targetingsystems

1453 N. Cuyamaca St, El Cajon, CA 92020
Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
www.targetingsystems.com

TARGEFECT-RAW Product Citations

Highly efficient and cost-effective transfection of RAW264.7 cells and primary Kupffer cells
(Please also review the Targefect-RAW notes as they give citations and summarize how different labs have transfected RAW cells for different applications using our reagents
Proven performance (over 10 citations (see partial list on page 6)

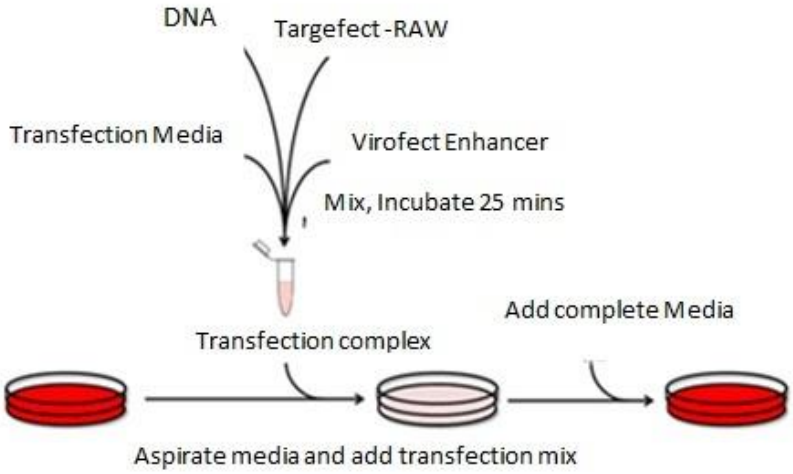
KIT COMPONENTS
Targefect-RAW- Store at 4 ^{0} C
Virofect enhancer- Store at -80 ^{o} C

Figure 1: Transfection of Raw 267.4 cells with the Targefect -RAW reagent. Transfection efficiency approx. 60%. **Data courtesy of Dr Jennifer Sullivan, Genzyme Corporation, MA.**

The kit contains two components Targefect-RAW (Targefect F-2) and Virofect**.** Each kit contains sufficient reagent for 500-1000 transfections (depending on amount of enhancer used) in 24-well dishes

ADVANTAGES

- Increased transfection efficiency results from a unique adenovirus –derived enhancer (Virofect), which complexes with plasmid DNA via the Targefect, an efficient cationic transfection reagent. Virofect enhances gene transfer by using adenoviral receptors on the cell surface to enhance intracellular delivery of transfection complexes. Following internalization, Virofect helps escape of the transfection complexes from degradation in the lysosome, and enhances the duration of transgene expression.
- Cost-effective. Each kit contains sufficient transfection reagent for approximately 500-1000 transfections in 24-well dishes.
- Simple transfection protocol.



targetingsystems

1453 N. Cuyamaca St, El Cajon, CA 92020
Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
www.targetingsystems.com

TARGEFECT-RAW PROTOCOL: For transfection of Raw 267.4 cells

Store the transfection reagents at the following temperatures immediately upon arrival.

Reagent	Volume	Temp
Targefect-RAW (Targefect-F2)	1.2ml	4°C
Virofect	600 µl	-80°C

You can also store the above at – 80°C if desired.

Preparation of Cells (Step 1)

Please use antibiotic-free DMEM for forming transfection complexes . Please use antibiotic-free growth media . Cells should be approximately 65-70% confluent at the time of transfection. A higher density will result in reduced transfection efficiency. As an example plate 400,000 cells per well of a 6-well dish the day before transfection. You may culture RAW cells in DMEM with 10% serum or medium of your choice.

PROTOCOL 1: (Fast Protocol, transfection complexes are added directly to cell culture media covering cells)

Preparation of Transfection Complex (Step 2)

Mix the Targefect-RAW (Targefect-F2) reagent gently by inverting the tube a few times just before use.

Please use serum-free high glucose DMEM. For preparation of transfection complexes

1. Add DNA to 0.6 ml of serum-free high glucose DMEM in an eppendorf tube, mix well by flicking the tube 10-12 times to create a vortexing action (do not vortex)
2. Add Targefect to diluted DNA as shown in Table 1.
3. Mix thoroughly by gently flicking 10 times.
4. Mix Virofect enhancer (VE) by inverting tube 10 times.
5. Add Virofect to the Targefect-DNA mixture as shown in Table 1.
6. Mix thoroughly by gently flicking 10 times.
7. Incubate the Transfection Complex at 37°C for 25 minutes to form transfection complexes

TABLE 1

Tube #	High Glucose DMEM (Serum free)	DNA	Targefect-RAW	Virofect Enhancer Reagent
1	0.6 ml	6 µg	12 µl	25 µl

Add DMEM first. Add DNA, mix well by flicking the tube about 12 times to create a vortexing action (do not use vortexer) . Add Targefect next, mix well again by flicking the tube. Incubate the tubes at 37°C for 25 minutes to form the transfection complexes.

Condition 1: Transfection in 6-well dishes

At the end of the 25 min incubation period transfection complexes (250 ul) were added to wells containing 2 ml of fresh culture meida and incubated overnight at 37°C after which the media was replaced with fresh culture media Assay at 36-48 hours post transfection.

When transfecting a 12- well dish transfect as above adding 0.125 ml of transfection complexto 1 ml of culture medium in each well. When transfecting a 24-well dish, transfect as above mixing 0.075 ml of transfection complex with 0.5ml of complete media per well of a 24-well dish.

targetingsystems

1453 N. Cuyamaca St, El Cajon, CA 92020
Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
www.targetingsystems.com

PROTOCOL 2: Transfection in the absence of serum

I. FORMATION OF TRANSFECTION COCKTAIL

A. Preparation of DNA (Step 1)

1. Dilute the plasmid DNA with Transfection Medium serum-free high glucose DMEM or OptiMEM1) as shown in Table 1.
2. Mix thoroughly by flicking 10 times.

B. Preparation of transfection Complex (Step 2)

1. Mix Targefect by inverting tube 10 times.
2. Add Targefect to diluted DNA as shown in Table 1.
3. Mix thoroughly by gently flicking 10 times.
4. Mix Virofect enahancer by inverting tube 10 times.
5. Add Virofect to the Targefect-DNA mixture as shown in Table 1.
6. Mix thoroughly by gently flicking 10 times.
7. Incubate the Transfection Complex at 37°C for 25 minutes.

II. TRANSFECTION OF CELLS

A. Addition of Transfection Complex (Step 3)

1. Aspirate off antibiotics-Free Growth Media from cell culture.
2. Add the appropriate amount of Transfection Complex to each well as shown in Table 2 by gently pipetting the Transfection mix along the side of the well so as not to disrupt the cells. Incubate cells with the Transfection mix in 37°C, 5% CO_2 humidified incubator for 2 hours.

B. Replacement of Transfection Complex with Antibiotic-Free Growth Medium (Step4)

1. Aspirate off the Transfection Complex from cells.
2. Add Antibiotics-Free Growth Medium to the transfected cells as shown in Table 2.
3. Incubate the transfected cells in 37°C, 5% CO_2 humidified incubator for 24 hours.
4. Change to fresh growth medium and assay at 24-36 hrs post transfection

Table 1: Formation of Transfection Complex

	STEP 1: Preparation of DNA			STEP 2: Preparation of Transfection Complex					
Tissue Culture Plate	**DNA (µg)**	**Transfection Medium (µl) DMEM**	***Gently* Flick 10X**	**ADD Targefect (µl)**	**Flick 10X**	**ADD Virofect (µl)**	***Gently* Flick 10X**	**37°C for 25 mins**	**Approx Total Transfection Complex (µl)**
96-well	0.06	60		0.20		0.4			61
24-well	0.2	200		0.8		1.6			204
12-well	0.4	400		1.6		3.2			408
6-well	1.0	1000		4.0		8.0			1020

Table 2: Transfection of RAW 264.7l Cells with Targefect-Virofect Transfection Complex

	Step 3: Addition of Transfection Complex			STEP 4 Replacement of Transfection Complex with Antibiotic-Free Growth Medium		
Tissue Culture Plate	**Aspirate off Antibiotic-Free Growth Medium**	**ADD Transfection Complex (µl)**	**37°C 5% CO_2 for 2 hrs**	**Aspirate off Transfection Complex**	**ADD Antibiotic-Free Growth Medium (µl)**	**37°C 5% CO_2 for 24 hrs**
96-well		61			100	
24-well		204			500	
12-well		408			1000	
6-well		1020			2000	

Important: Please try both protocols 1 and 2 as there is a lab to lab variation as to which works better.

Protocol 2 above uses a 1:4 ration of DNA to targefect. In case you observe toxicity please use less Targefect (1:3 ratio of DNA to Targefect). The amoun of Virofect is always twice the amount of Targefect.

targetingsystems

1453 N. Cuyamaca St, El Cajon, CA 92020
Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
www.targetingsystems.com

CITATIONS AND PRODUCT REVIEW

References citing use of Targefect reagents for transfecting RAW cells:

1. Nary Veal, Chih-Lin Hsieh, Shigang Xiong, Jose M. Mato, Shelly Lu, and Hidekazu Tsukamoto (2004) Inhibition of lipopolysaccharide-stimulated TNF-{alpha} promoter activity by S-adenosylmethionine and 5'-methylthioadenosine Am J Physiol Gastrointest Liver Physiol, Aug 2004; 287: G352 - G362.

2. Ainhoa Iglesias Ara,1 Meng Xia,1 Komal Ramani,1 José M. Mato,2 and Shelly C. Lu1 (2008). S-adenosylmethionine inhibits lipopolysaccharide-induced gene expression via modulation of histone methylation†. Hepatology. 2008 May; 47(5): 1655–1666.

3. Domenico Galati*1, Satish Srinivasan*1, Haider Raza*, Subbuswamy K. Prabu*, Michael Hardy†, Karunakaran Chandran†, Marcos Lopez†, Balaraman Kalyanaraman† and Narayan G. Avadhani*2 (2009) 420 (439–449) (Printed in Great Britain) (2009) Role of nuclear-encoded subunit Vb in the assembly and stability of cytochrome c oxidase complex: implications in mitochondrial dysfunction and ROS production. Biochem. J. (2009) 420 (439–449)

Product Review - Biocompare:
Targefect-RAW From
Targeting Systems

Monday June 08, 2009

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 extremely difficult to transfect by calcium phosphate, electroporation, and lipid-based delivery methods. Thus, reproducible transient transfection of reporter plasmids and cDNAs proves difficult, if not nearly impossible, 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 (also available from Targeting Systems) is a replication deficient, adenovirus-derived enhancer formulation that greatly augments transfection efficiency in a variety of cell types when used in combination with additional Targefect reagents, as well as other cationic reagents such as Lipofectamine (from Invitrogen) and Superfect (from Qiagen). 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.

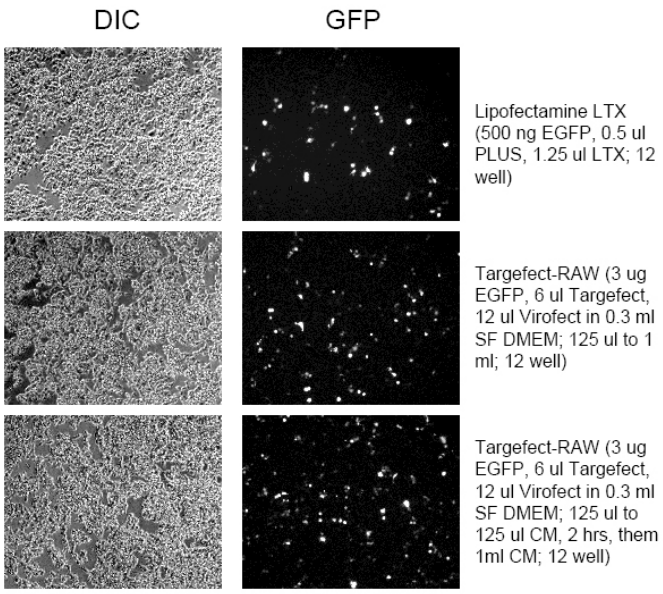
For transfection, cells are grown to 70% confluence in 12-well plates. Complexes are then prepared by combining 6 ug DNA with 12 ul Targefect-RAW and 24 ul Virofect in 600 ul of serum-free DMEM. After incubation for 20 minutes at 37„aC, 125 ul of complexes are then diluted in an equal amount of complete medium and added to the cells for 2 hours at 37„aC. 600-800 ul complete medium is then added to cells, and they are further incubated for 24 ¡V 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.

targetingsystems

1453 N. Cuyamaca St, El Cajon, CA 92020
Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
www.targetingsystems.com

We have successfully used Targefect-RAW to overexpress cDNAs and reporter plasmids in RAW cells, with transfection efficiencies usually within the 60 ¡V 80 % range. Most other commercial reagents transfect around 0.1 ¡V 1 % of cells in our hands, so the effectiveness of Targefect-RAW is clear. Even compared head-to-head with Lipofectamine LTX, which performs much better than Lipofectamine 2000 or Fugene 6 (from Roche), Targefect-RAW is substantially more effective (see FACS and IF data below). In addition, Targefect-RAW out-performs Amaxa Nucleofector technology in transient transfections, with significantly decreased toxicity and user cost.

Overall, Targefect-RAW is an affordable and highly efficient RAW cell transfection reagent that provides reproducible transfections on the order of those seen in fibroblast cell lines. Since Virofect contains replication-deficient adenovirus, users must practice care when working with the reagent; however, this inconvenience is heavily outweighed by the stellar performance of the product.



Phillip West
PhD Student
Department of Immunobiology
Yale University School of Medicine

APPLICATIONS:
The Targefect-RAW reagents has been used to deliver plasmid DNA, siRNA, the CRISPER complex (plasmid DNA plus guide RNA plus Cas9 protein) in RAW 264.7 cells (see citation list on next page. The Targefect-RAW reagent has also been used to transfect primary mouse Kupffer cells

Targefect-RAW Catalog #	Quantity	Price
RAW-01	1	$350
RAW-10	10	$2500

targetingsystems

1453 N. Cuyamaca St, El Cajon, CA 92020
Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
www.targetingsystems.com

REFERENCES CITING USE OF TARGEFECT-RAW REAGENTS TO TRANSFECT RAW 264.7 CELLS:

1. Li TL, Wang Z, You H, Ong Q, Varanasi VJ, Dong M, Lu B, Paşca SP, Cui B (2019) Engineering a Genetically Encoded Magnetic Protein Crystal. Nano Lett. 19(10):6955-6963
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7265822/
(Co-transfected 3 plasmids into RAW264.7 cells)

2. Walia R, Transfection Protocol for Raw 264.7 (Mouse Leukaemic Monocyte Macrophage Cell Line) in Targefect Handbook of Transfection Protocols (2011) Nature protocol exchange.
https://doi.org/10.1038/protex.2011.227

3. Storek KM, Gertsvolf NA, Ohlson MB, Monack DM. cGAS and Ifi204 cooperate to produce type I IFNs in response to Francisella infection (2015) J Immunol. 194(7):3236-45.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4367159/
(This paper cites use of the Targefect-RAW reagent to deliver Cas9, guide RNA and plasmid DNA into RAW cells)

4. Ara AI, Xia M, Ramani K, Mato JM, Lu SC.(2008) S-adenosylmethionine inhibits lipopolysaccharide-induced gene expression via modulation of histone methylation Hepatology. 47(5):1655-66.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2408693/
(Transfeciton of DNA into RAW cells

5. Song K, Kwon H, Han C, Chen W, Zhang J, Ma W, Dash S, Gandhi CR, Wu T. (2020) Yes-Associated Protein in Kupffer Cells Enhances the Production of Proinflammatory Cytokines and Promotes the Development of Nonalcoholic Steatohepatitis. Hepatology. 72(1):72-87.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7153981/
(Transfection of DNA into primary mouse Kupffer cells)

6. Galati D, Srinivasan S, Raza H, Prabu SK, Hardy M, Chandran K, Lopez M, Kalyanaraman B, Avadhani NG.(2009). Role of nuclear-encoded subunit Vb in the assembly and stability of cytochrome c oxidase complex: implications in mitochondrial dysfunction and ROS production. Biochem J. 420(3):439-49.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2735414/
(Transfection of DNA into RAW cells).

7. Venter G, Oerlemans FT, Wijers M, Willemse M, Fransen JA, Wieringa B. Glucose controls morphodynamics of LPS-stimulated macrophages. PLoS One. 2014 May 5;9(5):e96786
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4010488/
Transfected linearized DNA into RAW cells)

8. Venter G, Oerlemans FT, Willemse M, Wijers M, Fransen JA, Wieringa B (2014) NAMPT-mediated salvage synthesis of NAD+ controls morphofunctional changes of macrophages. PLoS One. May 13;9(5):e97378
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4019579/

9. Nary Veal, Chih-Lin Hsieh, Shigang Xiong, Jose M. Mato, Shelly Lu, and Hidekazu (2004) Inhibition of lipopolysaccharide-stimulated TNF- promoter activity by S- adenosylmethionine and 5'-methylthioadenosine Tsukamoto Am J Physiol Gastrointest Liver Physiol, Aug 2004; 287: G352 - G362.
https://journals.physiology.org/doi/full/10.1152/ajpgi.00316.2003
Transfected DNA in RAW264.7 cells using only Taregfect-F2 (same as Targefect-RAW) component of Taregfect-RAW kit