BioResearch

Unaltered Differentiation Potential of Leukemia and Lymphoma Cell Lines HL-60, THP-1 and U-937 after Nucleofection™

Technical Reference Guide

Introduction

Leukemia and lymphoma cell lines are widely used in immunology and hematology labs to study immunological pathways, such as differentiation or phagocytosis. Suspension cell lines, HL-60 (acute promyelocytic leukemia), THP-1 (acute monocytic leukemia) and U-937 (histiocytic lymphoma), represent frequently used model systems in blood cancer research for these purposes. Though easy to culture, these cells are considered difficult to transfect. With the aid of the Nucleofector™ Technology, suspension cell lines like HL-60, THP-1 and U932 can be transfected with high efficiencies and viabilities, making the investigation of these cells less cumbersome.

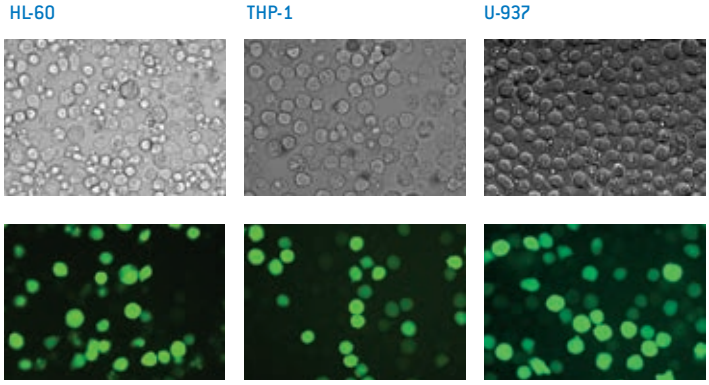

Figure 1: Efficient transfection of HL-60, THP-1 and U-937 cells. HL-60, THP-1 and U-937 cells were transfected by Nucleofection™ with the pmaxGFP™ Vector according to the respective Optimized Protocol. 5 hours post Nucleofection™, cells were analyzed by light and fluorescence microscopy.

Nucleofection™ of HL-60, THP-1 and U-937 Cells

Lonza offers Optimized Protocols, including culture tips and transfection parameters, for HL-60, THP-1 and U-937 cells for the different Nucleofection™ Platforms (please refer to www.lonza.com/nucleofection for more information). Transfection efficiencies between 50 and 65% can be achieved using Nucleofection™ (Figure 1).

As HL-60, THP-1 and U-937 cells are more sensitive to the amount of DNA used in transfection than most other cell lines, one important suggestion when transfecting these cells is to titrate the amount of the DNA substrate. When it comes to the Optimized Protocol for the above mentioned cells, only 0.5 µg DNA (THP-1) and 1 µg DNA (HL-60, U-937) should be used for a 100 µl Nucleocuvette™ Vessel to obtain high viability. In contrast, the majority of Optimized Protocols use 2 µg of pmaxGFP™ Vector.

Higher DNA amounts used in a transfection result in drastic increases in cell death in these cells (Figure 2). This effect on viability needs to be taken into consideration when working with Lonza's pmaxGFP™ Vector or any other plasmid of interest. A simple titration curve of your plasmid is all that is needed to find the optimal amount.

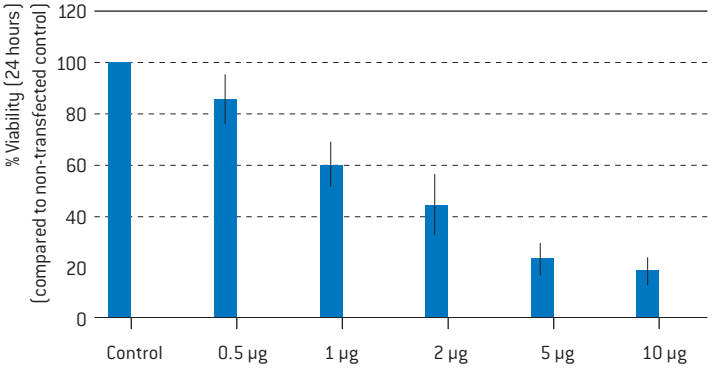

Figure 2: Increasing DNA amounts lead to lower cell viability in THP-1 cells transfected by Nucleofection™. THP-1 cells were transfected by Nucleofection™ using increasing amounts of the pmaxGFP™ Vector. 24 hours post Nucleofection™, viability of cells was analyzed (% of living cells compared to untransfected control cells).

How Can HL-60, THP-1 and U-937 Cells Be Differentiated?

Once HL-60, THP-1 or U-937 cells are transfected by Nucleofection™, they can be induced to differentiate easily using any common differentiation protocol. We recommend adding 10 – 100 nM PMA (Phorbol Myristate Acetate) immediately following Nucleofection™ to induce differentiation. Cells are usually fully differentiated within 1 – 4 days after induction. Successful differentiation of HL-60, THP-1 and U-937 cells after Nucleofection™ has been proven in several publications. [References 1.-3.]

What Indicates Differentiation?

Undifferentiated HL-60, THP-1 and U-937 cells are grown in suspension and are round in shape. Once the cells differentiate, they become adherent to the surface and take on an amoeboid or ovoid shape (Figure 3). Using PMA, HL-60, THP-1 and U-937 cells can all be differentiated into macrophage-like cells. HL-60, THP-1 and U-937 cells transfected by Nucleofection™ and differentiated show higher GFP expression levels than non-differentiated cells at 72 – 96 hours post-transfection. The cell count of differentiated cells is lower than for non-differentiated cells, as there is no proliferation during the process of differentiation. However, the percentage of living cells at 72 – 96 hours following transfection is similar between the two populations.

What Factors May Affect Differentiation?

Passage number can have a crucial effect on differentiation of suspension cell lines. THP-1 cells that are at very high passage number can require higher concentrations of PMA and longer incubation to achieve differentiation. These "old" cells can differentiate but will not actually become adherent, whereas younger properly cultured THP-1 cells that have been differentiated look more amoeboid in shape and adhere to their substrate (Figure 4). [Reference 4.]

Conclusions

Leukemia and lymphoma cell lines HL-60, THP-1 and U-937 can be transfected with high efficiencies using Nucleofection™, while differentiation potential is not affected. This makes these difficult-to-transfect cell lines even more tangible for use in immunology and hematology research.

References

1. Urahama N et al., 2005 Genes Cells 10(12), 1127-1137
2. Liang F et al., 2006 J Biol Chem 281(37), 27526-27538
3. Zada A et al., 2006 Leukemia 20(12), 2137-2146
4. Tsuchiya S et al., 1982 Cancer Res 42, 1530-1536

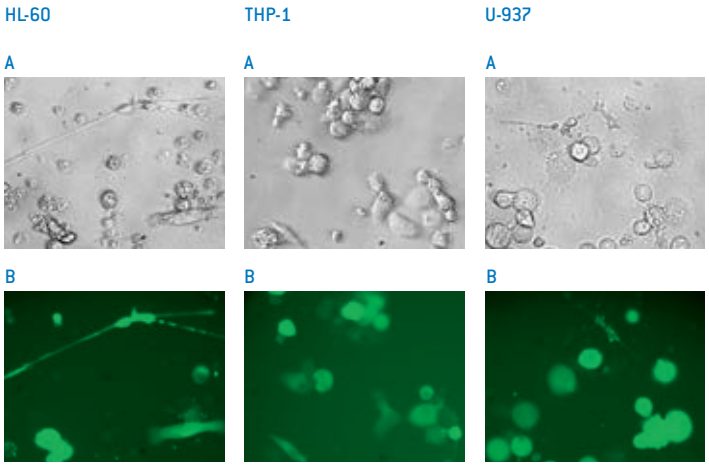

Figure 3: HL-60, THP-1 and U-937 cells can be differentiated after Nucleofection™. Cells were transfected by Nucleofection™ using the following conditions: HL-60 (1 µg pmaxGFP™ Vector), THP-1 (0.5 µg pmaxGFP™ Vector) and U937 (1 µg pmaxGFP™ Vector) following the respective Optimized Protocol. Immediately after transfection 10 nM (HL-60, U-937) or 20 nM (THP-1) PMA was added to the culture medium. Cells were analyzed by light (A) and fluorescent microscopy (B) 72 hours (THP-1) to 96 hours (HL-60, U-937) post Nucleofection™. maxGFP™ Reporter Protein expression levels were higher in differentiated than in non-differentiated cells.

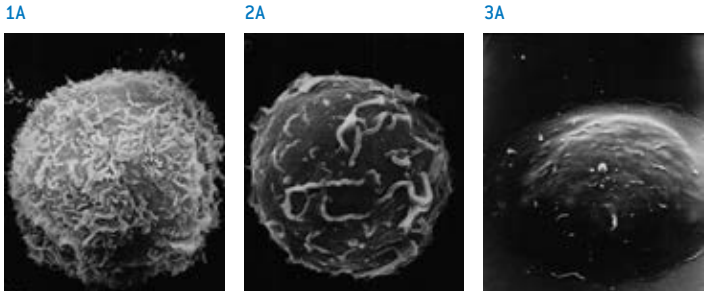

Figure 4: Passage number can affect differentiation of THP-1 cells. Differentiation of monocytic cell line THP-1 can be induced by treatment with TPA (12-o-tetradecanoylphorbol 13-acetate). Original THP-1 cells show characteristic microvilli and flaps (1A). After treatment with 160 nM TPA, THP-1 cells which have been continuously cultured without cryopreservation for 26 months show decreased number of microvilli and flaps, but still have a round shape (2A). In contrast, original THP-1 cells treated with 160 nM TPA became adherent by showing a macrophage-like flat shape (3A). (Data by permission of The American Association for Cancer Research, Inc.)

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