Electroporation & Electrofusion Products

Systems for Hybridoma Production, Vaccine Development & Large Volume Electroporation
MAXIMUM EFFICIENCY DNA DELIVERY

Effectively introduced DNA vaccines represent a powerful and safe means for stimulating an immune response that recognizes and eliminates target molecules in the body. However, traditional DNA vaccine delivery systems, such as gene gun delivery, or injection alone suffer from poor efficiency. The BTX® AgilePulse™ In Vivo System provides an intra-dermal/muscular electroporation solution to produce maximum transfection efficiency. The AgilePulse In Vivo System can be purchased with software supporting intra-dermal (ID) or intra-muscular (IM) applications.

For vaccine applications, DNA vaccination through the dermal layer is preferred since it is an easily accessible site that is immunologically active. After direct injection of plasmid DNA into layers of skin or muscle and target cells for high efficiency gene delivery.

Safety – Each needle electrode array comes with a safety cover and easy grip sides to make the electrode insertion procedure simple and safe.

Simple, Fast Treatment – Simple yet effective intra-dermal or intra-muscular electrode design allows for shorter delivery times of less than a second. The electrodes provide a uniform pulse to cover and target larger areas of tissue using a single pulsing application.

Optimal Design – Miniature, 2-mm length needles easily penetrate the layers of skin or muscle and target cells for high efficiency gene delivery.

At a Glance – Electroporation Increases the Immune Response

Dermal delivery of DNA vaccine along with electroporation using the AgilePulse™ In Vivo System increases the immune response of PSA-specific CD8+ T-cell response when standard protocols do not.

Methods: C57Bl/6 mice were immunized once with 10 pVAX-PSA/20 ml PBS intradermally (ID) or intramuscular injection (IM), with or without electroporation (See Figure 1 on facing page).

Temperature 10 to 40 ºC

Mains Voltage 100 to 250 VAC

Fuse 5 Amp Sl-Bio®, 5 mm x 20 mm

Software IM (Intra-muscular) or ID (Intra-dermal)

Figure 1

IN VIVO APPLICATIONS
• Intra-muscular gene delivery
• Intra-dermal vaccine applications
• Electrochemotherapy
• Drug delivery

WAVEFORM GENERATOR SPECIFICATIONS

User Interface Touch Screen Display, Footswitch

Voltage Range 50 to 1000 volts

Pulse Width Range 0.050 to 10 ms

Pulse Interval 0.200 to 1000 ms (5 kHz to 1 Hz)

Data Export USB Flash Key

Dimensions (with handle) 32 cm w x 20 cm h x 40 cm

(12.6 in w x 7.9 in h x 15.7 in)

Weight 25 pounds, 11.3 kg

OPERATING INFORMATION

Catalog # Description

AgilePulse ID System
47-0400N AgilePulse ID System Includes:
• AgilePulse ID Generator 47-0401N
• Handle for Parallel Needle Array 47-0000
• Parallel Needle Array, 4 Needle x 4 mm gap, 2 mm length, 3 each
• Parallel Needle Array, 6 Needle x 4 mm gap, 2 mm length, 3 each

AgilePulse IM System
47-0500N AgilePulse IM System Includes:
• AgilePulse IM Generator 47-0501N
• Handle for Parallel Needle Array 47-0000
• Parallel Needle Array, 4 Needle x 4 mm gap, 5 mm length, 3 each
• Parallel Needle Array, 6 Needle x 6 mm gap, 2 mm length, 3 each

ID Needle Arrays
47-0040 4 Needle Array x 4 mm gap, 2 mm length
47-0050 6 Needle Array x 4 mm gap, 2 mm length
47-0060 6 Needle Array x 6 mm gap, 2 mm length

IM Needle Arrays
47-0043 3 Needle Array x 4 mm gap, 3 mm length
47-0045 4 Needle Array x 4 mm gap, 5 mm length
47-0070 6 Needle Array x 6 mm gap, 10 mm length
47-0086 6 Needle Array x 6 mm gap, 16 mm length
47-0080 6 Needle Array x 6 mm gap, 25 mm length

Accessories
47-0320 Footswitch
Hybrimune™ - Hybridoma Production System

Hybrimune Production—10-Fold Greater Efficiency with Hybrimune™

The Hybrimune™ System is designed for fast, efficient hybridoma production as a first step in monoclonal antibody production. Electrofusion combines cell positioning and electroporation into a single, robust process for maximum efficiency. The innovative fusion chamber design permits direct scale-up of pulse parameters to production volumes. A 10-fold greater efficiency over standard polyethylene glycol (PEG) fusion has been demonstrated for Hybrimune™ E-Fusion (see Table 1). Transgenic, human-Ab producing mice immunized with tetanus toxoid (TT) provided spleens for fusion to SP2/O mouse myeloma cells.

HYBRID YIELDS GENERATED BY ELECTROFUSION ARE 10-FOLD GREATER THAN PEG

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>E-Fusion</th>
<th>PEG</th>
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<tbody>
<tr>
<td>1</td>
<td>20</td>
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<tr>
<td>2</td>
<td>10</td>
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<tr>
<td>Mean</td>
<td>145</td>
<td>11</td>
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</table>

Table 1: Four different transgenic mice expressing Abs to human Ag were used to compare the efficiency of E-fusion to PEG fusion.

FUSION CHAMBER SPECIFICATIONS

Both the optimization and production chambers have been engineered to have identical electrical characteristics to facilitate direct scale-up to production, once pulse parameters have been optimized. In addition, the small chamber has a transparent bottom to permit visualization of the cell alignment by inverted or regular microscope.

The fusion chamber is designed to have identical electrical characteristics to facilitate direct scale-up to production. In addition, the small chamber has a transparent bottom to permit visualization of the cell alignment by inverted or regular microscope. Compatible with ETQ, HiP, and HiP 1500 systems.

Electrofusion combines cell positioning and electroporation into a single, robust process for maximum efficiency. The innovative fusion chamber design permits direct scale-up of pulse parameters to production volumes. A 10-fold greater efficiency over standard polyethylene glycol (PEG) fusion has been demonstrated for Hybrimune™ E-Fusion (see Table 1). Transgenic, human-Ab producing mice immunized with tetanus toxoid (TT) provided spleens for fusion to SP2/O mouse myeloma cells.

ELECTROFUSION APPLICATIONS

- Monoclonal Antibody Production
- Dendritic – Tumor Cell Fusions

WAVEFORM GENERATOR SPECIFICATIONS

The Hybrimune™ Waveform Generator is programmed using the Application Software.

The following parameters are available:

- **Pulse Function**: Constant, linear, non-linear
- **Pulse Amplitude**: 100 to 1000 V
- **Pulse Width Range**: 20 to 1000 ms
- **AC Start Peak Range**: 5 to 75 V
- **AC Stop Peak Range**: 5 to 75 V
- **AC Frequency**: 0.2 to 2.0 MHz
- **AC Duration**: 0 to 60 sec

For reuse, the fusion chamber can be cleaned with NaOH, sterilization by EtOH, or Spor-Klenz® for spores and mycoplasma.

License Requirements

The use of the Hybrimune as a commercial and therapeutic system requires a license from Cellectis. Please contact BTX for more information.

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<td>Hybrimune™ Electrofusion System Includes: Hybrimune waveform generator, 2 ml and 9 ml coaxial chambers, BTXpress Cytofusion® Medium C, user interface software, cables and manual. Requires Windows based laptop or PC (not included).</td>
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<tr>
<td>47-0301</td>
<td>User-Interface Application Software</td>
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<tr>
<td>47-0030</td>
<td>2 ml Optimization Chamber</td>
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<td>47-0020</td>
<td>9 ml Production Chamber</td>
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<td>BTXpress Cytofusion® Medium C, 500 ml</td>
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The system includes a user-friendly, programmable waveform generator engineered to provide uniform electric fields in a stable temperature environment, for excellent cell viability.

Cells and polynucleotide are suspended in our proprietary BTXpress Cytoporation® Medium T and transferred via sterile syringe to the large-volume electroporation chamber where a programmed sequence of electric pulses is applied. First, a sequence of short, high-intensity pulses open pores in the cell membranes, followed by long, low-intensity electric pulses to further drive transfectants into bioreactors.

Simple User Interface  All controls are operated with the 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.

Pulse Agile® Advantage  Transfection efficiency and cell viability are enhanced by specialized, programmable electrical pulse waveforms, particularly important for larger polynucleotide delivery such as DNA plasmids. The patented Pulse Agile® 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 cell viability.

Features & Benefits

Scale-up  – Transfection protocols readily scale-up from standard laboratory cuvettes to large-volume transfection in the AgilePulse MAX™ system.

Maximal Efficiency with Cytoporation® Medium  – BTXpress® Cytoporation® Medium T used with the AgilePulse MAX™ system has been optimized for maximal efficiency with a number of cell lines, including K562, A20, HEK293 and CHO-K1. It is compatible with a large range of transfectants including DNA, RNA, siRNA, and oligonucleotides. It can be directly diluted in complete growth medium for post-electroporation cell culture.

User Interface  – All controls are operated with the 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.

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At 24 hours post-electroporation, the percent transfection was comparable for all three volumes. At 48 hours post-electroporation, the percent transfection was determined by flow cytometry.

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At 24 hours post-electroporation, the percent transfection was comparable for all three volumes. At 48 hours post-electroporation, the percent transfection was determined by flow cytometry.

Efficiency of GFP RNA Delivery is the same in 4 mm Cuvettes (0.5 ml) and AP MAX Chamber (0.5 and 6 ml). The large volume transfections were performed with the AgilePulse MAX™ using a 4 mm gap large volume chamber. Identical pulse parameters were applied to all three volumes.

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WAVES • AGILE

BTX Electroporation & Electrofusion Products

Effects & Benefits

- Transfect 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 immunotherapy
- Transfect eukaryotic cells for protein production in bioreactors
- Large-scale production of replication-deficient viruses
- Large-scale peptide production

AT A GLANCE SCALE-UP OF K562 CELL mRNA TRANSFECTION

The figure below demonstrates the simple direct scale-up of transfection using the AgilePulse MAX™ System. K562 cells (myelomonocytic cells commonly used for natural killer target cell assays) were transfected with GFP+ mRNA in both laboratory cuvettes (0.5 ml) and the large-volume AgilePulse MAX™ System (0.5 and 6 ml). The efficiency of transfection was comparable for all three volumes.

Methods: Cells were suspended to a cell density of 20 million cells/mL in Cytoporation® Medium. GFP mRNA was added to the cell suspension to a final concentration of 40 pg/mL. The small 0.5 mL volume transfections were carried out using standard 4 mm gap electroporation cuvettes. The large volume transfections were performed with the AgilePulse MAX™ using a 4 mm gap large volume chamber. Identical pulse parameters were applied to all three volumes.

At 24 hours post-electroporation, the percent transfection was comparable for all three volumes. At 48 hours post-electroporation, the percent transfection was determined by flow cytometry.

Efficiency of GFP RNA Delivery is the same in 4 mm Cuvettes (0.5 ml) and AP MAX Chamber (0.5 and 6 ml).

Volume of Treated Cells

Percent GFP+ Efficiency of GFP RNA Delivery is the same in 4 mm Cuvettes (0.5 ml) and AP MAX Chamber (0.5 and 6 ml).
BTXpress® Cytofusion®

FEATURES & BENEFITS
- Optimized for eukaryotic electrofusion applications
- Maximum fusion efficiency, high cell viability
- Stable environment for cell alignment
- Low conductivity means minimal heating
- Physiological pH and Balanced Osmolarity
- Contains no animal products

ADVANCED BUFFER FOR HIGH PERFORMANCE CELL FUSION

BTXpress Cytofusion® Medium C is an advanced electrofusion buffer designed for use with the BTX Hybrimune System for high performance cell fusion applications. The low conductivity buffer is specially-formulated to minimize cell turbulence during cell alignment and heating during electrofusion for robust cell fusion efficiency and high cell viability.

BTXpress Cytofusion® Medium C is sterile filtered from the highest quality non-animal, medical-grade reagents. It is the buffer of choice for many commercial biotech and pharmaceutical companies in their standard hybridoma production process for monoclonal antibody discovery.

CYTOFUSION® MEDIUM SPECIFICATIONS

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>500 ml</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>270-290 mOsm/L</td>
</tr>
<tr>
<td>Conductivity @ 25°C</td>
<td>0.080 ± 0.005 mS/cm</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>&lt; 0.25 EU/ml</td>
</tr>
<tr>
<td>Sterility</td>
<td>Sterile filtered</td>
</tr>
<tr>
<td>Storage</td>
<td>2 to 8 °C</td>
</tr>
<tr>
<td>Shelf-life</td>
<td>18 months from production date (shipped within 6 months of production)</td>
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RECOMMENDATIONS FOR USE

Maintain a sterile environment. Standard aseptic techniques are recommended to avoid contamination during use.

Thorough, repeated washing. BTXpress Cytofusion® Medium C is a low conductivity medium designed for efficient electrofusion. Trace amounts of high conductivity solutions such as PBS or tissue culture growth medium can disrupt the fusion process. Therefore, it is critical to wash the cells thoroughly with BTXpress Cytofusion® Medium C prior to the fusion process. For up to 5 x 10^7 cells, at least two washes in BTXpress Cytofusion® Medium C are recommended. For more than 5 x 10^7 cells, at least three washes are recommended.

Thoroughly clean electrofusion chamber. To avoid other sources of ionic contamination, clean the electrofusion chamber after each use and rinse thoroughly with sterile, deionized water.

Room Temperature Electrofusion. For maximum efficiency cell fusion, use BTXpress Cytofusion® Medium C at room temperature. Cell washes prior to the final wash may be carried out at 4°C.

Minimize time in buffer. While Cytofusion® Medium C is non-toxic, it does not contain nutrients to support cell viability over long periods of time. For best results, minimize the time that cells are suspended in BTXpress Cytofusion® Medium C. It is not recommended that cells remain in BTXpress Cytofusion® Medium C longer than 30 minutes subsequent to the final wash.

5:1 Direct Dilution. Post-electrofusion, cells in BTXpress Cytofusion® Medium C can be diluted in cell culture medium without washing the cells. A minimum dilution of five parts complete culture medium to one part BTXpress Cytofusion® Medium C is recommended. Alternatively, cells may be washed in growth medium to completely remove BTXpress Cytofusion® Medium C prior to culturing.

STORAGE INFORMATION

Store at 2-8°C after opening. Short term storage (i.e. for shipping) at -20°C to +50°C for 7 days is acceptable. Contents may separate upon freezing. If frozen, mix well before use.

WARNINGS & DISCLAIMERS

Do not use if tamper-proof seal is missing or bottle is damaged. Damage to the bottle or deliberate tampering may result in contamination of this product. Check product for clarity before use.

BTXpress Cytofusion® Medium is intended for research and investigational purposes only. It is not intended for human use. This product is not considered to be hazardous based on evaluations made under OSHA Hazard Communication Standard 29 CFR 1910.1200.

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<tr>
<td>47-0001</td>
<td>BTXpress® Cytofusion® Medium C</td>
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BTX Electroporation & Electrofusion Products

toll free 800.272.2775 • local 508.893.8999 • email support@hbiosci.com • www.btsxonline.com
BTXexpress® Cytoporation®

CYTOPORATION® MEDIA SPECIFICATIONS

<table>
<thead>
<tr>
<th>Specification</th>
<th>T Media</th>
<th>T4 Media</th>
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<tbody>
<tr>
<td>Volume</td>
<td>500 ml</td>
<td>500 ml</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>270-290 mOsm/L</td>
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</tr>
<tr>
<td>Conductivity @ 25°C</td>
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<td>3.45 ± 0.05 mS/cm</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 ± 0.2</td>
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</tr>
<tr>
<td>RNase</td>
<td>None Detected</td>
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Do not use if tamper-proof seal is missing or bottle is damaged. Damage to the bottle or deliberate tampering may result in contamination of this product. Check product for clarity before use.

Cytoporation® Media T & T4 is intended for research & investigational purposes only. It is not intended for human use. This product is not considered to be hazardous based on evaluations made under OSHA Hazard Communication Standard 29 CFR 1910.1200.

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<td>47-0002</td>
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</tr>
<tr>
<td>47-0003</td>
<td>BTXpress™ Cytoporation® Media T4</td>
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RECOMMENDATIONS FOR USE

- Maintain a sterile environment. Standard aseptic techniques are recommended to avoid contamination during use.
- Thorough, repeated washing. Cytoporation® Media T & T4 are low-conductivity solutions designed for efficient electroporation. Trace amounts of high conductivity solutions such as PBS or tissue culture growth media can disrupt the electroporation process. Therefore, at least two full washes in Cytoporation® Media T or T4 are recommended prior to electroporation.
- Minimize time in buffer. While Cytoporation® Media T & T4 are non-toxic, they do not contain nutrients to support cell viability over long periods of time. For best results, minimize the time that cells are suspended in Cytoporation® Media T or T4.
- 5:1 Direct Dilution. Post-electroporation, Cytoporation® Media T & T4 can be directly diluted in cell culture media without washing the cells. A minimum dilution of five parts complete culture media to one part Cytoporation® Media T or T4 is recommended. Alternatively, cells may be washed in growth media to completely remove Cytoporation® Media T or T4 prior to culturing.

FEATURES & BENEFITS
- Optimized for eukaryotic electroporation applications
- Maximum transfection efficiency, high cell viability
- Low conductivity means minimal heating or electrode arcing
- Two formulations available – optimized conductivity to cell type
- Physiological pH and balanced osmolarity
- Contains no animal products
- Certified RNase and DNase free

ADVANCED BUFFER FOR HIGH PERFORMANCE TRANSFECTION

BTXpress Cytoporation® Media T & T4 are advanced electroporation buffer designed for use with the AgilePulse MAX™ Large Volume Electroporation System for ex vivo in vitro delivery of DNA, RNA, oligonucleotides, and siRNA. The low conductivity buffer is specially formulated to minimize heating of solution during large volume electroporation for maximum transfection efficiency and high cell viability.

BTXpress Cytoporation® Media is sterile filtered from the highest quality non-animal, medical-grade reagents. Two formulations with different conductivities are available for optimum conductivity for each eukaryotic cell types. Buffer can be directly diluted in complete growth media for post-electroporation cell culture.

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- Maintain a sterile environment. Standard aseptic techniques are recommended to avoid contamination during use.
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For further references regarding specific applications and optimization, please contact BTX Technical Support:

BTX-Division of Harvard Apparatus
84 October Hill Road Holliston, MA 01746
call 1-508-893-8999 • toll free 1-800-272-2775
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website www.btxonline.com

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