Xfect™ Transfection Reagent Protocol-At-A-Glance (PT5003-2)

This protocol is provided for transfection with Xfect Transfection Reagent (Cat. Nos. 631317 & 631318). The following protocol assumes a transfection in a well of a 6-well plate (see Figure 1). Please see Table I on page 2 for other formats. Transfections can be carried out entirely in the presence of serum.

Notes

Storage & handling

- Thaw Xfect Polymer at room temperature just prior to use. Once thawed, store Xfect Polymer at 4°C for up to 12 months.
- Thaw Xfect Reaction Buffer at room temperature just prior to use. Vortex after thawing. Once thawed, store Xfect Reaction Buffer at 4°C for up to 12 months.
- After each use make sure that the cap for the Xfect Polymer is closed tightly and return to the supplied foil pouch containing desiccant.

Mock transfections

- Use a plasmid that does not contain your gene of interest. You should include a source of nucleic acids to assemble with the Xfect Polymer.

Transfection Protocol

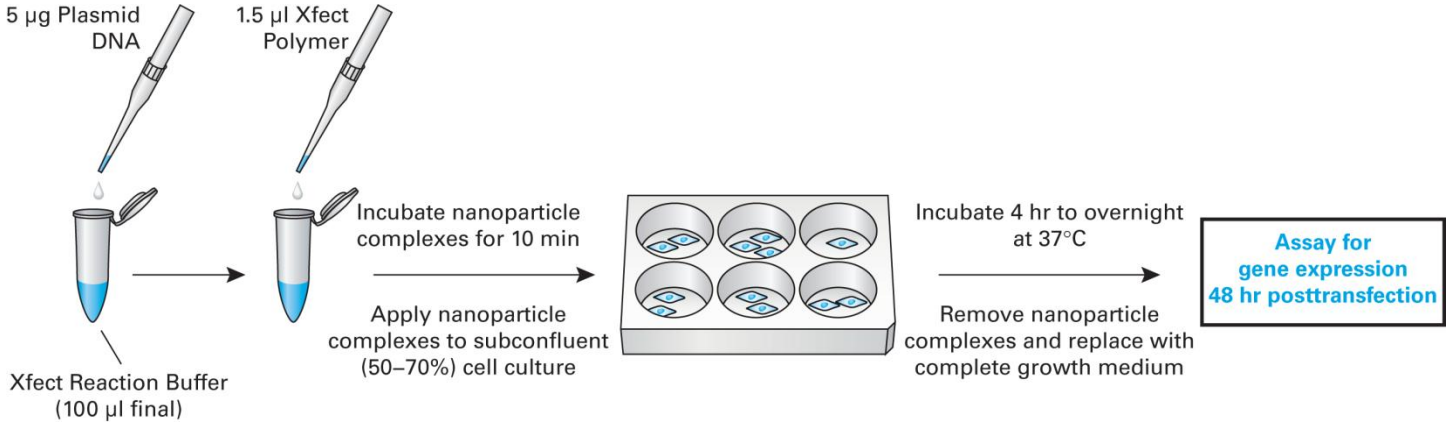

Figure 1. Simple Xfect protocol.

1. Prepare cells for transfection
   - **Adherent cells:** One day prior to the transfection, plate cells in 1 ml of complete growth medium so that the cells will be 50–70% confluent at the time of transfection.
   - **Suspension cells:** Just prior to preparing complexes (step 2), plate 5 x 10^5–1.25 x 10^6 cells in 1 ml of growth medium.
2. Thoroughly vortex Xfect Polymer.
3. In a microcentrifuge tube, dilute 5 µg of your plasmid DNA with Xfect Reaction Buffer to a final volume of 100 µl. Mix well by vortexing for 5 sec at high speed.

   NOTES:
   - Always add your plasmid to the buffer *before* adding Xfect Polymer.
   - At least 50 µl of the solution must be Xfect Reaction Buffer.
   - Do not use less than 2.5 µg of DNA per well of a 6-well plate. However, the first time you use Xfect, we recommend testing 2.5 µg, 5 µg, and 7.5 µg. Using less than 2.5 µg per well in a 6-well plate may result in a low transfection efficiency. See Figure 2 on page 2.

4. Add 1.5 µl Xfect Polymer to the diluted plasmid DNA. Mix well by vortexing for 10 sec at high speed.
   NOTE: Always keep the ratio of Polymer:DNA the same. Use 0.3 µl of Xfect Polymer per 1 µg of plasmid DNA.
5. Incubate for 10 min at room temperature to allow nanoparticle complexes to form.
   NOTE: It is recommended that the Xfect Polymer does not remain in aqueous solution for longer than 30 min.
6. Spin down for 1 sec to collect the contents at the bottom of the tube.
7. Add the entire 100 µl of nanoparticle complex solution dropwise to the cell culture medium. Rock the plate gently back and forth to mix.
   NOTE: It is not necessary to remove serum from your cell culture medium. It is normal for the medium to change color slightly upon addition of the nanoparticle complex solution.
8. Incubate the plate at 37°C for 4 hr to overnight.
   NOTE: 4 hr incubation with Xfect-DNA nanoparticles is sufficient for optimal transfection. Incubation overnight is possible for convenience but does not generally increase transfection efficiency. If you have sensitive cells we recommend incubating for no more than 4 hr.
9. Remove nanoparticle complexes from cells by aspiration, replace with 2 ml fresh complete growth medium, and return the plate to the 37°C incubator until time of analysis. Peak expression is typically reached 48 hr posttransfection.

Table I. Scaling Xfect Transfections Up or Down

Culture Vessel	Surface Area/ Well	Growth Medium	DNA	Final Dilution Volume (in Xfect Reaction Buffer)	Xfect Polymer Volume
24-well plate	2 cm^2	250 µl	0.5–1 µg	25 µl	Always use 0.3 µl of Xfect Polymer for every 1 µg of plasmid
12-well plate	4 cm^2	500 µl	1–2.5 µg	50 µl	
6-well plate	10 cm^2	1 ml	2.5–7.5 µg	100 µl	
10 cm dish	60 cm^2	10 ml	20–40 µg	600 µl	

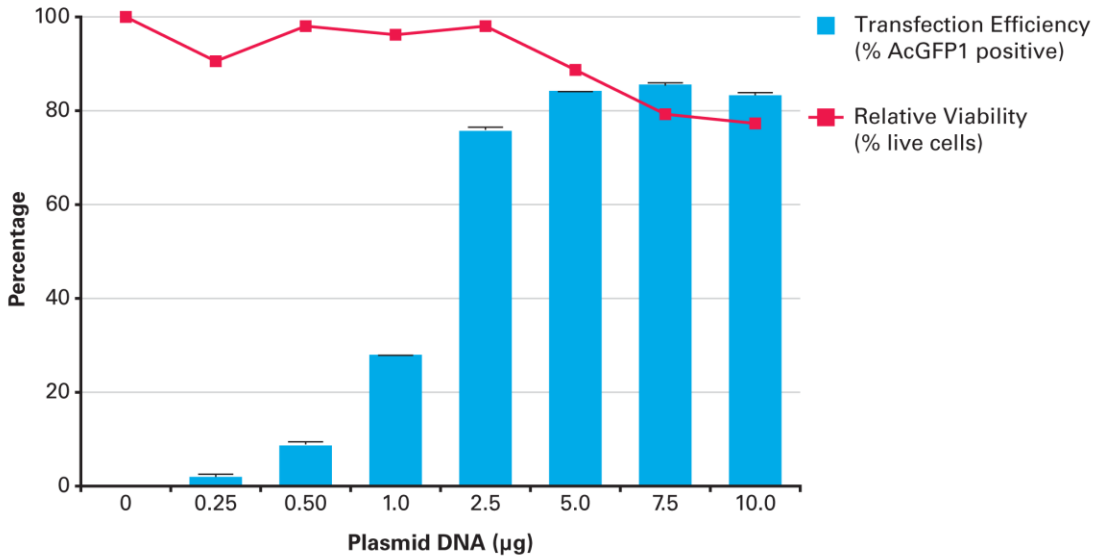

Figure 2. Transfection of Hela Cells in a 6-well plate using Xfect. Increasing the amount of DNA + Xfect Polymer significantly increases efficiency without affecting viability. 5 µg of plasmid DNA works best for most cell lines.

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: www.clontech.com	web: www.clontech.com
e-mail: orders@clontech.com	e-mail: tech@clontech.com

Notice to Purchaser

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at http://www.clontech.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

This document has been reviewed and approved by the Clontech Quality Assurance Department.