Ultrafect™ Reagent System

Cat. No.: SM502-1000 Size : Ultrafect Reagent: 1 ml TransPlus Reagent: 1 ml
Cat. No.: SM502-0100 Size : Ultrafect Reagent: 100 µl TransPlus Reagent: 100 µl
Store at 4°C (Do not freeze)

Description

UltraFect carries most advanced lipid nanoparticle technology to provide superior transfection performance with improved application outcomes and reproducible results. It delivers superior transfection efficiency and improved cell viability.

Note:

- Do not add the antibiotics to the medium during transfection as this causes cell death.
- Maintain the same seeding conditions between experiments.
- For most cell lines, transfection in the absence of serum is optimal. For HeLa cells, high level transfection requires the presence of serum in the medium during transfection. This option should be tested if optimization does not yield high-level transfection.
- Some no serum medium might inhibit the transfection of cation ion and plasmid. We recommend using the reduced serum medium without serum.
- Different cell types and number of passages might lead to different transfection efficiency, and we recommend using at least two different concentrations of transfection reagent as control in new transfect experiments to optimize experimental conditions.

Kit contents

Catalog number	SM502-1000	SM502-0100
UltraFect Reagent	1 ml	100 µl
TransPlus Reagent	1 ml	100 µl

Required Materials

- Cell line
- CO_2 incubator
- Medium without serum (or other reduced serum medium)

Quality Control

The quality of the Simply UltraFect™ Reagent System is tested on a lot-to-lot basis to ensure consistent product quality.

Plasmid DNA Transfection protocol

Use the following procedures to transfect mammalian cells in a 96- well format. For other formats, see Scaling Up or Down Transfections. All amounts and volumes are given on a per well basis. Prepare complex using a DNA (µg) to UltraFect reagent ratio of 1:1.5 to 1:2.5 for most cell lines. Transfect cells at high cell density for high efficiency, high expression levels, and to minimize cytotoxicity. Optimize transfections especially if you are transfecting a mammalian cell line for the first time.

1. One day before transfection, plate cells in growth medium without antibiotics so that cells will be 80-90% confluent at the time of transfection.
2. For each transfection sample, prepare complexes as follows:
   a. Dilute 0.2 µg DNA and TransPLUS reagent 0.4 µl (2 µl /µg DNA) in 25 µl of medium without serum (or other reduced serum medium). Mix gently. Incubate for 5 minutes at room temperature.
   b. Mix UltraFect reagent gently before use, then dilute 0.3 or 0.5 µl (1.5 or 2.5 µl /µg DNA) in 25 µl of medium without serum (or other reduced serum medium). Incubate for 5 minutes at room temperature.
   c. Combine the diluted DNA and diluted UltraFect reagent (total volume = 50 µl). Mix gently and incubate for 20 minutes at room temperature.
3. Add the 50 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth. Medium changed after 4-6 hours.
4. Incubate cells at 37°C in a CO2 incubator for 18-48 hours prior to testing for transgene expression.

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as the DNA and UltraFect reagent system concentrations. Make sure that cells are greater than 90% confluent and vary DNA (µg): UltraFect reagent (µl) ratios from 1:1.5 to 2:5.

Scaling Up or Down Transfections

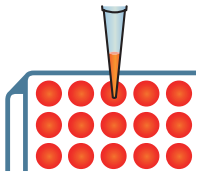
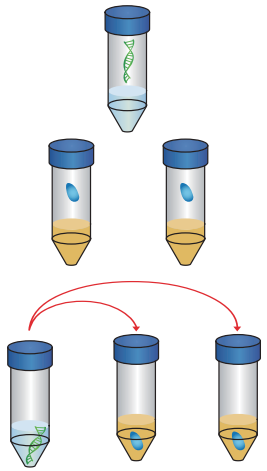
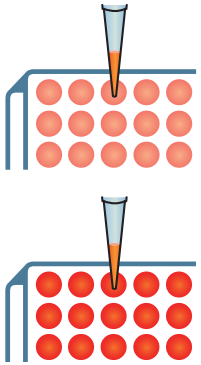
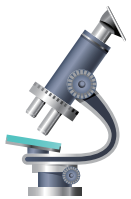
To transfect cells in different tissue culture formats, vary the amounts of UltraFect reagent system, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table. With automated, high-throughput systems, a complexing volume of 50 µl is recommended for transfections in 96-well plates.

Note: Perform rapid 96-well plate transfections by plate transfections by plating cells directly into the transfection mix. Prepare complexes in the plate and directly add cells at twice the cell density as in the basic protocol in a 100 µl volume. Cells will adhere as usual in the presence of complexes.

Culture vessel	Surf. Area per well a	Shared Reagent		DNA -TransPlus c omplex			Ultrafect Reagent mix	
		Vol. of plating medium (µl)	Vol. of dilution mediumb (µl)	DNA (µg)	TransPLUS (µl)	Medium (µl)	Ultrafect reagent (µl)	Medium (µl)
96-well	0.3 cm^2	50	50	0.2	0.4	25	0.3 or 0.5	25
24-well	2 cm^2	100	100	1	2	50	1.5 or 2.5	50
6-well	10 cm^2	500	500	2	4	250	3 or 5	250

a. Surface areas may vary depending on the manufacturer.
b. Volumes of dilution medium in step 2a & 2b of Plasmid DNA Transfection protocol.

Note: The volume of medium, DNA, transfection reagents are each well (dish), and the amount of nucleic acid transfection reagent need to optimize in new transfect experiments.

Day 0		Before transfection, plate cells in growth medium without antibiotics so that cells will be 80-90% confluent at the time of transfection
Day 1		Prepare master mix of DNA by diluting DNA in medium, then add TransPLUS reagent and mix well Incubate for 5 minutes at room temperature
		Dilute UltraFect reagent in medium (2 tubes) and mix well Incubate for 5 minutes at room temperature
		Add diluted DNA to each tube of diluted UltraFect reagent (1:1 ratio) Incubate for 20 minutes at room temperature
		Add the DNA-lipid complex to cells
		Change the medium after 4-6 hours
Day 2~3		Incubate cells for 18-48 hours at 37°C. Then, analyze transfected cells

Troubleshooting

Problem	Cause	Solution
Low transfection efficiency	Plasmid DNA or UltraFect reagent diluted in media containing serum or complexes formed in the presense of serum	Use serum-free medium for dillutions of plasmid DNA and transfection reagents. We recommend using reduced serum medium to dilute UltraFect and DNA before complexing.
	DNA: transfection reagent ratio sub-optimal for cell line	Prepare complexes using a DNA (µg) to UltraFect ratio of 1:1.5 to 2.5 for most cell lines. Optimization may be necessary. If using a different transfection reagent, please consult the product manual.
Reduced cell viability following transfection	Plasmid DNA preparation contains high levels of endotoxin	Ensure that the plasmid DNA used for transfection is of high quality.
	Antibacterial agents were used in growth medium during transfection	Do not use antibiotics in growth medium because during transfection, cells are more permeable to antibiotics, which may cause toxicity.
Transfection results not reproducible	Transfections performed at d ifferent cell confluencies, or at different DNA: transfection reagent ratios	Transfection performance reproducibility is dependent on day-to-day consistency in cell splitting, plating and transfecting with a consistent protocol (same DNA: transfection reagent ratios). Different DNA preparations or media changes may also change transfection performance. Optimize transfections especially if you are transfecting a mammalian cell line for the first time.

Related Ordering Information

Cat. No.	Description	Size
CC002-1000	Insulin-Transferrin-Selenium Mixture (ITS-M), 500X	1 ml
SD010-R600	1Kb DNA Ladder, RTU	600 µl