Ultra293™ Transfection Reagent

Cat. No.: SM506-1000 Package: 1 ml
Cat. No.: SM506-0100 Package: 100 µl
Store at 4°C (Do not freeze)

Description

The Simply Ultra293™ is specifically optimized to provide exceptional transfection efficiency of plasmid DNA in HEK 293 and associated cell lineages. It provides all the attributes of the trusted transfection reagents: high transfection efficiency, low toxicity, simplicity of use and reproducibility. Ultra293™ is suitable for both transient and stable transfection and can be used for many applications. After Ultra293™ and plasmid mixed, the Ultra293™ / plasmid complexes protect DNA from degradation and facilitate efficient plasmid delivery into eukaryotic cells. It provides effectively, reproducibly, and affordable benefits for scientific research. The entire procedure can be completed in 35-40 minutes.

Kit contents

Catalog number	SM506-1000	SM506-0100
Ultra293™ Transfection Reagent	1 ml X 1 vial	100 µl X 1 vial

Quality Control

The quality of the Ultra293™ Transfection Reagent is tested on a lot-to-lot basis to ensure consistent product quality.

Required Material

- Incubator
- 1.5 ml centrifuge tube
- Serum free medium

Ultra293™ Transfection Reagent Protocol

Step 1. Cell Seeding:

1. Cell should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 70-80% confluency at the time of transfection.
2. Complete culture medium is freshly added to each well 4 hours prior to transfection.

Step 2. Preparation of Ultra293™ / DNA complex and transfection procedures

- For different cell types, the optimal ratio of Ultra293™ (µl): DNA (µg) is around 1 to 1. We recommend starting with this ratio which usually gives very high transfection efficiency with less cytotoxicity.
- To optimized size of complex particles, we recommend using serum free medium to dilute DNA and transfection reagent.

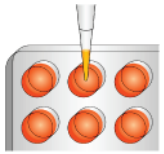
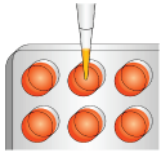
The following protocol is given for transfection in 6-well plates; refer to Table 1 for transfection in other culture formats.
The optimal transfection condition may vary in different adherent cell lines.
A general starting point for optimization in 293 cells is given in the standard protocol described below.

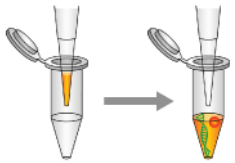
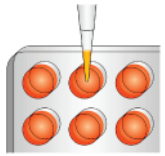
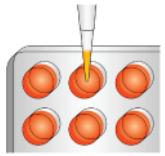
1. For each well, add 2 ml of complete medium freshly 2-4 hours before transfection.
2. For each well, dilute 2-4 µg of DNA into 150 µl of serum free medium. Vortex and spin down.
3. For each well, dilute 2-4 µl of Ultra293™ Transfection Reagent into 150 µl of serum free medium. Vortex and spin down.
4. Incubate at room temperature for 10 minutes.
5. Add the diluted Ultra293™ Transfection Reagent drop-wise to the diluted DNA solution. Vortex immediately and spin down. (Do NOT mix the solution in the reverse order!)
6. Incubate at room temperature for 15-20 minutes.
7. Add the Ultra293™ Transfection Reagent / DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
8. For sensitive cell lines, remove Ultra293™ / DNA complex-containing medium and replace with fresh complete medium 4 to 12 hours post-transfection to minimize cytotoxicity.
9. Check transfection efficiency and virus titer 24 to 48 hours post-transfection. 48 hours gives better titers.

Table 1.Recommended Amounts for Different Culture Vessel Formats

Vessel	Medium (ml)	DNA (µg)	Ultra293™ Transfection Reagent (µl)	Serum Free Medium (µl)
12-well	0.5	1-2	1-2	100
6-well	1-2	2-4	2-4	150
60 mm	2.5-5	6-12	6-12	250
10 cm	5-10	12-24	12-24	500

Note: We recommend using the lower amount of DNA as starting condition and 1 µl of Ultra293™ Transfection Reagent per 1 µg of DNA; however the amount of transfection reagent may be adjusted from1-2 µl per µg of DNA depending on the cell line to be transfected.

Day 0		Before transfection, plate cells in growth medium so that cells will be 70-80% confluent at the time of transfection. Complete culture medium is freshly added to each well before transfection.
Day 1		For each well, add complete medium freshly 2-4 hours before transfection.
		Prepare master mix of DNA by diluting DNA in serum free medium, then vortex and spin down.
		Prepare master mix of Ultra293™ Transfection Reagent solution by diluting Ultra293™ in serum free medium, then Vortex and spin down.

Day 1	 10 minutes	Incubate at room temperature for 10 minutes.
		Add the diluted Ultra293 ™solution drop-wise to the diluted DNA solution. Vortex immediately and spin down. (Do NOT mix the solution in the reverse order!)
	 20 minutes	Incubate at room temperature for 15-20 minutes.
		Add the Ultra293™ / DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
		For sensitive cell lines, remove Ultra293™ / DNA complex-containing medium and replace with fresh complete medium 4 to 12 hours post-transfection to minimize cytotoxicity.
Day 2~3		Check transfection efficiency 24 to 48 hours post-transfection.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did transfect DNA with the kit.

Problem	Cause	Solution
Low transfection efficiency	DNA: transfection reagent ratio sub-optimal for cell line	To follow the standard protocol as recommend.
Low cell viability	Plasmid DNA preparation contains high levels of endotoxin	To ensure that the plasmid DNA used for transfection is of high quality. For plasmid DNA purification kits, we recommend using our Plasmid *midi*PREP Kit (SN205-0020).
Transfection results not reproducible	Transfections performed at different cell confluencies, or at different DNA: transfection reagent ratios	1. 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. 2. Optimize transfections especially if you are transfecting a mammalian cell line for the first time.

Related Ordering Information

Cat. No.	Description	Size
SN022-0100	Genomic DNA Isolation Reagent Kit	100 Reactions
SN023-0100	Genomic DNA Isolation Kit (Blood / Cultured Cell / Fungus)	100 Reactions
SN205-0020	Plasmid *midi*PREP Kit	20 Reactions
SM508-1000	UltraTRAX Transfection Reagent	1000 µl
SM521-0100	FAM Annexin V PI Apoptosis Detection Kit	100 Reactions
CC804-0100	Ultrafect-MEM	100 ml

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- All products are for research use only.