



Cyto Pulse Sciences, Inc.

A medical device and treatment development

Data Sheet

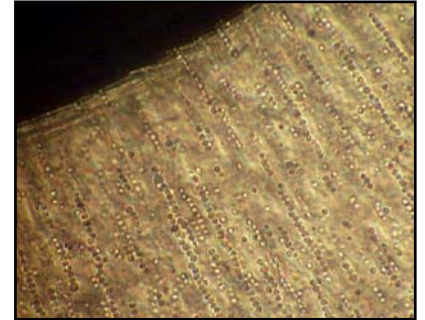
Hybrimmune™ - The Hybridoma Production System

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

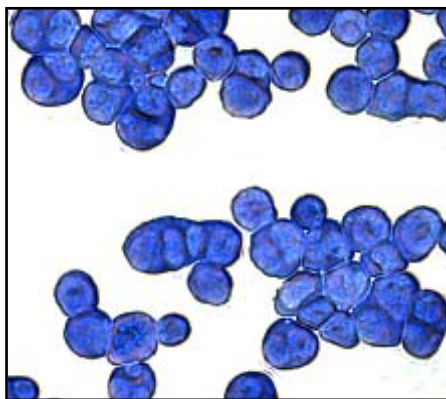
mRNA delivery
siRNA delivery
plasmid delivery

Repeatable Large Volume Cell Fusion by Electrofusion

Electrofusion is a technique to fuse different cell types. Electrofusion uses non-uniform alternating current (AC) fields to apply a force to a neutral particle and move that particle. This process, called dielectrophoresis, causes the cells to align. Cyto Pulse technology permits user control of the force that is applied to the cells during alignment. First, a minimum force is applied to cause the cells to align in an orderly manner without producing disruptive phenomena such as turbulence and heating. After the cells are aligned, additional force can then be applied to compress the cells into tight contact with a large common surface area. After alignment and compression, an electroporation pulse is immediately applied to disrupt the cell membranes allowing the contents of the cells to mix and fuse. Finally a post fusion pulse AC waveform is applied to hold the cells in place allowing the fusion process to mature. This process is carried out in low conductivity medium and requires no additional chemicals.



Hybridoma Production (clone efficiency at least 10X greater than PEG)



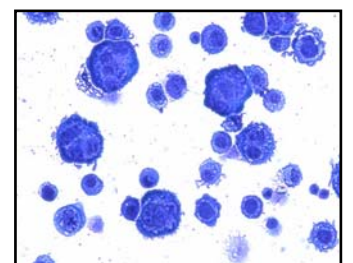
Experiment Number	Antigen Specific Clones	
	E-Fusion	PEG
1	20	0
2	10	0
3	400	23
4	151	21
Mean	145	11

E-fusion versus PEG: Transgenic human Ab producing mice were used in experiments comparing efficiencies of e-fusion to PEG fusion. Each experiment used a portion of the same splenocyte preparation from mice immunized with tetanus toxoid (TT) for comparison of the E-fusion and PEG fusion methods. Results shown are the number of TT antigen-specific clones generated by each method normalized to the same number of cells. These data are representative of additional experiments (not shown) utilizing four different antigens. Taken together, all experiments showed E-Fusion generated approximately ten-fold more antigen specific antibody clones relative to PEG fusion. Data provided courtesy of M. Coccia, Ph.D., Platform Development Group at Medarex, Inc., Milpitas, CA.

Image shows Cytospin™ prepared and Wrights-Giemsa stained SP2/0 cells 30 minutes following large scale e-fusion. In these e-fusion experiments more than 75% of cells in the final population were fused with 2 to 3 nuclei per cell.

Tumor-Dendritic Cell Hybrid Production

Electrofusion of mature dendritic cells with tumor cells is a method for introducing tumor antigen directly into dendritic cells. The physical process is similar to that used for hybridoma production. The picture on the right shows electrofusion of dendritic cells with A549 human lung carcinoma cells. Fusion efficiencies of 10% were obtained. Cells were fused at 8 million cells/ml. Data provided courtesy of Dr. Katrina Trevor, Arizona Cancer Center.



Headquarters:
810 Cromwell Park Dr, Suite T
Glen Burnie, MD 21061, USA
Voice: 410-787-1890
Fax: 410-787-1891
Web: www.cytopulse.com

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The Cyto Pulse Hybrimmune Electrofusion System Includes

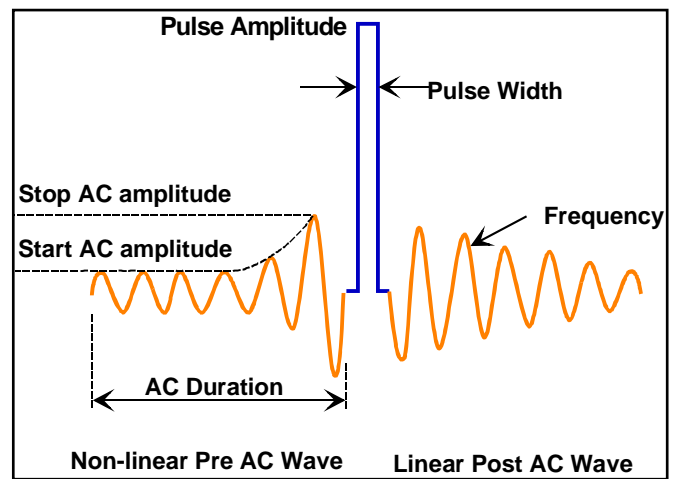
- CEEF-50B Waveform Generator
- User Interface Application Software, Windows® based
- Two Chambers
 - One 2 ml chamber for Optimization (use inverted or regular microscope)
 - One 9 ml production chamber
- One 500 ml bottle Cytofusion® Low Conductivity Medium, CPS-LCMC
- User Manual
- Laptop or PC required, not included

Waveforms

The waveform parameters are set using the software interface. The following parameters for pulse, pre-fusion pulse AC and post-fusion AC are available:

Pulse amplitude 100-1000 v
Pulse width 20 to 1000 µs

Start AC 5-75 v-peak
 Stop AC 5-75 v-peak
 Function Constant
 Linear change
 Non-linear change
 Frequency 0.2 to 2.0 Mhz
 Duration 0 to 60 sec



Chamber Characteristics

	Optimization CHNF-2R-381-8333	Production CHNF-9R-381-8333
Volume	2 ml	9 ml
Outer ID	45.72 mm	45.72 mm
Inner OD	38.10 mm	38.10 mm
Gap	3.81 mm	3.81 mm
Well Height	5 mm	18 mm
r ₁ /r ₂	0.8333	0.8333

The electrical characteristics of the two chambers are identical. The small chamber has a transparent bottom; cell alignment can be observed by inverted or regular microscope. The large chamber is used after waveforms have been optimized. Cleaning is accomplished using NaOH, sterilization by EtOH, and Spor-Klenz® for spores and mycoplasma.

Cyto Pulse License Required

Cyto Pulse has patents pending and other intellectual property on the chambers, waveforms and medium. The system is only supplied directly by Cyto Pulse under license agreement for commercial applications.

Cytospin is a Trademark of Shandon Inc.

Specifications may change without notice



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