



## Hybrimune™

### THE HYBRIDOMA PRODUCTION SYSTEM TECHNICAL SPECIFICATIONS

#### 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 Cytofusium® 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 Cytofusium® 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 Cytofusium® medium is a specially formulated low conductivity solution for robust cell fusion efficiency.

#### Hybridoma Production— 10-Fold Greater Efficiency with Hybrimune™

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

The Hybrimune™ System is ideally suited 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). Transgenic, human-Ab producing mice immunized with tetanus toxoid (TT) provided spleens for fusion to SP2/O mouse myeloma cells.

**Fusion Methods:** PEG fusion was performed with standard protocols. For E-Fusion, mouse splenocytes and SP2/O cells were washed twice in Cytofusium 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. Image shows cells Cytospin™ prepared and Wright-Giemsa stain 30 minutes following E-Fusion.

# Hybrimmune™ TECHNICAL SPECIFICATIONS

## Tumor-Dendritic Cell Hybrid Production for Immunotherapy

(Data courtesy of Dr. Katrina Trevor, Arizona Cancer Center)

The Hybrimmune™ System is ideally suited for fast, efficient electrofusion of human dendritic cells with human tumor cells removed from a patient to produce a personal immunotherapeutic vaccine against a tumor. The E-Fusion process is similar to that used for hybridoma production (see above for details). The picture on the right shows the remarkable 10% efficiency of electrofusion of dendritic cells with A549 human lung carcinoma cells. Cells were fused at a concentration of 8 million cells/ml.

Trevor, et al., 2004 Cancer Immunology, Immunotherapy, 53(8): 705-714.

## HYBRIMUNE™ ELECTROFUSION SYSTEM INCLUDES

- Hybrimmune™ System (47-0300)  
System Includes:
  - Hybrimmune Generator
- User-Interface Application Software (47-0301)
  - Requires Windows® based Laptop or PC (not included)
- Hybrimmune™ Fusion Chambers
  1. Optimization chamber (2 ml, with transparent bottom) (47-0030)
  2. Production Chamber (9 ml) (47-0020)
- BTXpress Cytofusion® Medium C, 500 ml (47-0001)
- User Manual

## WAVEFORM SPECIFICATIONS

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

The following parameters are available:

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

## FUSION CHAMBER SPECIFICATIONS

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
<b>Volume</b>	2 ml	9 ml
<b>Outer ID</b>	45.72 mm	45.72 mm
<b>Inner OD</b>	38.10 mm	38.10 mm
<b>Gap</b>	3.81 mm	3.81 mm
<b>Well Height</b>	5 mm	18 mm
<b>Inner/Outer Radius</b>	0.8333	0.8333

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

## License Requirements

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

Cytospin is a Trademark of Shandon Inc.

## TECHNICAL & CUSTOMER SERVICE

For further references regarding specific applications and optimization, please contact BTX Technical Support:

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