



## Hybrimmune Electrofusion System

### Hybridoma Production - E-Fusion vs. PEG Medarex Results

The data described in this document was produced by Marco Coccia, Ph.D. in the Platform Development Group, Medarex, Inc., Milpitas, CA using the Cyto Pulse Sciences Hybrimmune Commercial Electrofusion System. Cyto Pulse has permission from Medarex to release this information.

In preparation for the fusion, mice were immunized intraperitoneally or subcutaneously every 2-4 weeks with an antigen plus Ribi adjuvant. The mice were bled periodically to determine if an adequate antibody titer had developed. Mice were given an intravenous antigen boost 3-4 days before harvesting spleens to activate B cells and increase the number of antigen positive cells located in the spleen. Spleens were collected from adequately immunized mice just prior to the electrofusion.

For the fusion, mouse spleen cells and SP2/O mouse myeloma cells were washed twice in Cytofusion medium. The cells were at a concentration of 10 million cells per ml. A volume of 4.5 ml of each cell suspension was mixed then placed into a 9 ml volume Cyto Pulse electrofusion chamber. Cells were aligned and fused using the following parameters.

Pre-pulse sine wave: 40 V to 60 V, 1.4 MHz for 15 seconds  
Pulse: Amplitude 800 volts, width 40  $\mu$ s, one pulse  
Post pulse sine wave: 60 V to 5 V, 1.4 MHz for 30 seconds

After electrofusion, cells were left in the fusion chamber for 30 minutes. Samples of cells from wells were then analyzed by Wrights stain of Cytospin preparations of the samples. The bulk of the cells were cultured in HAT medium. The cells were cultured in 96 well plates at 5000 cells/ml.

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 poisson 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 differed fusion.

Symbols used:

$\gamma$	The immunoglobulin heavy chain for IgG antibody (includes IgG 1-3)
$\kappa$	One of two immunoglobulin light chains
$\#_{\gamma\kappa}$	The number of wells that contained IgG antibody of any specificity (the number of wells with hybridoma clones secreting IgG antibody)
$\#_{Ag,\gamma}$	The number of wells with hybridoma clones secreting IgG antibody with specificity for the antigen of interest
Ag	antigen, the specific antigen used in the screening
TT	Tetanus toxoid

The table shows the results of 12 experiments. 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 the average electrofusion produced approximately 9 times as many IgG producing hybridomas and 8 times as many antigen specific hybridomas. However, there were many cases where E-fusion produced results and PEG did not.

## Experimental Results

Experiment	Ag	E-fusion		PEG Fusion	
		No. $\gamma, \kappa$	No. Ag, $\gamma$	No. $\gamma, \kappa$	No. Ag, $\gamma$
1	TT	336	96	ND	ND
2	TT	170	40	ND	ND
3	TT	208	20	0	0
4	TT	1400	10	150	0
5	TT	< 1100	< 400	83	23
6	TT	582	151	69	21
7	Ag 1	456	65	8	1
8	Ag 2	ND	166	ND	18
9	Ag 3	493	101	128	56
10	Ag 4	71	0	0	0
11	Ag 5	323	0	47	0
12	Ag 5	246	0	36	0