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TARGEFECT-HEPATOCYTE

Efficient transfection of plasmid DNA into primary hepatocytes (Over 30 product citations)

The Targefect-Hepatocyte kit contains two components: Targefect-Hepatocyte (Targefect F-1), and Virofect. Each kit contains sufficient reagent for 600 transfections in 24-well dishes, price $275.00.

Note: For siRNA transfection or Co-delivery of siRNA with DNA we recommend the Targefect F-2 reagent with the Virofect enhancer

Gene delivery into rat, mouse and human hepatocytes using Targefect-Hepatocyte:

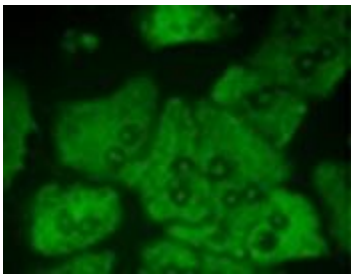


Figure 1:
Mouse hepatocytes transfected with Targefect-Hepatocyte: **Data courtesy of Dr. Suzanne Lyman, Dr. Behren's lab, University of North Carolina at Chapel Hill, NC**

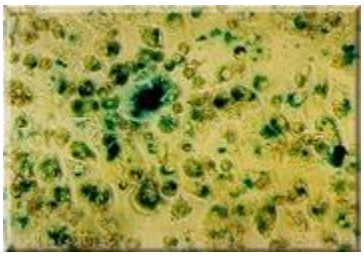
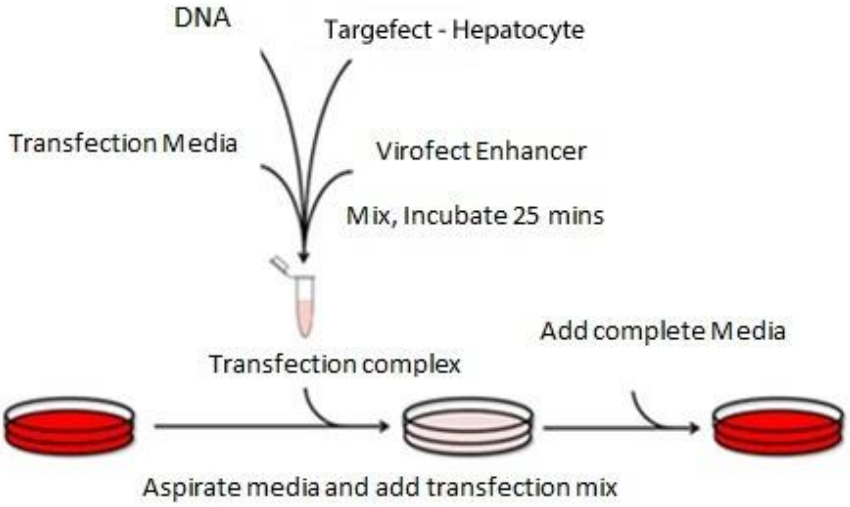


Figure 2:
Human hepatocytes transfected with Targefect-Hepatocyte: **Targeting Systems, CA**

ADVANTAGES

1. Increased transfection efficiency results from the inclusion of a unique enhancer Virofect, which works in conjunction with the Targefect F-1 reagent to enhance transgene delivery and expression. Virofect is an adenovirus-derived enhancer, 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.
2. Low toxicity - Excellent cell survival, a major advantage over using the AMAXA or other electroporation-based systems.
3. Cost-effective. Each kit contains sufficient transfection reagent for approximately 500-1000 transfections in 24-well dishes.
4. Simple transfection protocol.
5. Citation list of over 40 citations in reputable journals (see list below).



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TRANSFECTION PROTOCOL I: Transfection using Targefect-Hepatocyte and Virofect enhancer:

Please use antibiotic-free media as this reduces toxicity.

If using serum-free media we recommend supplementation with 10% serum (not required but recommended). Also if possible, do not coat with collagen or matrigel . Sometimes (for eg in case of rat hepatocytes) you can get good growth using Primaria tissue culture dishes from BD Falcon (see link below)

http://www.gogenlab.com/products/primaria-treated-tissue-culture-dishes-bd-falcon

The protocol below also works well for hepatocytes plated out on collagen-coated dishes but the transfection efficiency may be slightly lower.

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

Reagent	Volume	Temp
Targefect-Hepatocyte (Targefect-F1)	600 µl	-20°C
Virofect	600 µl	-20°C

Thaw the Targefect-F1 reagent, vortex at full speed for 30 seconds 2-3 times just before use. Cells should be approximately 70% confluent at the time of transfection. A higher density will result in reduced transfection efficiency **Please use OptiMEM 1 (GIBCO BRL, now Invitrogen) or serum-free high glucose DMEM for complex formation.**

B. Preparation of Transfection Complex (Step 2)

1. Add Targefect to diluted DNA as shown in Table 1.
2. Mix thoroughly by gently flicking 10 times.
3. Mix Virofect enhancer (VE) by inverting tube 10 times.
4. Add Virofect to the Targefect-DNA mixture as shown in Table 1.
5. Mix thoroughly by gently flicking 10 times.
6. Incubate the Transfection Complex at 37°C for 25 minutes. Aspirate culture media and add transfection complexes to cell Swirl the dish of cover cells well and Incubate at 37°C for 2 hrs. Then add the recommended amount of cell culture media (complete media with serum but no antibiotics

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) OptiMEM1**	***Gently* Flick 10X**	**ADD Targefect (µl)**	**Flick 10X**	**ADD VE (µl)**	***Gently* Flick 10X**	**37°C for 25 mins**	**Total Transfection Complex (µl)**
96-well	0.20	40		0.4		0.8			41.5
24-well	0.75	150		1.5		3.0			157
12-well	1.5	300		3.0		6.0			316
6-well	3.0	600		6.0		13.0			627

Table 2: Transfection of hepatocytes with Targefect-PE 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**		**ADD Antibiotic-Free Growth Medium (µl)**	**37°C 5% CO_2 for 24 hrs**
96-well		41.5			80	
24-well		157			400	
12-well		316			800	
6-well		628			1400	

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TRANSFECTION PROTOCOL II: Transfection with Targefect-Hepatocyte in the absence of serum:

We recommend you also try this protocol first as it is cost effective, gives a transfection efficiecncy which is acceptable for most applications and is a preferred protocol in some labs

The amounts below are given for a 6-well plate format. Use Table 2 to adjust the reagent volumes for other plate sizes. Please use antibiotic-free media as these reduce toxicity

Protocol:

Note: Please use OptiMEM 1(antibiotic –free) or high glucose DMEM as the complexing medium. We first recommend performing the following optimization experiment to determine the optimal transfection condition for primary hepatocytes used in your lab. The optimal DNA:Targefect ratios are different for mouse, rat and human hepatocytes and also depend on whether or not you are plating on collagen-coated dishes. In general for rat and mouse hepatocytes we find that condition 1 or 2 work well and condition 3 works well for human hepatocytes. Add OptiMEM1 first, and then add DNA mix well by flicking the tube with your hand about 12 times to create a vortexing action, add Targefect-Hepatocyte, and mix well again

- Plate cells so they reach a confluence of 70% at the time of transfection.
- For each transfection well, add 2 µg of plasmid DNA into 1 ml OptiMEM1 in a micro centrifuge tube.
- Vortex the Targefect-hepatocyte transfection reagent at full speed for 30 seconds twice just before pipetting.
- Add 2 ul, 4 ul or 6 ul of Targefect-Hepatocyte Transfection Reagent to each tube and mix well by flicking the tube. Incubate at room temperature for 20-30 minutes to form the transfection complexes.
- Wash cells once with serum-free medium.
- Aspirate the culture medium from the cells and immediately replace with the 0.8 ml of the transfection mixture. Swirl the plate gently in order to evenly disperse the complex mixture.
- Return the plate to the incubator and incubate cells for 2-3 hours.
- Add 2 ml of complete growth medium containing serum on top of the transfection complex incubate overnight. Replace with fresh complete medium the next morning. Assay at 24 hours-48 hours post-transfection or as needed to maintain healthy cells.

Table 1: Formation of transfection complexes:

Tube #	DMEM or OptiMEM	DNA	Targefect-Hepatocyte
1	1 ml	2ug	2 ul
2	1 ml	2 ug	4 ul
3	1 ml	2 ug	6 ul

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Table 2 : Volume of transfection complex for different culture vessels

Culture vessel	Volume of transfection complex	Volume of complete medium
96-well dish	50 ul	100 ul
48-well dish	150 ul	500 ul
12-well dish	300 ul	1 ml
6-well dish	0.8 ml	2 ml
60 mm dish	1.6 ml	5 ml
10 cm dish	3.2 ml	10 ml

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List 1: Citations for transfection primary hepatocytes using Targefect reagents:

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