Efficient Electrofusion of Human B Cells and Humanized SP20 Myeloma for Hybridoma Production Applications

Hybridoma technology methods offer the ability to create immortalized lines of cells which produce specific monoclonal antibodies targeting antigens of interest. The resulting monoclonal antibody products are powerful tools for a variety of downstream applications which include basic biological or translational research, assay development and target discovery, diagnostics, and potential therapeutics.

Electrofusion instruments such as the BTX ECM 2001+ and the BTX Hybrimune offer an ideal solution for Hybridoma applications. These instruments utilize combinations of precise electrical pulses to efficiently align cells into pearl chain formations, fuse cytoplasmic compartments to create hybrid cells, and stabilize the hybrid cells post-fusion. This process is efficient, reliable, and offers flexibility to scale from a few cells at a time up to 100 million cells. Unlike PEG-based cell fusion methods, electrofusion is less affected by lot-to-lot based variation of reagents or cytotoxicity issues. A comparison of hybridoma production efficiency with electrofusion versus efficiency of PEG based fusion is shown in Table 1. Additionally, a benefit of electrofusion is that the researcher may use an optically clear microslide or coaxial fusion chamber to view the cell alignment and fusion as it is happening for QC and optimization purposes.

Table 1 below shows the results of 12 experiments for Hybrimune Electrofusion vs PEG chemical fusion run side by side with several different antigens of interest. Total clones were counted by screening the wells in 96 well plates by eye for hybridoma growth on day 7 to 9. The number of clones was mathematically calculated using a poison distribution analysis. Wells were screened for presence of IgG antibody and antigen specificity using ELISA or an automated fluorescent screening system (HTRF). Data collected during the screening was normalized to 100 million cells to allow direct comparison of different fusion methods. For electrofusion, the average number of wells in the 12 experiments that produced IgG-secreting hybridomas was 542. Of those, 91 bound to the antigen of interest. For the PEG fusions, the average number of wells in the 12 experiments that produced IgG-secreting hybridomas was 58 and 12 of those were antigen specific. This means that on average electrofusion produced approximately 9 times as many IgG producing hybridomas and 8 times as many antigen specific hybridomas. However, additionally there were many cases where E-fusion produced results and PEG did not.

		Electrofusion		PEG Fusion	
Experiment	Antigen	IgG secreting clones	clones with IgG specific for antigen	IgG secreting clones	clones with IgG specific for antigen
1	Tetanus Toxoid	336	96	ND	ND
2	Tetanus Toxoid	170	40	ND	ND
3	Tetanus Toxoid	208	20	0	0
4	Tetanus Toxoid	1400	10	150	0
5	Tetanus Toxoid	<1100	<400	83	23
6	Tetanus Toxoid	582	151	69	21
7	Antigen 1	456	65	8	1
8	Antigen 2	ND	166	ND	18
9	Antigen 3	493	101	128	56
10	Antigen 4	71	0	0	0
11	Antigen 5	323	0	47	0
12	Antigen 6	246	0	36	0

Table 1: Electrofusion vs. PEG Chemical Fusion

Hybridoma Efficiency Results with BTX Electrofusion Instruments

Mouse monoclonal antibodies are an issue for therapeutic applications on human patients due to potential immunogenic reactions of the subject to the mouse antibody. These immune responses can reduce the effect of the therapeutic by preventing the mouse antibody from reaching the intended target and can also cause allergic reactions in the patient. Thus, it is necessary to create less allergenic Human monoclonal antibodies or humanized chimeric Human-Mouse antibodies. In this study, electrofusion pulse settings from BTX protocol 1016 (originally developed for mouse spleen B cell to mouse myeloma cell fusion) were utilized for Human B cells isolated from PBMC's hybridized with humanized mouse SP20 Myeloma cells. This method was found to be efficient for both BTX ECM 2001+ and Hybrimune electrofusion instruments (Table 2).

Instrument	# Hybridomas produced per 10 million Human B cells	Ratio of Hybridomas : Human B cells
ECM 2001+	~125,000	1:80
Hybrimune	~143,000	1:70

Table 2: Hybridoma Efficiency Results with BTX Electrofusion Instruments

ECM® 2001+ and Hybrimune Electrofusion Protocol

Note: Please refer to BTX protocol 1016 for detailed procedures.

Cell Line: Human B cells isolated from PBMCs, fused with Humanized SP20 mouse myeloma cells

Application: Antibody Secreting Hybridoma Production

Electrode: 2 ml Coaxial Chamber for Optimization, Item 47-0030

Electrofusion Medium: Cytofusion Medium C, Item 47-0001

Pre-Electrofusion Preparation: Clean and sterilize the Coaxial Chamber with 1N NaOH, Sporklenz, DI Water, and 70% Ethanol (as described on page 27 of the Hybrimune User Manual). Count each cell type, then gently mix B cells and myeloma cells in cell culture medium at a 1:1 ratio. Pellet the cells by centrifuging for 5 to 7 minutes at 1400 rpm, then wash the cells two times in electrofusion medium (pre-warmed to room temperature). The final cell suspension in electrofusion medium should be at a density of 10 million cells per ml.

Sample Volume: For each electrofusion condition, load 2 ml cell suspension at a density of 10 million cells per ml into 2 ml Coaxial Chamber. Each 2 ml cell suspension consists of 10 million Human B cells isolated from PBMC's and 10 million Humanized SP20 myeloma cells (mixed at a ratio of 1:1). Immediately proceed to Electrofusion Pulses and avoid having the cells settle at the bottom of the chamber. Cell alignment may be viewed on a microscope during electrofusion if desired.

Electrofusion Pulse Settings:

Step-1 Pre-AC

Initial Voltage (V0) = 40 V
Final Voltage (VF) = 60 V
Time (T) = 15 s
Frequency (F) = 1.4 MHz

Step-2 Pre-AC

Initial Voltage (V0) = 70 V
Final Voltage (VF) = 70 V
Time (T) = 20 s
Frequency (F) = 1.4 MHz

Step 3 DC – PULSE

Voltage (V) = 800 V
Time (T) = 40 µs
Number of Pulses (N) = 1
Interval time after Pulse (I) = 0 ms

Step 4 Post-AC

Initial Voltage (V0) = 60 V
Final Voltage (VF) = 5 V
Time (T) = 30s
Frequency (F) = 1.4 MHz

Post-Electrofusion Cell Treatment:

Following electrofusion, dilute cells in a pre-warmed HAT medium and plate at a density of 20,000-40,000 cells, in a volume of 100 µl in each well of 96 well plates. Incubate cells for 5 days, and then observe for fused outgrowth. For the data presented in this application note, total clones were counted by eye, and numbers of clones were mathematically calculated using a Poisson distribution analysis.

Electrofusion efficiency experiments were conducted by Radhika Dixit Ph.D., BTX Applications Scientist, in collaboration with Baron C. Heimbach Ph.D., Senior Research Scientist, Immunome, Exton, PA.