BTX Applications

Tech-Trends

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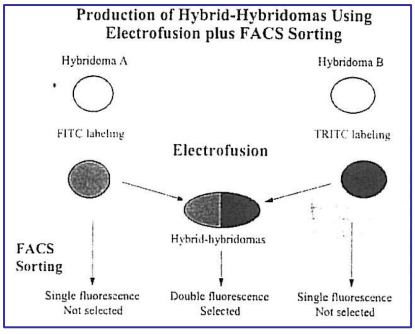
Quadroma generation by BTX Electrofusion

A New Method to Generate Quadromas by Electrofusion and FACS Sorting

Electro-FACS-fusion is a simple, rapid method for the generation of high-frequency hybrid-hybridomas (quadromas). This new technique uses fluorescence activated cell sorting (FACS) following electrofusion which eliminates the need for double resistant cell lines*. The two parent hybridomas are labeled with fluorescein isiothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC) prior to the fusion. After the electrofusion, cells exhibiting dual fluorescence are selected by FACS. The fused cells can be directly plated in microplates for clonal growth.



Under optimum conditions, it was demonstrated that electrofusion of 2 x 10^5 cells resulted in 30 hybridomas in the mouse system and 10 hybridomas in the human system. These efficiencies were about **10 times higher than those by PEG fusion*.** Electrofusion offers several distinct advantages over the use of PEG in the generation of hybridomas. It is less labor intensive, eliminating the need for repeated washings, and may be more efficient in producing a large number of fused cells. In addition, fewer cells are required. It is very difficult to perform a successful PEG fusion with fewer than 10^6 cells, but electrofusion can theoretically be performed with as few as two cells*. Cells undergoing electrofusion are subject to localized membrane breakdown; on the other hand, cell membranes undergoing fusion with PEG are uniformly affected, which may cause a greater loss of cellular constituents*. Visualization of the fusion process makes the production of hybrids theoretically possible, even without the use of a selection system. Another potential application of this method is in the development of primary hybridomas. Myeloma cells can be labeled with TRITC, and antigen-specific B-cells could be selected using the antigen labeled with FITC.

TABLE 1. RELATIVE PERCENTAGE OF DOUBLE POSITIVE CELLS BEFORE AND AFTER ELECTROFUSION

Hybridoma combination	*Unfused double positive (%)*	*Fused double positive (%)*
B43.13 × P92	1.6	3.0
B43.13 × P84	2.1	3.7
B80.1 × P92	2.5	6.2

Combined data from three different fusions showing the percentage of double positive cells before (unfused) and after electrofusion (fused).

BTX Catalog: 450010 Electro Cell Fusion System includes ECM 2001 Generator, Micro-slides 450, 453, Meander Fusion Chamber 454, Flat Electrode/Divergent Field 484, Electrode Adapter, Connection Cable, Safety Stand 630B, Cuvettes 1 mm, 2 mm, 4 mm, pkg. of 30 (10 each), Cuvette Rack 660

Electrofusion Procedure:
The labeled cells were resuspended in the electrofusion solution at approximately 10^7 cells/ml. ~2 x 10^6 (200 µl) cells from the FITC-labeled hybridoma were mixed with the same amount of cells from the TRITC-labeled hybridoma then transferred to a 2 mm gap cuvette.

Cell Preparation:
All the cell lines were maintained in RPMI-1640 medium supplemented with 2mmol/L L-glutamine, 50,000 units/L penicillin, 509 mg/L streptomycin, and 10% V/V of fetal bovine serum.

Electrofusion Settings:

Set Alignment Amplitude (AC):	40 V
Set AC Time:	15 s
Set DC Voltage:	200 V
Set DC Pulse Width:	15 µs
Set Number of Pulses:	3
Chamber:	2 mm gap
Desired Field Strength:	1000 V/cm

Electrofusion Procedure:

Temperature:	4ºC for 5 min prior to fusion
Pulse:	Press Automatic **Start** to Activate Pulse Sequence
Plate:	Cells were diluted 1:30 in RPMI 1640 medium containing 20% FBS.

Kreutz, F. T. et al., A New Method to Generate Quadromas by Electrofusion and FACS Sorting, Volume 17, Number 3, 1998, *Hybridoma*

* See publication for citations.

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