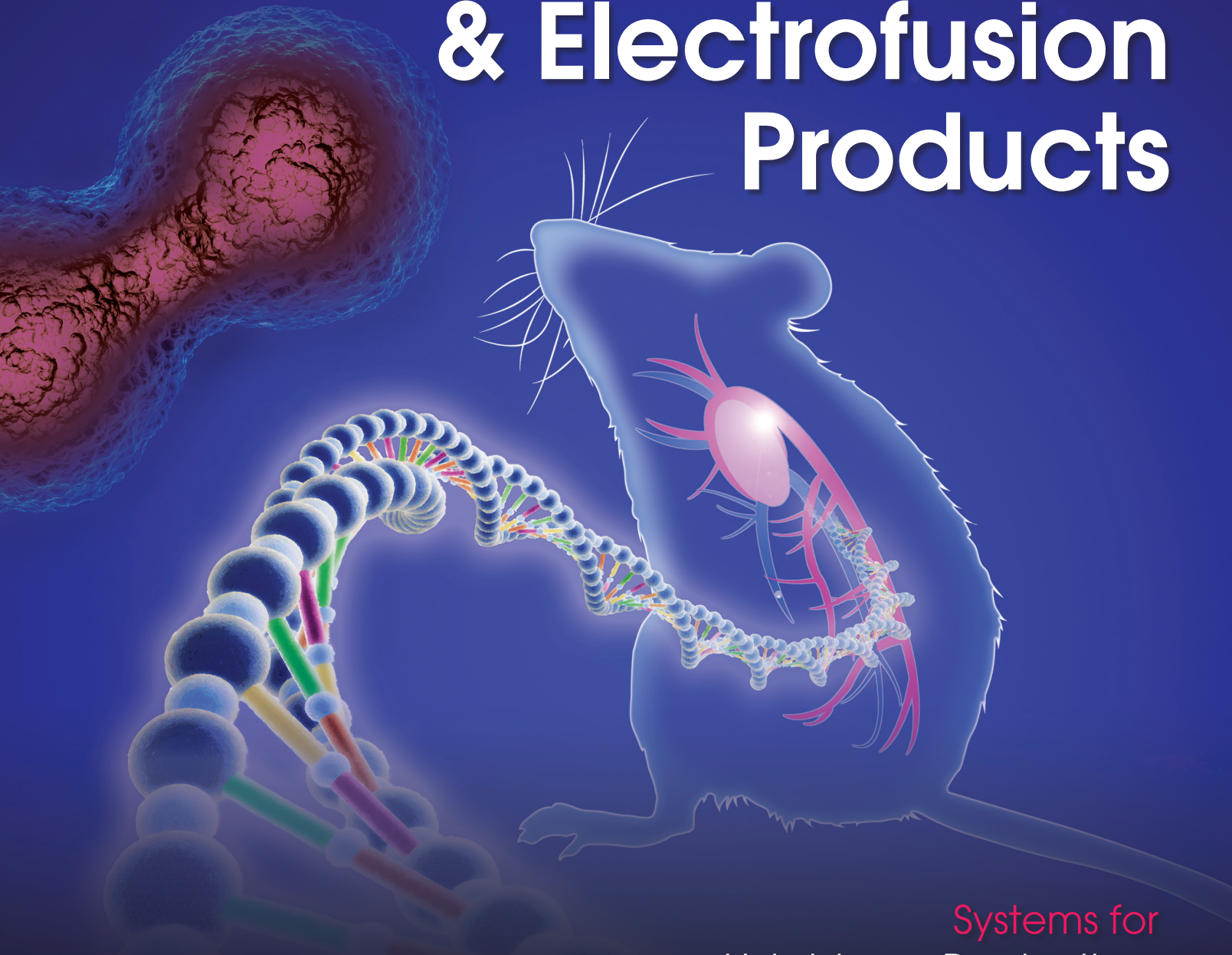


BTX[®]

The Electroporation Experts

Electroporation & Electrofusion Products



Systems for
Hybridoma Production,
Vaccine Development &
Large Volume Electroporation

AgilePulse™ In Vivo System for Vaccine Development



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 in the dermal layer, a programmed sequence of electric pulses is applied through a miniature parallel-needle electrode array to promote cellular uptake and transfection. Cells in the surrounding tissue are transfected, including dendritic antigen-presenting cells and mesenchymal origin cells.

Gene expression stimulates the immune system to respond to the secreted antigen. Gene expression in skin is 100-fold higher when delivery is enhanced by electroporation compared to simply injecting plasmid DNA.

FEATURES & BENEFITS

The AgilePulse™ In Vivo System is well-suited for applications requiring robust immune responses includes gene therapy and cancer vaccines. The AgilePulse In Vivo System includes a user-friendly, programmable waveform generator with patented Pulse Agile® technology and a miniature parallel-needle electrode array.

Simple User Interface – Touch screen user interface, USB key data storage, and Windows® Mobile 6.0 operating system. The system automatically stores a digitized record of all pulse parameters for quality control. EN 60601-1, EN 60601-1-1 EN 60601-1-2, UL60601-1 and CSA 601.1 compliant.

Pulse Agile® Advantage – The patented Pulse Agile® technology combines a unique sequence of short, high-intensity pulses to open pores in cell membranes, followed by long, low-intensity pulses to further drive the DNA into cells via electrophoresis. The adjustable pulses improve efficiency while maintaining cell viability. The specialized use of the Pulse Agile® waveform has shown to significantly enhance antigen specific CD8+ T-cell response when standard protocols do not.

Uniform, Reliable Electroporation – AgilePulse parallel-needle arrays produce uniform electric fields across the treatment area for a more thorough transfection of the tissue. Electrode impedance is monitored through software analysis to ensure reliable electrode placement in tissue each and every time.

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.

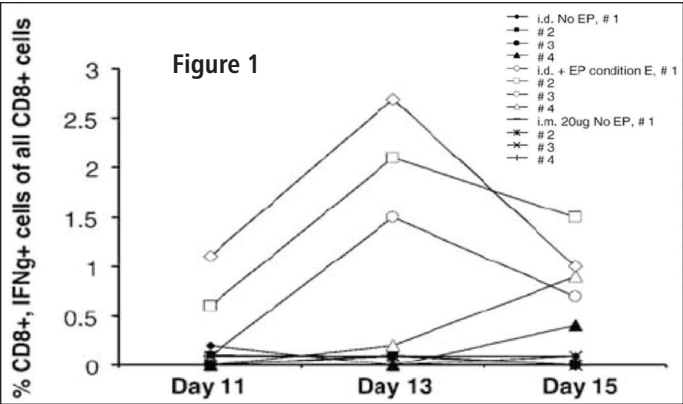
Safety – Each needle electrode array comes with a safety cover and easy grip sides to make the electrode insertion procedure simple and safe.

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 cells over intradermal (id) alone or intramuscular injection (im), with or without electroporation (See Figure 1 on facing page).

Methods: C57Bl/6 mice were immunized once with 10 pVax-PSA/20 ml PBS intradermally (id) on each flank with or without electroporation (EP) or intramuscular (im) in each TA muscle. Blood was collected on days 11, 13 and 15 and effector cells were stimulated for 4 h with 100 nM PSA-derived peptide psa65-73 or a control peptide GP33. The activated CD8+ T cells were quantified by intracellular cytokine staining for IFN-γ and analyzed by flow cytometry.

Roos, A-K, et al., 2006 Molecular Therapy 13(2):320-327.



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 Temperature	10 to 40 °C
Mains Voltage	100 to 250 VAC
Fuse	5 Amp Slo-Blo®, 5 mm x 20 mm
Software	IM (Intra-muscular) or ID (Intra-dermal)



ORDERING INFORMATION

Catalog #	Description
AgilePulse ID System	
47-0400N	AgilePulse ID System Includes: <ul style="list-style-type: none">• 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, 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: <ul style="list-style-type: none">• 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



Hybridoma 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

Antigen Specific Clones		
Experiment Number	E-Fusion	PEG
1	20	0
2	10	0
3	400	24
4	151	21
Mean	145	11

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

Source: Data courtesy of M. Coccia, PhD, Platform Development Group, Medarex Inc, Milpitas, CA)

Fusion Methods: PEG fusion was performed with standard protocols. For E-Fusion, mouse and SP2/O cells were washed twice in Cytofus medium then mixed in the fusion chamber and tri-phasic pulse applied. Cells were recovered after 30 min and cultured in 96 well plates at 5000 cells/ml. Antigen-specific clones were counted using ELISA or HTRF, normalized to 100M cells.

FEATURES & BENEFITS

Hybrimune Advantage – Hybridoma production efficiency and cell viability are enhanced by specialized waveforms. The patented Ramp-K™ feature enhances cell compression, resulting in high fusion rates and excellent cell viability.

Non-Uniform Waveform – Provides rapid cell alignment and compression for increased fusion.

Scale-up – Direct scale-up from 2 ml to 9 ml to large-volume hybridoma production in the Hybrimune system.

Programmable – Easy programmable user-friendly windows based software. Data logs are stored and retrieved easily.

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

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.

Parameter	Optimization Chamber	Production Chamber
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
Inner/Outer Radius	0.8333	0.8333

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 Collectis. Please contact BTX for more information.

ORDERING INFORMATION

Catalog #	Description
47-0300N	Hybrimune™ Electrofusion System Includes: Hybrimune waveform generator, 2 ml and 9 ml coaxial chambers, BTXpress Cytofus medium C, user interface software, cables and manual. Requires Windows based laptop or PC (not included).

Accessories:

47-0301	User-Interface Application Software
47-0030	2 ml Optimization Chamber
47-0020	9 ml Production Chamber
47-0001	BTXpress Cytofus medium C, 500 ml

ADVANCED ELECTROFUSION FOR MAXIMUM EFFICIENCY CELL FUSION

The BTX Hybrimune™ System is an advanced electrofusion solution for fast, efficient cell fusion in hybridoma production, hybrid cell formation or nuclear transfer applications. The Hybrimune™ system includes an innovative fusion chamber design, proprietary BTXpress Cytofus medium and sophisticated, tri-phasic electric field pulses that quickly position cells and disrupt cell membranes for maximum cell fusion efficiency with short cycle-times and minimal heating or turbulence for excellent cell viability.

Cells are combined in the proprietary BTXpress Cytofus medium C and transferred to the coaxial fusion chamber under sterile conditions. A tri-phasic sequence of programmed pulses is applied. First, an AC waveform positions the cells into “pearl-chain” alignment using dielectrophoretic force. A gradual increase in AC amplitude compresses the cells for maximal cell-cell contact. Then, a short, robust DC pulse porates the cell membranes to permit cell content exchange and cell fusion. A final AC waveform holds the cells in place and stabilizes the fusion as the force is gradually reduced. The waveform generator is fully-programmable for pulse parameter optimization to maximize efficiency and cell viability.

The Hybrimune™ system consists of a user-friendly, programmable waveform generator controlled through the user-interface application software running on a Windows-based computer system (not included). The fusion chamber uses coaxial electrodes designed for optimal electric field stimulation, independent of bath height. In this way, pulse parameters defined with the low volume optimization chamber are directly applicable for the large volume production chamber. The optimization chamber includes a transparent bottom for microscope viewing during the process optimization. BTXpress Cytofus medium is a specially formulated low conductivity solution for robust cell fusion efficiency.

AgilePulse MAX™ - Large Volume Transfection System



EFFICIENT TRANSFECTION FOR UP TO 6 ML VOLUMES

The AgilePulse MAX™ System is an advanced electroporation solution for fast, efficient transfection up to 10 mL of cell suspension. Specifically engineered for large-volume applications, our system maximizes cellular uptake with minimal heating and short cycle-time to ensure high cell viability in further cell processing.

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 opens pores in the cell membranes, followed by long, low-intensity pulses that drive the material into cells via electrophoresis. The patented Pulse Agile® technology maximizes efficiency and cell viability.

The system includes a user-friendly, programmable waveform generator with patented Pulse Agile® technology, the patent-pending large-volume electroporation chamber, and proprietary BTXpress Cytoporation® Medium T; optimized for large-volume electroporation. The system is engineered to provide uniform electric fields in a stable temperature environment, for excellent 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.

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.

LARGE-VOLUME ELECTROPORATION APPLICATIONS

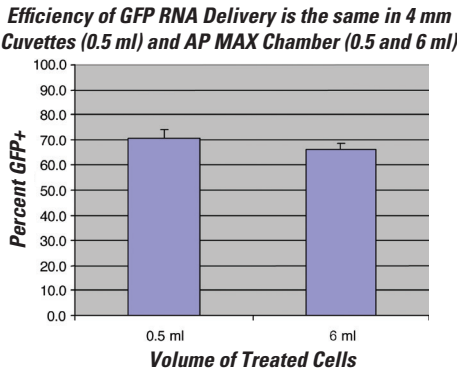
- Transfect bone marrow cells to produce or replace a missing protein
- Deliver siRNA to suppress gene expression
- Deliver genes for permanent gene correction
- Load cells with a drug for drug delivery
- Cancer immunotherapy
- Transfect eukaryotic cells for protein production in bioreactors
- Large-scale 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 µg/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 determined by flow cytometry.

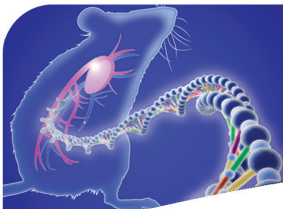


WAVEFORM GENERATOR SPECIFICATIONS

User Interface	Touch Screen Display
Chamber Electrode Gap	1 mm, 4 mm or 6 mm
Chamber Volume	0.5 to 10 mL
Chamber Connector	Male and Female Luer Locks with Vent Filter (only for 6 mm gap chamber)
Pulse Amplitude	100 to 1200 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

ORDERING INFORMATION

Catalog #	Description
47-0200N	AgilePulse MAX™ Electroporation System Includes: AgilePulse waveform generator, large volume chamber stand, safety stand, 6 ml chamber - 2 each, Cytoporation® Medium T - 500 mls, 4 mm gap cuvettes
Accessories:	
47-0202N	Large Volume Chamber Stand
47-0203	Safety Stand for Cuvettes
47-0204N	6 ml, 6 mm gap Chamber
45-0126	4 mm gap Cuvettes
47-0002	Cytoporation® Medium T



BTXpress™ Cytofusion®

Medium C



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 Hybrimmune 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

Volume	500 ml
Osmolarity	270-290 mOsm/L
Conductivity @ 25°C	0.080 ± 0.005 mS/cm
pH	7.2 ± 0.2
Endotoxin	< 0.25 EU/ml
Sterility	Sterile filtered
Storage	2 to 8 °C
Shelf-life	18 months from production date (shipped within 6 months of production)

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 10⁷ cells, at least two washes in BTXpress Cytofusion® Medium C are recommended. For more than 5 x 10⁷ 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.

ORDERING INFORMATION

Catalog #	Description
47-0001	BTXpress™ Cytofusion® Medium C

BTXpress™ Cytoporation®

Media T & T4



CYTOPORATION® MEDIA SPECIFICATIONS

Volume	500 ml
Osmolarity	270-290 mOsm/L
Conductivity @ 25°C	
T Media:	0.080 ± 0.005
T4 Media:	3.45 ± 0.05 mS/cm
pH	7.2 ± 0.2
RNase:	None Detected
DNase:	None Detected
Endotoxin:	< 0.25 EU/ml
Sterility:	Sterile filtered
Storage:	2 to 8°C

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.

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.

ORDERING INFORMATION

Catalog #	Description
47-0002	BTXpress™ Cytoporation® Media T
47-0003	BTXpress™ Cytoporation® Media T4

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.



BTX TECHNICAL & CUSTOMER SERVICE

For further references regarding specific applications and optimization,
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The Electroporation Experts

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