



Cyto Pulse Sciences, Inc.

A medical device and treatment development company

Data Sheet

CytoLVT-S™ Large Volume Transfection System

Cyto Pulse Electroporation and Electrofusion Systems for:
In vivo therapeutic delivery
Gene therapy
Immunotherapy
Hybridoma production
Vaccine delivery
Nuclear transfer

mRNA delivery
siRNA delivery
plasmid delivery

Efficient Large Volume Transfection of Cells

Laboratory-scale transfection systems based on electroporation are limited to treatment volumes that fit into standard cuvettes of 0.7 ml or less. The CytoLVT-S™ transfection system overcomes this limitation and allows simultaneous treatment of up to 20 ml of cell suspension in a single chamber. Successful large volume electroporation of cells is made possible by a combination of patent pending chamber dimensions and proprietary low conductivity medium. These advances in technology avoid the excessive heating problem typically found in large volumes, thus retaining high cell viability necessary for clinical and industrial applications. Fast sub-second runtimes mean less time that cells are out of culture medium.

Potential Clinical Uses:

- Transfect cells such as bone marrow cells to produce or replace a missing protein
- Deliver siRNA to suppress gene expression
- Deliver genes for permanent gene correction
- Load cells with a drug for drug delivery
- Cancer immunotherapy¹

Industrial Uses

- Transfect eukaryotic cells for protein production in a fermenter
- Use in helper virus production of non-replicating viruses



Direct Scale-up – Transfection protocols readily scale-up from research level transfections in 4 mm electroporation cuvettes to 20 ml large volume transfection without modification. For all practical purposes regarding cell handling and electroporation procedures, the large volume chamber is simply treated as a standard cuvette. No new complicated processes need to be learned.

Cytoporation® Medium Synergy – Cyto Pulse electroporation medium used with the CytoLVT-S™ system has proven efficiency with a number of cell lines. It is compatible with a large range of transfectants including DNA, RNA, siRNA, oligonucleotides, etc. and can be directly diluted with complete medium in post-electroporation culture.

<u>Cell Line</u>	<u>Percent DNA GFP+</u>	<u>Pulse protocol</u>
K562	62%	2 Pulses, 1600 V/cm, 400 µS plus 2 Pulses, 800 V/cm, 800 µS
A20	60%	2 Pulses, 2000 V/cm, 200 µS plus 2 Pulses, 800 V/cm, 800 µS
HEK293	80%	2 Pulses, 1600 V/cm, 200 µS plus 4 Pulses, 400 V/cm, 800 µS
CHO-K1	77%	2 Pulses, 2000 V/cm, 400 µS plus 2 Pulses, 1000 V/cm, 800 µS

Smooth Usability – All operating controls are located on a simple touch screen on the front panel. Data is quickly retrieved by USB key and can be analyzed for detailed pulse characteristics including pulse voltage and pulse current samples. The system design contains no moving parts (e.g. pumps, fans, etc.) to increase maintenance reliability.

Pulse Agile® Advantage – Transfection efficiency and cell viability are enhanced by specialized electrical pulse waveforms, particularly for larger polynucleotide delivery such as DNA plasmids. Cyto Pulse patented *PulseAgile®* technology combines a unique sequence of short high-intensity pulses to porate cell membranes, followed by long low-intensity pulses to further drive transfectants into cells via electrophoresis while maintaining viability.

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¹ Markovic S.N., Dietz A.B., et al. Preparing clinical-grade myeloid dendritic cells by electroporation-mediated transfection of in vitro amplified tumor-derived mRNA and safety testing in stage IV malignant melanoma. *Journal of Translational Medicine*. 2006 Aug; 4:35.

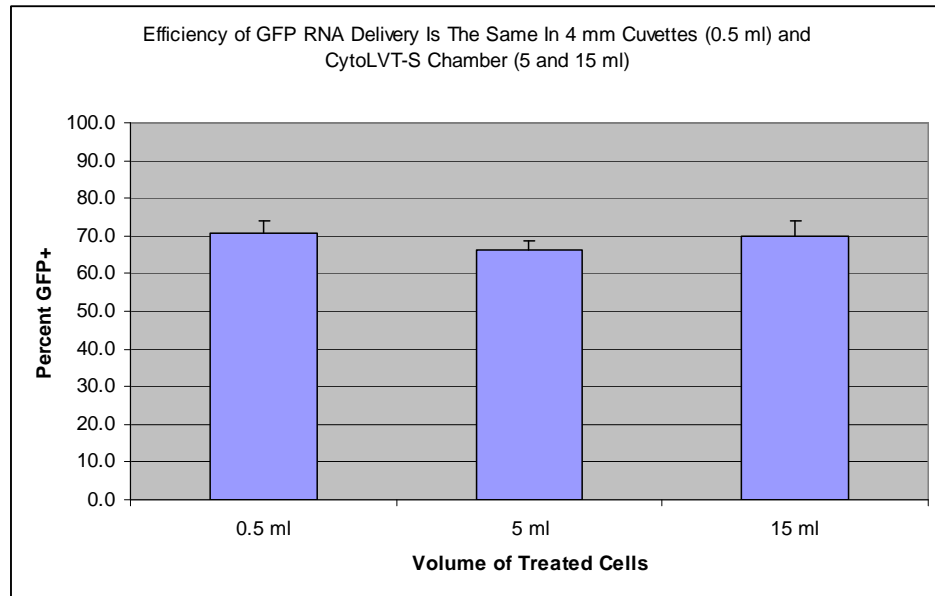
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At a Glance – Scale-up of K562 Cell mRNA Transfection

K562 cells are myelomonocytic cells commonly used for natural killer target cell assays. These cells were used in transfection experiments to demonstrate the ability to scale-up transfections from laboratory cuvettes to volumes commonly used in the CytoLVT-S™ system. Reporter gene mRNA (GFP) was prepared for the experiments. Cells were suspended at a cell density of 20 million cells/ml and 40 µg/ml of GFP mRNA was added to the cell suspension. The small, 0.5 ml, volume transfection was done using standard 4 mm gap cuvettes. The remainder of transfections were done using the CytoLVT-S™. 24 hours later, the percent transfection was determined by flow cytometry.

Transfection efficiency was the same with all three volumes. The pulse parameters (1900 V/cm, 100 µs duration, one pulse) were the same for all three volumes. This shows that pulse parameters can be optimized in smaller volumes to conserve material and directly scaled-up to the CytoLVT-S™ chamber.



The CytoLVT-S™ Electroporation System Includes

- CCEP-100 Waveform Generator
- Large Volume Chamber holder
- Chamber Electrode
- Electrode Connector Cable
- One 500 ml bottle Cytoporation® Low Conductivity Medium
- User Manual

Specifications

User Interface	Resistive Touch Screen Display, Footswitch
Chamber Electrode Gap	1mm; 4 mm; or 6 mm
Chamber Volume	2 to 30 ml
Chamber Connector	Male and Female Leur Locks with Relief Valve
Pulse Amplitude	50 to 1000 volts
Pulse Width	0.050 to 10 ms
Pulse Interval	0.200 to 1000 ms (5 kHz to 1 Hz)
Data Access	USB Flash Key

Cyto Pulse License Required

Cyto Pulse has patents, patents pending, and other intellectual property on the CytoLVT-S™ system and it is only supplied directly by Cyto Pulse under license agreement for commercial and clinical applications.

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