Transfection Protocol for Raw 264.7 (Mouse Leukaemic Monocyte Macrophage Cell Line) in Targefect Handbook of Transfection Protocols

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Method Article

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Abstract

The Targefect-Raw reagent is combined with the Virofect enhancer for optimal transfection of Raw 264.7 macrophages and other macrophage cell lines. The Targefect -Raw reagent enhances gene transfer by allowing the trasnfection complexes to escape degradation in the lysosomes. The Virofect enhancer is a unique adenovirus-derived formulation which greatly enhances the efficeincy of gene transfer by exploiting adenoviral receptors on the cell for efficient intracellular delivery of DNA containing trasnfection complexes. The efficiency of gene transfer into Raw 264.7 cells \(Mouse Leukaemic Monocyte Macrophage Cell Line) is around 50-60%.

Introduction

Targefect-RAW is a new reagent from Targeting Systems specifically designed for the transfection of the RAW 264.7 macrophage-like cell line. Although these cells are quite useful for studying innate immune responses and numerous other processes, they are resilient to transfect by calcium phosphate, electroporation, and lipid-based delivery methods. Thus, reproducible transient transfection of reporter plasmids and cDNAs proves difficult, and the creation of stable RAW transfectants is limited. To address these limitations, Targefect-RAW combines lipid-based transfection with a novel component, Virofect, to significantly enhance delivery and subsequent expression of plasmid DNA. Virofect enhances gene expression by utilizing adenoviral receptors on the cell surface to increase uptake and intracellular delivery of transfection complexes, while also preventing lysosomal degradation of the complexes. When used in combination with Targefect-RAW reagent, Virofect dramatically enhances plasmid delivery and expression, providing an easy to use, highly superior tool for RAW cell transfection.

Reagents

Targefect-Raw transfection reagent form Targeting Systems composed of two components , Targefect-Raw and Virofect enhancer, Cells were maintained in hgih glucose DMEM supplemented with 5% serum. Serum free DMEM is used for formation of trasnfection complexes

Procedure

For transfection, cells are grown to 70% confluency in 12-well plates. , **Preparation of Transfection Complexes**: 1. Complex 6 µg DNA with 12 µl of targefect and 24 µl of Virofect. in 0.6 ml of serum-free high glucose DMEM. 2. Incubate at 37 °C for 20 minutes to form transfection complexes, shake well after each addition. *Addition of transfection complexes to cell: Transfection in 12-well dishes*: 1. Dilute 125 ul of complexes in an equal amount of complete medium and add to the cells for 2 hours at 37„aC. 2. Add 600-800 ul complete medium to cells, and incubate for 24 - 36 hours before assay. Targefect-RAW transfections can be easily scaled for any size plate or dish and are comparable in time and difficulty to other commercial transfection protocols. **Transfection in 6-well dishes**: We also recommend aspirating of all the media from the 6-well dish leaving 500 µl of supernatant on the cells. Add 250 µl of

transfection complex to 500 µl of the cell supernatants mix well and incubate overnight \(the latter condition gave some improvement). Assay at 36-48 hrs post transfection For transfection in 24-well dishes transfect as above adding 70 ul of transfeciton complex per well of a 24-well dish

Timing

30 mins for preparation of transfection complexes. 2 hr incubation of transfeciton complexes with cells before addition of complete medium

Troubleshooting

Please note that when scaling up the amount of trasnfsection complex added to the cells does not increase in proportion to the area of the dish. The amount of transfection complex \(diluted) should be sufficient to cover cells well for a 3 hr period without allowing the cells to dry. Toxicity may be seen is a very low cell density \(less than 40% is seen) or too much trasnfection complex is added to the cells.

Anticipated Results

Transfection eficiencies of 50-80% have been achieved in Raw 264.7 cells usign the Targefect-Raw reagen

References

Please see the product review on the Targefect-Raw reagetn published in biocompare at teh following link "http://www.biocompare.com/Articles/ProductReview/1200/Targefect-RAW-From-Targeting-Systems.html":http://www.biocompare.com/Articles/ProductReview/1200/Targefect-RAW-From-Targeting-Systems.html Product review is written by A. Phillip West PhD Student Department of Immunobiology Yale University School of Medicine United States References citing use fo the Taregfect reagents to transfect raw cells 1. Inhibition of lipopolysaccharide-stimulated TNF- promoter activity by S-adenosylmethionine and 5'-methylthioadenosine Nary Veal, Chih-Lin Hsieh, Shigang Xiong, Jose M. Mato, Shelly Lu, and Hidekazu Tsukamoto Am J Physiol Gastrointest Liver Physiol, Aug 2004; 287: G352 - G362. 2. S-adenosylmethionine inhibits lipopolysaccharide -induced gene expression via modulation of histome emthylation \(2008) Al Ara, M Xia, K Ramani, JM Mato and S Lu. Hepatology 47 \(5) 1655- 1666 3. Role of nuclear-encoded subunit Vb in the assembly and stability of cytochrome c oxidase complex: implications in mitochondrial dysfunction and ROS production. \(2009) Domenico GALATI**1, Satish Srinivasan, Haider Raza, Subbuswamy K. PRABU**, Michael Hardy, Karunakaran CHANDRAN†, Marcos Lopez, Balaraman KALYANARAMAN† and Narayan G. Avadhani. Biochem. J. \(2009) 420, 439–449 \(Printed in Great Britain) doi:10.1042/BJ20090214

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Figures

Figure 1

Transfection of Raw 264.7 cells using Targect-Raw Transfection of Raw 267.4 cells with the Targefect - Raw reagent. Transfection efficiency approx. 60%. Data courtesy of Dr Jennifer Sullivan, Genzyme Corporation, MA.

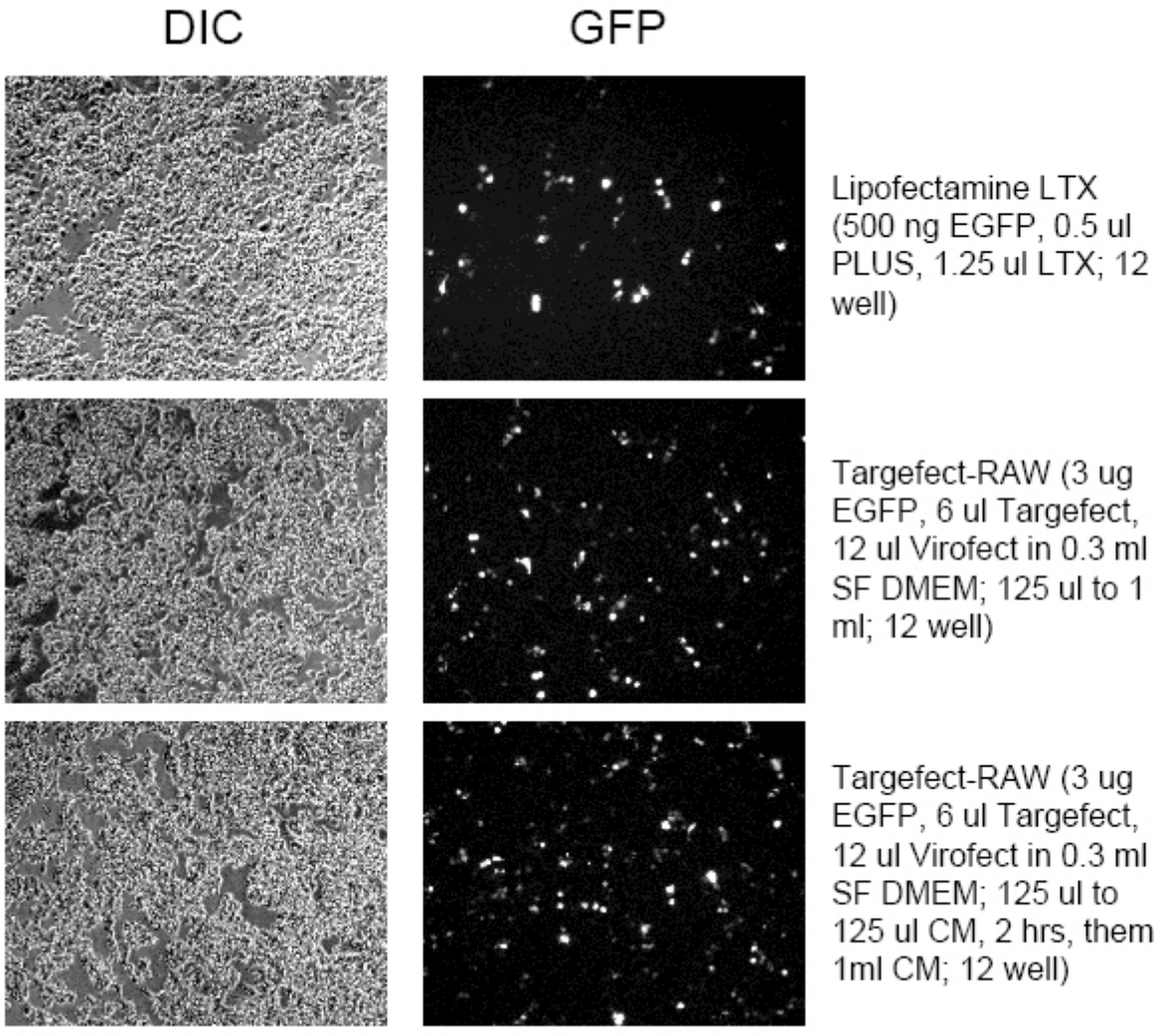

Figure 2

Comparision of Targefect-Raw with Lipofectamine LTX reagent Raw 264.7 cells were transfected with an EGFP expression vector using Targefect-Raw under two different conditions and comparision with transfection of Raw 264.7 cells usign the Lipofectamine LTX reagent. Data courtesy of Philip West, Yale University School of Medicine, USA. Data taken from the following link http://www.biocompare.com/Articles/ProductReview/1200/Targefect-RAW-From-Targeting-Systems.html