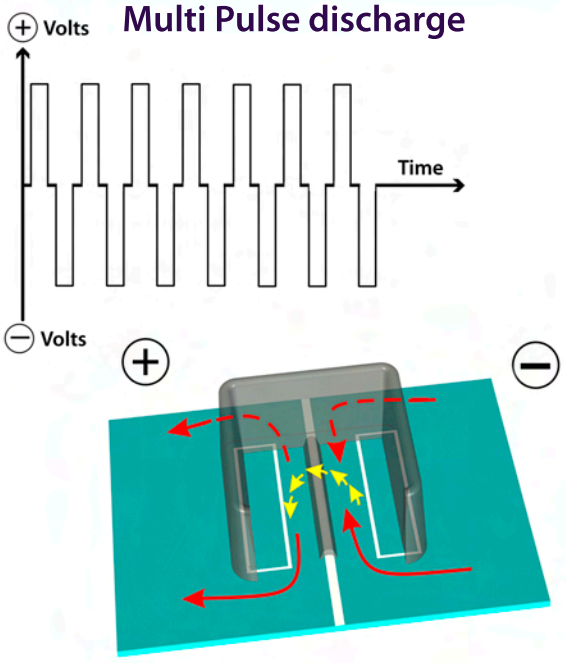
- By being able to use uniquely low voltages.
- Using multiple pulse discharges to dramatically reduce the electric shock effect.
- Alternating the polarity of the pulses to reduce the harmful ion build-up that commonly occurs in single polarity electroporation systems.

As a result, under the appropriate conditions the InSitu Porator can achieve essentially 100% efficiences (see photos) without disturbing the cells either physically or from the electric field.

Disposable chamber formats

The sterile InSitu Chamber format permits the simultaneous electroporation of two samples under identical conditions, for comparison purposes.





Voltage pulse along InSitu Chamber

Electroporation Analysis

Following electroporation, the InSitu Chamber can be transferred to a microscope for visual analysis using fluorescence or phase contrast illumination.

A quantitative assessment of dye transfer is shown on the next page, middle panel: The number of cells that the dye transferred into through gap junctions (dots) can be divided by the number of cells at the edge of the electroporated area (stars) to give a numerical value.

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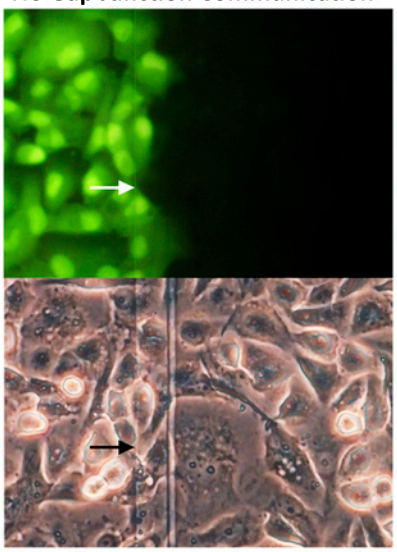
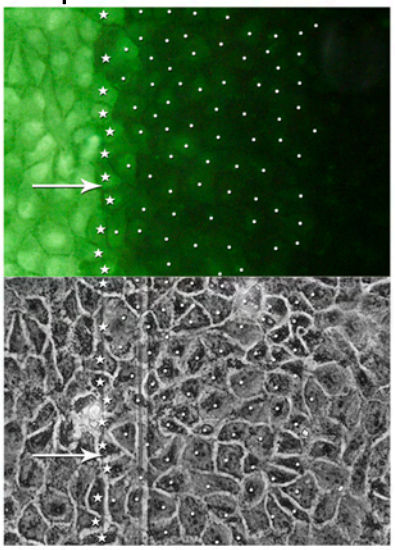
InSitu Electroporation

Gap Junction Studies

No Gap Junction Communication



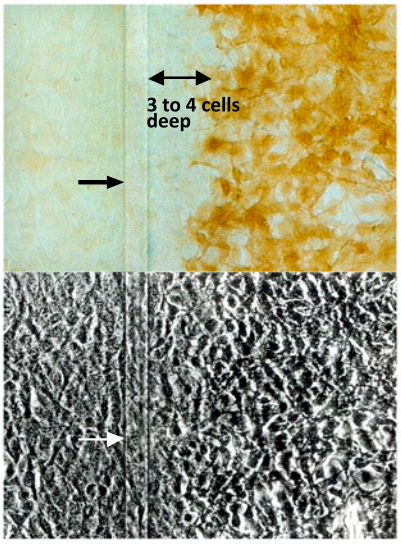
Gap Junction Communication



indicates electroporation edge

Peptides

Erk Inhibiting

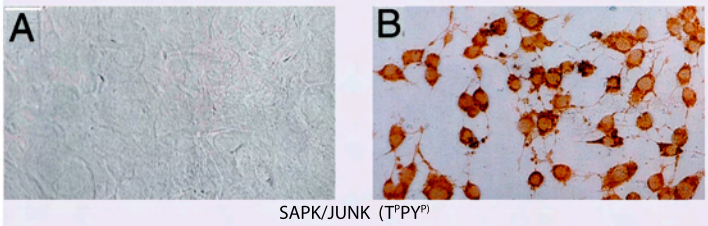


A549 lung carcinoma cells	T51B rat liver epithelial cells	NIH3T3 fibroblasts
Lucifer Yellow dye	**Lucifer Yellow dye**	**Grb2-SH2 blocking peptide**
Lucifer yellow was electroporated into A549 lung carcinoma cells that have no gap junctions. Following a 5 min incubation, cells were photographed under fluorescence and phase contrast illumination.	Lucifer yellow was electroporated into T51B rat epithelial cells that have extensive gap junctional communication. Following a 5 min incubation, cells were photographed under fluorescence and phase contrast illumination.	The peptide was electroporated into the cells growing on the left side of the slide. All cells were then stimulated with EGF for 5 minutes, fixed and probed for p-Erk. Note the dramatic reduction in staining of electroporated cells (left), compared to control cells grown on the non electroporated area (right).
Note the absence of dye transfer through gap junctions.	Note the extensive transfer through gap junctions. For quantitation, the number of cells that the dye transferred into through gap junctions (dots) can be divided by the number of cells at the edge of the electroporated area (stars).	**Note that the signal inhibition extends into 3-4 rows of cells on the non-electroporated area, due to movement of the peptide through gap junctions.**

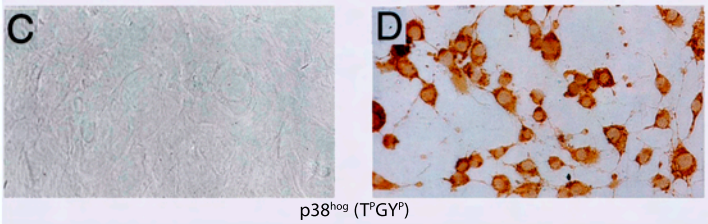
Note the absence of damage to the cells in the phase contrast images.

InSitu Electroporation does not activate stress kinases

Electroporated **UV irradiated**



SAPK/JUNK ($T^{P}PY^{P}$)



$p38^{hog}$ ($T^{P}GY^{P}$)

Electroporation *In Situ* opens pores on the cell membrane which permit the introduction of the material, then they rapidly reseal with no detectable damage to the cell.
To ensure the absence of stress we examined the stress pathways. Mouse NIH3T3 fibroblasts were electroporated (A,C) or UV irradiated (B,D) then fixed and probed for the stress-activated kinases SAPK (A,B) or $p38^{hog}$ (C,D).

Note the absence of activation of the stress kinases by the InSitu electroporation (A,C)

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Technical support for the InSitu Porator

We would be happy to assist in answering any scientific or technical queries you may have, for any aspect of the Insitu process either during set up, running or the post assessment microscopy phases. In fact we would positively encourage you to make contact so that we can run a search and provide key advise and technical tips from our extensive range of tested cell lines and molecules transported

Contact the Technical Support Team: info@cellprojects.com

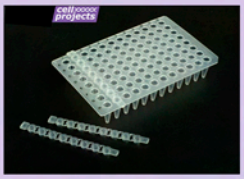
InSitu Porator Specifications

Power Requirement	110 to 240 Volts, 50/60 Hz, 3amp rating
Pulse Generator	
Size	254 × 178 × 132mm
Weight	1.5kg
LCD Display	4 Lines of 20 character backlit
Key Pad	16 Key membrane keypad
InSitu Holder	
Size	320 × 100 × 50mm
Pulse Parameters	
Volts	2 to 45 Volts
Pulses	1 to 255 pulses
Pulse duration	2 microseconds to 6.999 milliseconds
Pulse spacing	1 millisecond to 9.999 seconds
Pre programmed selections	Mild, Medium and Strong setting using combinations of the above pulse parameters.
Advanced manual settings	Yes using any of the available pulse parameters.
Safety detection	Electroporation can only occur in the protected compartment.

Ordering Information

Catalogue Number	Description
ACE-100	Pulse generator main unit (ACE-100-01) includes power supply, control circuitry and keypad, InSitu Holder (ACE-100-02), power cable and instruction manual
ACE-08-CC	25 x 8 well individually wrapped and sterilised InSitu Chambers
ACE-04-CC	25 x 4 well individually wrapped and sterilised InSitu Chambers
ACE-08-CC/L	25 X 8 well individually wrapped and sterilized low barrier InSitu Chambers
ACE-04-CC/L	25 X 4 well individually wrapped and sterilized low barrier InSitu Chambers
ACE-25-CC	25 mgs of *In Situ* poration quality tested High Grade Lucifer Yellow.

Other Products from Cell Projects



Suspended Cell Electroporation:

- **HiMax range of cuvettes** maximises efficiencies for Bacteria, Yeast, Insect, Plant and Mammalian cells, fits most electroporators.
- **ElectroBuffer** is designed to improve transfection efficiencies whilst improving survival rates post electroporation for Eukaryotic cells.

DNA Swabs and Sample handling:

- **Isohelix DNA Buccal Swabs** – for superior DNA Buccal cell collection using novel materials.
- **Isohelix DNA Isolation kits** - produce high quality DNA from buccal swabs.
- **Stabilisation and PCR kits** to check quality and stability of DNA.

PCR Plastic Products:

- Primary manufacturer of **96 well PCR plates**, **sealing caps** and **adhesive films**.

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