

Application Note 3

Sorting Transfected Cells Based on Gene Expression, Followed by Culture In Vitro

Introduction

Transfecting human genes into nonhuman cells is commonly used to study a given gene product in the absence of other human gene products.^{1,2} In this application note, we show that the FACSort™ can be used to enrich for transfected mouse cells expressing high levels of the human thrombin receptor.^{3*} The sorted fraction can then be cultured in vitro and reanalyzed 12 days later to show that it remains enriched for thrombin receptor-expressing cells.

* For research use only. Not for use in diagnostic or therapeutic procedures.

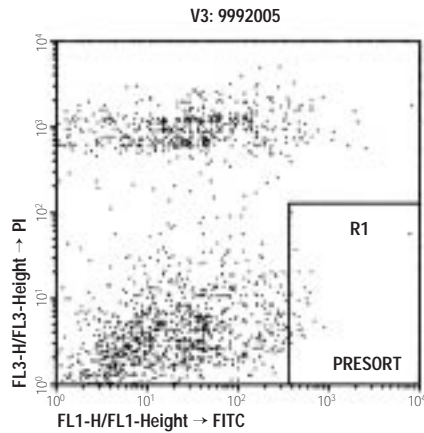


Figure 1 Mouse 3T12 cells transfected with the human thrombin receptor gene. (FL1, antithrombin receptor; FL3, propidium iodide). Shown here is the sort region used to sort cells expressing high amounts of human thrombin receptor.

Materials and Methods

Transfection

Balb/C mouse 3T12 cells (ATCC# CCL164) were transfected with the human thrombin receptor gene using a calcium phosphate transfection technique.⁴ The cells were co-transfected with the receptor gene cDNA inserted into a vector that contained the SR α promoter and the RSVneo gene.⁵ Stable transfectants were selected for survival in Dulbecco's Minimum Essential Medium (DMEM; GIBCO Laboratories, Grand Island, NY) containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 200 mg/mL G-418 sulfate (Geneticin[®]; GIBCO).⁶ Colonies appearing after 10 to 12 days were pooled and analyzed for expression of the thrombin receptor using flow cytometry.

Staining

Approximately 2×10^6 3T12 cells were incubated for 30 minutes on ice with a monoclonal antibody to the amino terminus of the thrombin receptor (Cor Therapeutics Inc., South San Francisco, CA) using 0.3 μ g antibody in 100 μ L of phosphate-buffered saline (PBS). The cells were washed twice with PBS containing 2.5% FBS (staining buffer), then resuspended in the same medium containing fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG (Cappel, Durham, NC). The cells were again incubated on ice for 20 minutes, and then washed twice with staining buffer and resuspended in 1 mL of the buffer containing 1 μ g/mL propidium iodide (PI; Calbiochem, San Diego, CA), which was added to aid in discriminating live from dead cells.

Flow Cytometry

The FL1 and FL3 detectors were used to detect FITC and PI, respectively. The amplifiers were set to LOG, and the detectors were adjusted to place the mean signal from the unstained 3T12 cells at approximately 10 on the linear intensity scale for FL1 and FL3. Flow cytometric analysis of the unsorted cells showed that there was a wide range of thrombin receptor expression within the population; therefore, the sort gate, R1 (Figure 1), was set to collect the top 5% of the FITC-positive, PI-negative cells.

Sorting was performed in the Exclusion sort mode, with the flow rate set to LO. Approximately 20,000 cells were collected into a BSA-coated (see *FACSort User's Guide*, Chapter 4) 50-mL polypropylene tube (Falcon[®], Becton Dickinson Labware, Franklin Lakes, NJ) in 45 mL of sheath fluid.

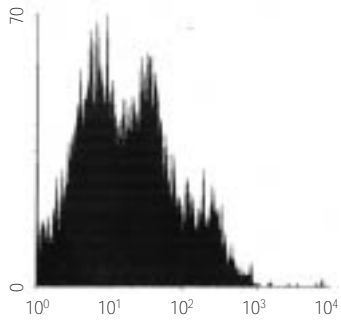


Figure 2a

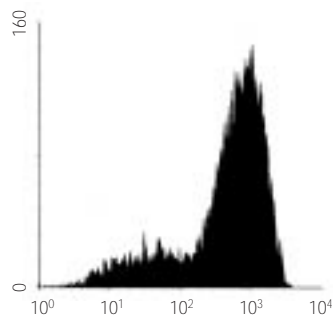


Figure 2b

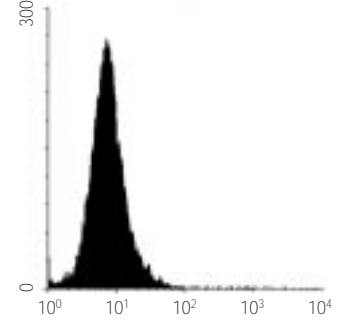


Figure 2c

Figure 2 Thrombin receptor expression of viable transfectants (see Figure 1 legend) (2a) before and (2b) 12 days after sorting. A nontransfected, stained control is shown (2c) for comparison.

Cell Culture

The sorted cells were centrifuged for 10 minutes at 900 x *g*, resuspended in 5 mL of culture medium containing G-418 sulfate, and plated into a 25-mm culture dish (Falcon). The cells were incubated humidified at 37°C in 5% CO₂. When the culture reached 75% confluency, it was transferred to a T75 (Falcon) culture flask. Twelve days after sorting, the cells were removed from the flask with 2-mM EDTA containing PBS, and assayed again for thrombin receptor expression using the FACSsort.

Results

Figures 2a and 2b are histograms of the FITC fluorescence (thrombin receptor expression) of the cells before sorting and 12 days after sorting, respectively. Figure 2c shows the fluorescence of the stained, nontransfected control. The dot plot in Figure 3 shows that the sorted, cultured cells were enriched for viable thrombin receptor-expressing cells.

Discussion and Summary

In this application note, we have shown that the FACSsort can sort transfected mouse 3T12 cells based on their expression of the human thrombin receptor. The sorted cells can be cultured for 12 days, then reanalyzed by flow cytometry to show that they continue to express high levels of human thrombin receptor. Cultures prepared in this manner retain this high-level expression of thrombin receptor for a minimum of 2 months after sorting.⁷

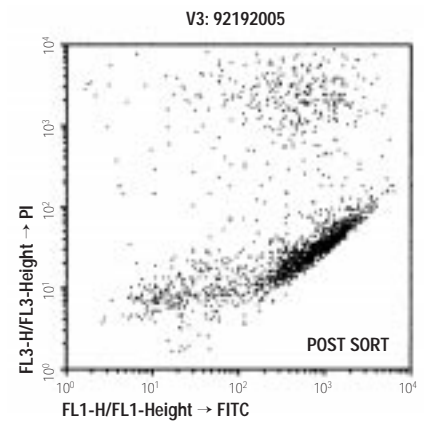


Figure 3 Thrombin receptor expression (FL1) and viability (FL3) of 3T12 transfectants 12 days after sorting.

BDIS publishes this method

as a service to investigators.

Detailed support for non-flow

cytometric aspects of this

procedure may not be

available from BDIS.

Hints

- Use Ca⁺⁺/Mg⁺⁺-free phosphate-buffered saline as sheath fluid while sorting to ensure maximum cell viability.
- When collecting multiple tubes of sorted cells, place each collection tube on ice immediately after it becomes full to maintain high cell viability.
- When collecting multiple tubes of sorted cells, for some cell types, it may be best to centrifuge the sorted sample immediately after it is collected, then place the cells in protein-containing medium.

References

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7. Unpublished data. Cor Therapeutics, Inc., 1992.

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