Celetrix

Electroporation

Large Scale and Small Scale

High efficiency and versatility

Genome Editing

CAR-T

CAR-NK

Antibody Production

Cell Line Development

Bacteria Yeast

Cell Fusion

Patented in US, China and Japan

www.celetrix.com

Products

The upgraded model: LE+

1. For 20ul, 100ul and 200ul electroporation tubes;
2. New Cell Line mode and PBMC mode;
3. Optimized for large plasmid electroporation.

The upgraded model: EX+

1. EX+ fits 20ul, 100ul, 200ul and 1ml electroporation tubes;
2. New Cell Line mode and PBMC mode;
3. Optimized for large plasmid electroporation;
4. Electro cell fusion add-on available for hybridoma.

The large-scale model: SLT

1. A 10ml tube electroporates 2 x 10^9 CHO cells instantly;
2. Same efficiency/ viability as small-scale models;
3. Single optimized program with adjustable level;
4. For 200ul, 1ml, 5ml and 10ml electroporation tubes;
5. Electro cell fusion add-on available for hybridoma.

Bacteria / Yeast model: UHV Transformer

1. Bacteria: 20ul, 100ul, 600ul electroporation tubes;
2. Yeast: 20ul, 100ul, 200ul, and 1ml electroporation tubes;
3. Pulse time adjustable at 1ms steps;
4. Voltage range 2.5KV-15KV;
5. Truly No Arcing (Not detection and shut-off).

Electroporation kits

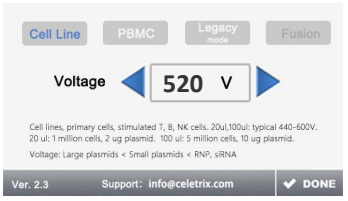
1. One buffer for all mammalian cells;
2. Each kit contains 48 samples;
3. Sealed electroporation tubes capable of reducing gas bubbles, great for efficiency and viability;
4. Easy change of adapters.

Introduction

Extremely Simplified Operation by Celetrix

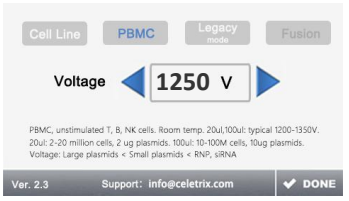
While there are so many mammalian cell types, Celetrix creatively categorizes these cell types into two groups. Each group only needs voltage micro-adjustment.

The first group is summarized as Cell Line. Suspension cell lines, adherent cell lines, adherent primary cells, CD34+ HSC and stimulated T cells, B cells and NK cells can use the Cell Line mode with small adjustments of voltage.



Cell Line mode

The second cell group includes PBMC (Peripheral blood mononuclear cell), unstimulated T cells, B cells and NK cells because of their similarly small cell diameter. These cells can be electroporated under the PBMC mode.



PBMC mode

Electro cell fusion can be done in electroporation tubes with the same electroporation buffer. Mix mouse splenocytes and fusion partner cells and load them to an electroporation tube to form a mixed cell pellet for fusion. Users only need to select a single parameter on the screen to perform fusion.

Fusion mode

Sealed Electroporation Tubes

1. Sealed electroporation tubes capable of reducing gas bubbles, great for efficiency and viability.
2. Volume from small 20ul to large 10ml.
3. Handling at ease, convenient change of adaptors.

Optimized Electroporation Buffer

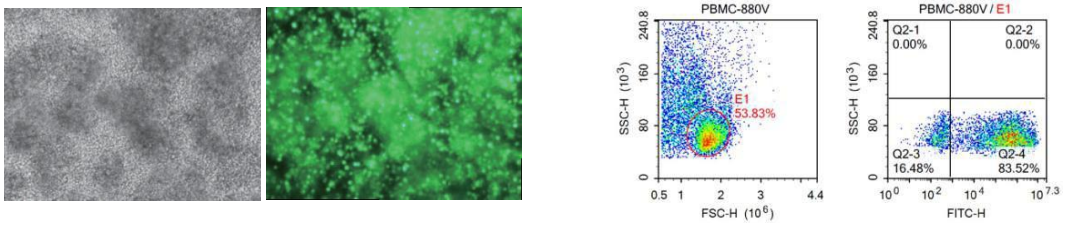
- One buffer for all mammalian cells.
- High efficiency.
- Low cytotoxicity.
- Long term storage.

New Era for T Cell Immunotherapy

Human T cells have been hard to transfect with other methods, especially for plasmids.

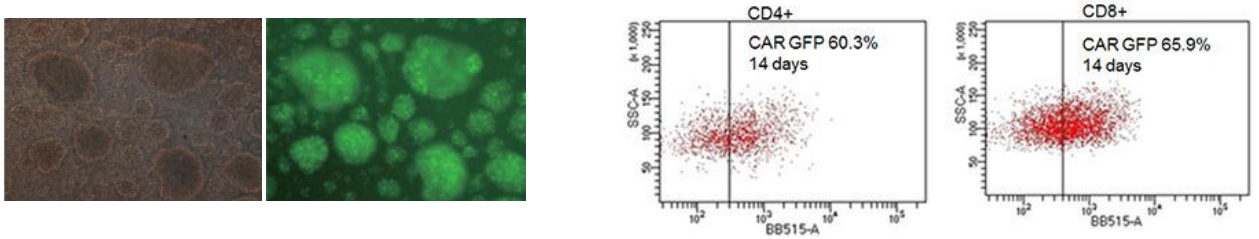
With Celetrix electroporation, T cells can be transfected any time from the fresh PBMC stage to the stimulated or cultured stage. The Celetrix technology can achieve high transfection efficiency while maintaining high level of cell survival and expansion, allowing immunotherapy applications such as CAR, TCR-T generation and CRISPR knock-down of T cell genes.

Human PBMC transfection with GFP plasmid



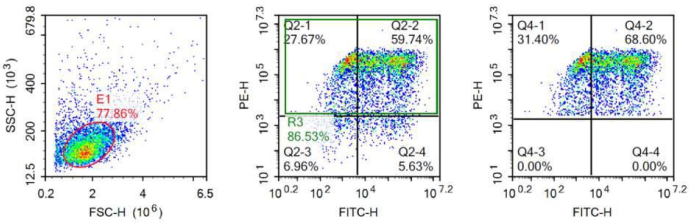
Transfection efficiency with GFP plasmid after 24 hours is over 80%

Human PBMC transfection of CAR-T2A-GFP with SB transposon



Human PBMC CAR expression at 65.9% after 14 days.
Cells form clusters and proliferate similar to un-electroporated cells.

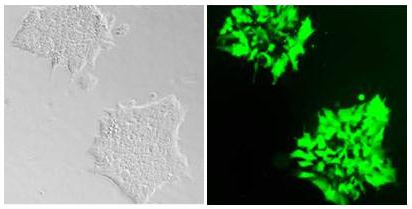
Expanded NK stably transfected with GFP transposon



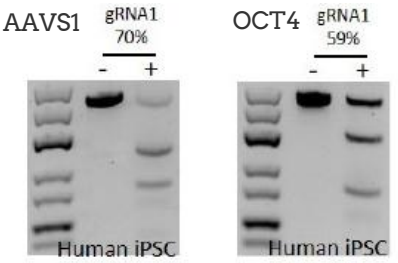
Expanded NK cells (Day 7) can be electroporated with high efficiency and the cells can continue growth and maintain expression of PiggyBac GPF transposon at Day 14.

Research and Industrial Applications

Genome editing by Cas9 RNP electroporation

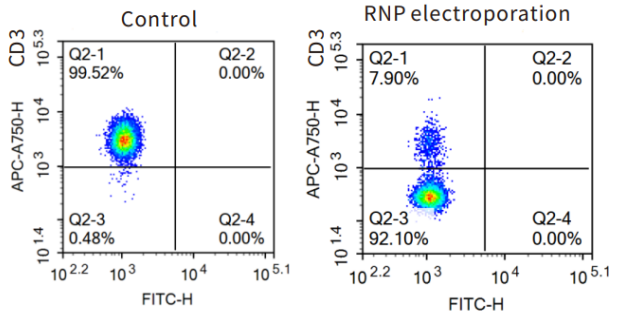


iPSC with GFP



T7E1 assay

The Celetrix technology can electroporate human iPSC with Cas9/gRNA. High knockdown efficiency is observed at the AAVS1 and OCT4 sites.



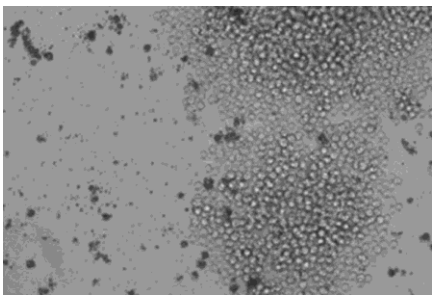
In activated T cells, knockdown of TCR can be achieved at over 90% efficiency. Three days after electroporation of RNP for TCR, CD3 has been mostly down regulated.

Cell Electrofusion

Model EX+ and Model SLT can be equipped with optional fusion accessories for hybridoma generation. Mouse spleen cells can be fused with myeloma cells such as SP2/0 and P3X63Ag8 with high efficiency.

The process is very simple. Mouse spleen cells are mixed with myeloma cells in the electroporation buffer to form a mixed cell pellet in the electroporation tube and the sample tube is inserted into the machine for fusion. After 3 days, colony growth is visible and supernatant is ready for assays in about 10 days.

Compared with PEG fusion, electrofusion is more stable with better efficiency and better reproducibility.



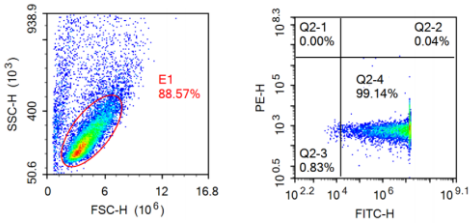
Small to Large Scale Protein Expression

Supporting Antibody Development

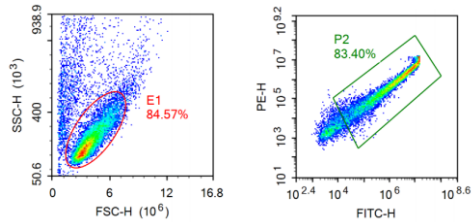
Model SLT (Single Large Tube) 10ml electroporator can electroporate 2 x 10^9 CHO cells instantly for 400ml Fed-batch culture to reach 1g/L antibody expression level in 7-12 days.

Model EX+ electroporator can process 1ml tubes for 2×10^8 CHO cells for 20-40ml Fed-batch culture to harvest about 10-20mg antibody. It is also suitable for stable pool generation and high expression clone screening.

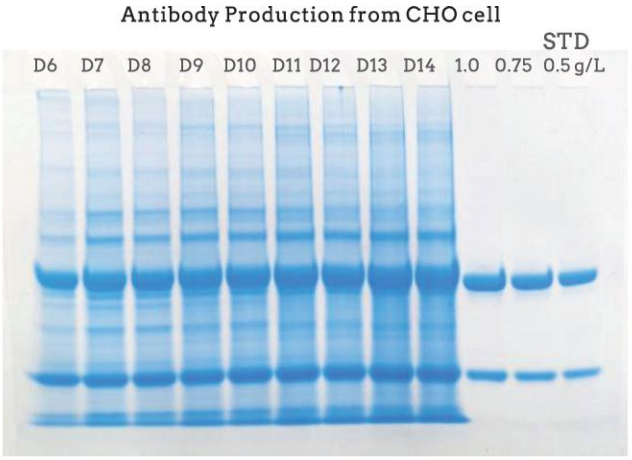
Both small scale and large scale Celetrix electroporators can reach 99% efficiency on CHO cells with small plasmids and over 80% efficiency with 11kb larger plasmids.



6kb plasmid: efficiency>99%



11kb plasmid: efficiency>80%



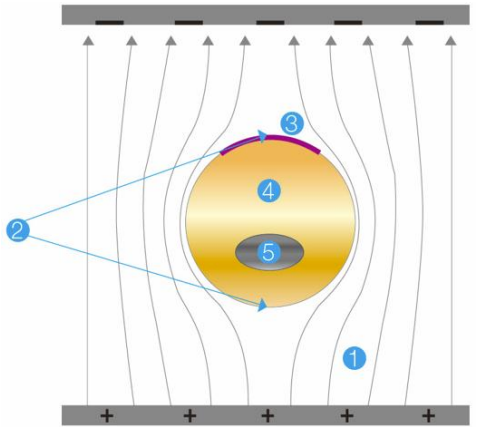
10ml electroporation tube can electroporate 2x10^9 CHO cells instantly, and reach more than 0.5g/L antibody expression level in 7 days.

	Celetrix 10 ml tube	Flow electroporation (other brands)
CHO cell number	2 X 10^9	1 X 10^{10} (100ml)
Electroporation time	1 second	20 minutes
HC + LC plasmids	1 mg	20 mg
Culture volume (pcDNA vector)	200 – 400 ml	2 L
Antibody amount	Up to 300 mg	Up to 1 gram
Culture volume (Transposon vector)	1 -2L	*
Antibody amount	Up to 2 gram	*
Sample cost	Very low	High

* Transposon vectors allow cell expansion and they usually need a smaller electroporation volume.

Mechanism of Electroporation

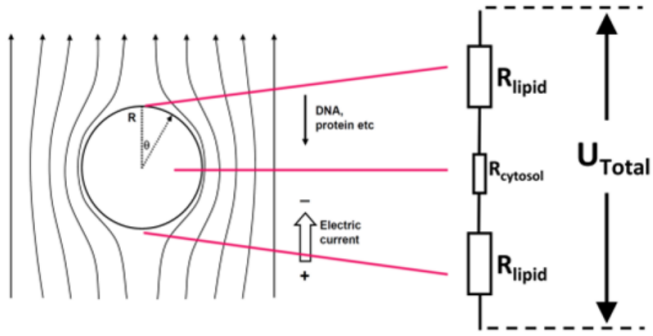
Electroporation is a widely used cellular delivery method especially for charged molecules such as DNA, RNA and proteins.



1. The cell membrane resembles an insulator and the electric current warps around a cell.
2. In a series circuit, voltage distributes in proportion to the resistance and the cell membrane shoulders most of the voltage applied on the cell.
3. Only one terminal surface is effective for electroporation of charged molecules such as DNA.
4. The voltage within the cell plasma is minimal and insufficient for DNA movement once it has crossed into the cell. Autonomous cellular mechanisms take over after electroporation for translocation of DNA within cell plasma.
5. The voltage on the cell nucleus is minimal and it is shouldered by the nucleus envelope. Inside the nucleus there is virtually no electric current and electroporation is not associated genotoxicity.

Nucleus Transfection is a Wrong Concept

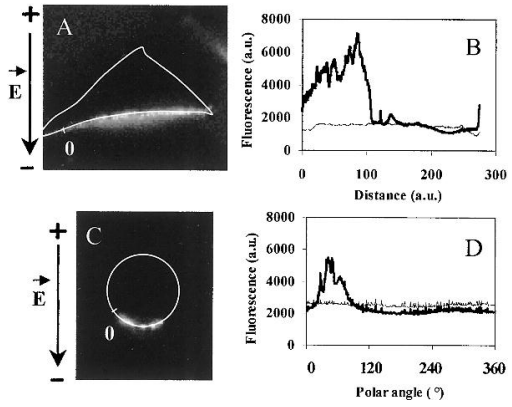
The cell membrane shoulders most of the electric potential applied on the cell and inside the cell, there is very little electric current or field distributed. The low internal potential is further distributed on the cell plasma and the cell nucleus, therefore the cell nucleus only gets a portion of the low electric field. Cell nucleus is smaller than a cell and require a higher potential to drive plasmids across, therefore it's not possible for the internal electric field to be high enough for plasmid delivery directly into the nucleus.



Certain vendor has claimed that their electroporation is special and plasmids can be delivered into the cell nucleus directly, without any experimental evidence.

The "direct-to-nucleus" concept is wrong as revealed by the mechanistic analysis. Also, Golzio et. al. published experimental findings that DNA is still near the cell membrane right after electroporation.

There is no need to worry about nucleus localization of plasmids as plasmids can travel to nucleus by themselves. The Celetrix system has been widely used on plasmid-based stable cell line generation, transposon mediated gene expression and Cas9 RNP mediated gene editing.

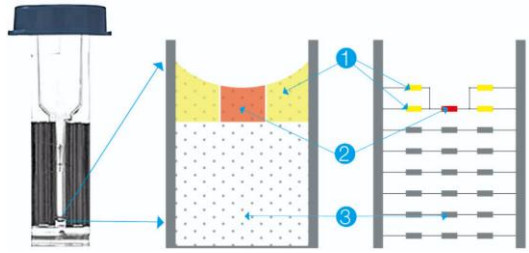


Direct visualization at the single-cell level of electrically mediated gene delivery Muriel Golzio, Justin Teissie, and Marie-Pierre Rols PNAS February 5, 2002. 99 (3) 1292-1297; https://doi.org/10.1073/pnas.022646499

A Farewell to Cuvettes

Surface Effect Warps Electric Field in Cuvettes

The liquid surface inside a cuvette is curved because of a small liquid-solid contact angle. The curvature alters the even distribution of voltage. The voltage distribution in the liquid can be simulated by a simplified equivalent circuit. The whole liquid is subdivided into resistors of roughly identical resistance. These resistors are connected into multiple series with the same end-to-end total voltage between the electrodes and the series are also arranged in parallel. In a series circuit, voltage is distributed in proportion to resistance. Each of these resistors would have the same voltage if the liquid surface is flat.

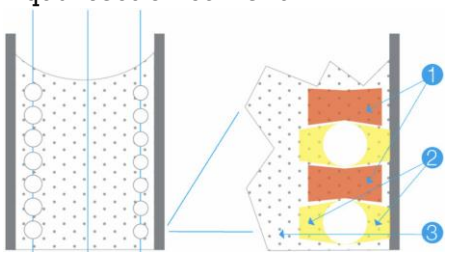


At the liquid corner (1), two resistors are first connected in parallel with the resistance cut in half and they are then connected in series to the center resistor (2). The corner resisters assume a lower voltage and the cells in the corners are insufficiently electroporated (1) . The center resistor is allocated a higher voltage and the cells in the center under the curved surface are killed by an excessive voltage (2). Therefore, the upper portion in the cuvette is a poor area for electroporation. Only the lower portion (3) is the good electroporation area unaffected by the surface warping effect.

1. Corner with lower resistance and voltage: no electroporation.
2. Center with higher voltage: cell death.
3. Lower portion: not affected by surface warping.

Bubbles Cause Current Turmoil in Cuvettes

Equal section current



1. High current between bubbles: cell death.
2. Low current before and after bubbles: no electroporation.
3. Away from bubbles: effective electroporation.

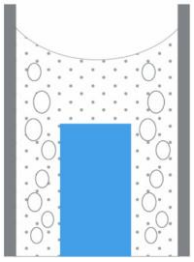
The electrode surface is quite large in cuvettes. Electrochemical reactions produce toxic agents and gas bubbles. The bubbles are a huge adverse factor in electroporation.

The bubbles are insulators and the electric current has to flow between the bubbles. The space between the bubbles thus has a higher current and the cells in this area are killed (1).

A bubble warps the electric current similar to the insulator cell shown in page 7. On the direction of total current, areas before and after the bubble (2) have a lower current and cells in these areas are not effectively electroporated. Only the middle portion of the liquid(3) away from the electrodes is the optimal area.

Limitation of Cuvettes

Within the cuvette, the upper portion is a poor area for electroporation as well as the two side portions adjacent to the electrodes.
After cutting out the poor areas, the real optimal area (shown as the blue block at the right) in the cuvette is small. For 1mm and 2mm cuvettes, there might be no optimal area at all since the bubbles can spread from the electrodes quite vigorously.



Advantages of Sealed Tubes

1. No surface warping effect with cylindrical liquid sample.
2. Small contact area between liquid and electrodes.
3. Very low production of toxic agents and air bubbles.
4. Bubbles compressed to prevent current disturbance.
5. All area in sealed electroporation tube optimal for electroporation.

The Celetrix™ Electroporation Tube

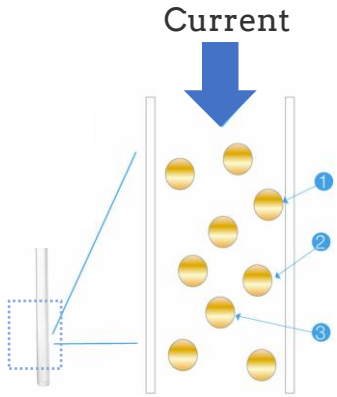
Limitations of capillary electroporation

The wall of the capillary is an insulator. When a cell is very close to the capillary wall, a narrow passage between the cell and the capillary wall is formed for the electric current to pass through. This narrow passage is allocated a higher voltage because liquid resistance is inversely proportional to the cross-sectional area. The effective electroporation surface on the cell is also increased. The capillary effect (CE) can be similarly achieved in a non-capillary solution by placing cells at a high concentration allowing them to be each other's next insulator.

The capillary effect is very sensitive to the distance between the cell and the capillary wall. When a cell is very close to the capillary wall (1), capillary effect (CE) is very strong and this cell would need a lower pulse voltage. As the cell-to-wall distance increases (2), capillary effect quickly weakens and this cell would need a higher pulse voltage. When the cell is placed about two cell diameters' distance from the capillary wall (3), there is almost zero capillary effect and this cell would require an even higher voltage.

The limitations of capillary electroporation:

1) Help is partial and unequal, inherently unsuitable for high efficiency;
2) Air bubbles not compressed, current disturbance and arcing;
3) Small volume, inconvenient and high cost with bulk cell samples;
4) Low power pulse generator, non-scalable.

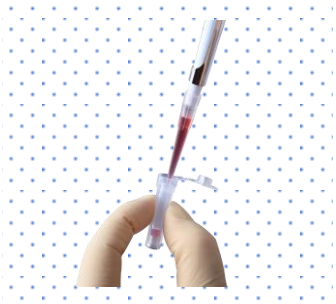


Since all cells in a capillary experience the same voltage pulse from a pulse generator, it is not possible for the pulse to be equally suitable for all three areas of cells. Therefore, the capillary mechanism is inherently limited.

Advantages of Celetrix™ Tube

	 Celetrix™ Electroporation tube	 Cuvette	 Capillary
Sample Volume	20ul - 10ml	100ul - 800ul or 20ul- 100ul	20ul- 100ul
Bubble Compression	Yes	No	No
Field Uniformity	High	Lowest	Lower
Cytotoxicity	Low	High	Medium
Precision	High	Low	Medium

The Celetrix™ Sealed Tube



1 . Uniform electric field for high efficiency electroporation.

2 .No limitations of cuvette or capillary.

3. Selection of sample volumes from small 20ul to large 10ml.

4.Fast and cost-effective for large samples.

Celetrix Citations (partial)

[1] Yin J, Lu R, Xin C, et al. Cas9 exo-endonuclease eliminates chromosomal translocations during genome editing.
Nat Commun, 2022, 13(1): 1204.doi:10.1038/s41467-022-28900-w

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Model

The basic model: SP100

1. Supports only 20ul and 100ul electroporation tubes;
2. Simplified single pulse for all cell types;
3. Voltage range 200- 1000V.

The upgraded model: LE+

1. For 20ul, 100ul and 200ul electroporation tubes;
2. New Cell Line mode and PBMC mode;
3. Optimized for large plasmid electroporation.

The upgraded model: EX+

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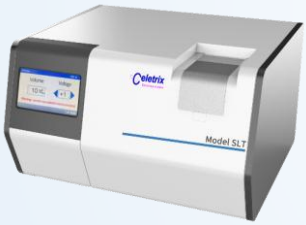

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2. Yeast: 20ul, 100ul, 200ul, 1ml electroporation tubes;
3. Pulse time adjustable 1ms steps;
4. Voltage range 2.5KV-15KV;
5. Truly No Arcing (Not detection and shut off);
6. Model UHV-Plus can electroporate cell, bacteria and yeast.

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Ver. 2.5