Protocol

Improved Protocol for Efficient Nonviral Transfection of Premature THP-1 Macrophages

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INTRODUCTION

The human monocytic leukemia cell line THP-1 is a widely used model for investigating monocyte and macrophage biology. Successful transfection of THP-1 monocytes with subsequent phorbol 12-myristate 13-acetate (PMA)-induced differentiation into macrophages is not a trivial matter, because according to previous transfection protocols, cell viability is lost almost completely within 24 h of PMA treatment following transfection. This protocol constitutes an optimized version of a previously published protocol by our group. It describes a procedure for transfecting premature THP-1 macrophages, which subsequently can be further differentiated into mature macrophages by PMA without a loss of cell viability. Transfection of THP-1 cells with plasmids or small interfering RNA (siRNA) is achieved by electroporation using the Lonza Nucleofector technology (Basel, Switzerland). This technique allows for the efficient nonviral delivery of plasmids, DNA, RNA, or siRNA into primary cells or cell lines even if the cells are not or are only slowly proliferating. Such cells are usually rather difficult to transfect by nonviral approaches. This means that only viral approaches would be left, which are expensive and labor-intensive and require laboratories complying with the respective safety regulations. The protocol described here is an efficient and convenient alternative.

RELATED INFORMATION

This procedure was originally described by Schnoor et al. (2009). For examples of successful application of this protocol, see Xie et al. (2006), Buers et al. (2009), as well as Robenek et al. (2009). For background information on the THP-1 cell line as a model of macrophage biology, see Auwerx (1991). The induction of differentiation of THP-1 monocytes by phorbol esters has been described by Tsuchiya et al. (1982), Schnoor et al. (2008), and Ansa-Addo et al. (2010).

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

β-Mercaptoethanol (β-ME)
Accutase I (PAA L11-007; Cölbe, Germany)
Amino acids, nonessential (100×) (PAA M11-003)
Fetal calf serum (FCS gold; PAA A15-151)
Human Monocyte Nucleofector kit (includes Human Monocyte Nucleofector Medium and Human Monocyte Nucleofector Solution) (Lonza VPA-1007)
Human serum off the clot (PAA C11-020)

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Iscove's Modified Dulbecco's Media (IMDM) with 25 mM HEPES and 25 mM L-glutamine (e.g., Lonza BE12-722F)
Penicillin/streptomycin/L-glutamine (PSG; 100×) (e.g., PAA P11-013)
Phorbol 12-myristate 13-acetate (PMA) (e.g., Fisher Scientific BP685)
Plasmid or siRNA for transfection
Roswell Park Memorial Institute 1640 medium (RPMI 1640) with L-glutamine (e.g., PAA E15-840)
Sodium pyruvate (100 mM)
THP-1 human leukemia monocytes (ATCC TIB-202)
Water (nuclease-free) or appropriate siRNA/plasmid buffer

Equipment

Cell counter (e.g., CASY Cell Counter and Analyser system, Schärfe System, Reutlingen, Germany; see Step 8)
Centrifuge
Centrifuge tubes
Flasks, tissue culture (75-cm² or 150-cm^2; see Step 3)
Incubator preset to 37°C
Micropipettor and tips
Nucleofector II (Lonza AAD-1001)
Pipettes
Plates (six-well or 12-well; see Step 20)
Transfection cuvettes and Pasteur pipettes from Human Monocyte Nucleofector kit (Lonza VPA-1007)
Water bath preset to 37°C

METHOD

Cultivation

1. Grow THP-1 cells in RPMI 1640 medium supplemented with 10% (v/v) FCS and 1% (v/v) PSG in a CO_2 incubator at 37°C.
2. 72 h prior to transfection, split the cells and transfer them to a new tissue culture flask.
3. 48 h prior to transfection, seed 1.5×10^7 cells into a 75-cm² tissue culture flask or 2.5×10^7 cells into a 150-cm² tissue culture flask in RPMI 1640 medium supplemented with 10% (v/v) FCS, 1% (v/v) PSG, 1% (v/v) sodium pyruvate, 1% (v/v) nonessential amino acids, 100 ng/mL PMA, and 50 μM β-ME.

Transfection (~1–2 h)

4. Prewarm Accutase I and all media in water bath at 37°C.
5. Remove culture medium from flask.
6. Add 6 mL (75-cm² flask) or 12 mL (150-cm² flask) of Accutase I and incubate for 30 min at 37°C in order to detach cells.

   Cells should regain a round appearance but may still appear to be attached to the flask; rinsing the flask gently with the suspension should complete detachment. Do not use a cell scraper, as this will reduce cell viability tremendously.

   See Troubleshooting.
7. Transfer suspension to a centrifuge tube and centrifuge for 5 min at 1000*g* at room temperature.
8. Resuspend cells in 5 mL of RPMI 1640 and determine cell count.

   If cell counting is performed automatically (e.g., using a CASY Cell Counter and Analyser system), then Steps 7 and 8 can be replaced by direct cell counting in Accutase I. Steps 7 and 8 are required to avoid prolonged exposure of cells to Accutase I and thus are essential with nonautomatic counting methods or with large sample numbers.
9. Split cell suspensions into aliquots containing $2.0–2.5 \times 10^6$ cells each (one aliquot for each scheduled transfection).
10. Centrifuge aliquots for 10 min at 250*g* at room temperature.

11. Remove RPMI 1640 from all aliquots.

12. Prepare appropriate dilutions of plasmids or siRNAs in nuclease-free water or an appropriate buffer.
The applied volume of plasmid or siRNA for transfection should be kept to a minimum.

13. Pipette 1 µg of siRNA or 0.5 µg of plasmid DNA directly into a Nucleofector cuvette.
Performing one transfection at a time (Steps 13–18) is recommended.

14. Resuspend pelleted cells of previously prepared aliquots in Nucleofector solution to yield a total volume of 100 µL.
Cells should not remain in pure Nucleofector solution any longer than necessary; avoid working time periods >15 min.

15. Transfer cells resuspended in Nucleofector solution directly into a Nucleofector cuvette.

16. Place the Nucleofector cuvette into the Nucleofector II device and perform program Y-001.

17. Use Nucleofector Pasteur pipettes to transfer the cell suspension into a reaction vial.

18. Immediately add 500 µL of Human Monocyte Nucleofector Medium supplemented with 1% (v/v) PSG, 1% (v/v) of nonessential amino acids, 1% (v/v) sodium pyruvate, and 5% (v/v) human serum (for siRNA) or 20% (v/v) human serum (for plasmid).

19. Repeat Steps 13–18 for each transfection sample.

20. Treat cell suspensions as follows:

    i. Thoroughly mix cell suspensions.

    ii. Transfer each suspension into one well of a six-well plate or into two wells of a 12-well plate.

    iii. Add Human Monocyte Nucleofector Medium supplemented with 1% (v/v) PSG, 1% (v/v) of nonessential amino acids, 1% (v/v) sodium pyruvate, and 5% (v/v) human serum (for siRNA) or 20% (v/v) human serum (for plasmid) to yield a total volume of 1.5 mL (for 12-well plate) or 3 mL (for six-well plate).

21. Four hours after transfection, completely remove culture medium and replace with 1.5 mL (for 12-well plate) or 3 mL (for six-well plate) of supplemented IMDM (1% [v/v] PSG, 1% [v/v] of nonessential amino acids, 1% [v/v] sodium pyruvate, 5% [v/v] human serum [for siRNA] or 20% [v/v] human serum [for plasmid], 100 ng/mL PMA, and 50 µM β-ME).
Cells should have reattached to the bottom of the plates again.

22. Continue cell differentiation for 24, 48, or 72 h according to the time required for maximum effect of the siRNA or plasmid.
For cultivation periods of 72 h, replacement of culture medium after 48 h by supplemented IMDM (see Step 21) is advised; under some experimental conditions, further optimization and validation of the applied medium may be necessary.

23. If incubation with additional effectors, e.g., cytokines, agonists, antagonists, and inhibitors, is intended, incubate the cells for the final 24 h of the siRNA or plasmid effective period in serum-free medium.
Do not maintain cells in serum-free medium for >24 h, as this will likely diminish cell viability considerably. Aside from this caveat, reducing the number of different media employed during the entire process has improved cell viability. Positive effects could be observed when all incubation periods after the transfection were performed either using supplemented IMDM or supplemented Human Monocyte Nucleofector Medium exclusively, thus avoiding the switch from one medium to another.

TROUBLESHOOTING

Problem: Cells do not detach properly upon Accutase I treatment.
[Step 6]
Solution: Consider the following:

1. Increase the incubation time.

2. Replace Accutase I with a fresh aliquot after the regular incubation time.

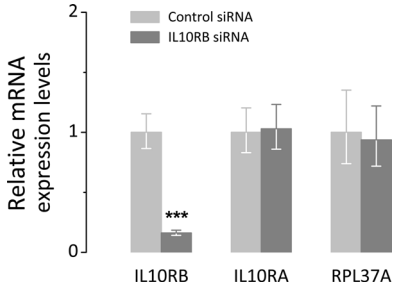

FIGURE 1. RNA-interference mediated knockdown of IL10RB mRNA. Expression of IL10RB mRNA was successfully knocked down by transfection with an IL10RB-specific siRNA versus transfection with a nonsilencing siRNA control in THP-1 macrophages 48 h after transfection of premature THP-1 macrophages according to the described transfection protocol. Specificity of siRNA-mediated knockdown was validated by expression data of the functionally closely linked IL10RA mRNA; RPL37A mRNA expression was applied as reference data in order to normalize expression across samples. (***) $P < 0.001$; n = 6.

Problem: There is insufficient knockdown/overexpression of the target gene.
Solution: Consider the following:

1. Determine the optimal incubation period for maximum siRNA or plasmid effect (usually 24–72 h).
2. Increase the amount of siRNA (2–3 µg) or plasmid (1–2 µg) used.
3. Pool several different siRNAs directed against the same target gene and transfect the cells using the pooled siRNA.

DISCUSSION

This is a revised and optimized protocol that was published originally by Schnoor et al. (2009). The technique was successfully used to study biogenesis of lipid droplets during macrophage foam cell formation (Buers et al. 2009; Robenek et al. 2009) and to investigate deactivation of macrophages with reduced IL10RB expression (Fig. 1). The most important advantage of this transfection approach is that it represents a method to rapidly and efficiently transfect premature THP-1 macrophages by a nonviral approach, while maintaining the cells' capability to differentiate upon PMA treatment. Thus, this method allows for examination of the effects of RNA interference-mediated knockdowns or plasmid expression within a widely used model of macrophage biology instead of only investigating these changes within undifferentiated monocytes, which are an inappropriate substitute for fully differentiated macrophages. In addition, this protocol can be applied for the transfection of undifferentiated THP-1 monocytes as well, if required subsequent differentiation of transfected monocytes by PMA is feasible. This is in strong contrast to previous transfection protocols that caused a total loss of cell viability when the cells were stimulated by PMA after transfection.

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