Xfect™ Transfection Reagent Protocol

General considerations

Thaw Xfect Polymer (reagent) and Xfect Reaction Buffer at room temperature just prior to use. Vortex after thawing.

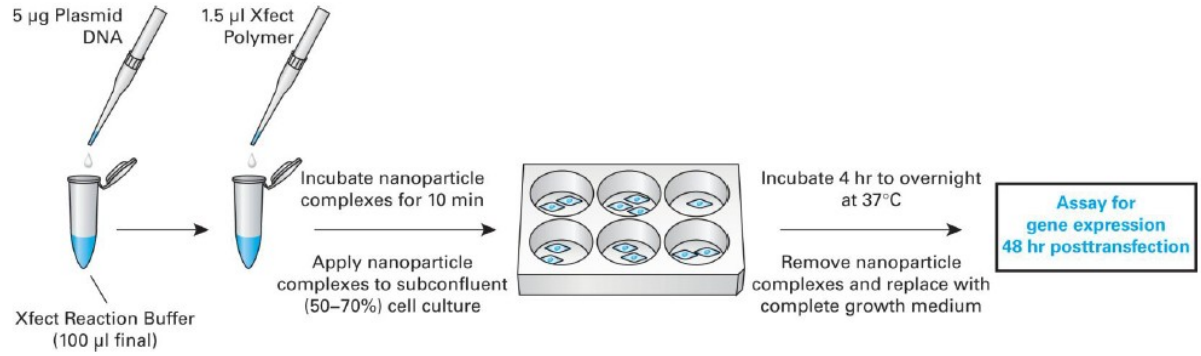
Day (24h) before Transfection

Prepare cells for transfection

Adherent cells: 24h 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.

Day of Transfection: 1hr before transfection, replace the complete growth medium with the antibiotic free medium by adding appropriate amount of media (see table below).

Transfection Protocol – 6 Well format



1. In a microcentrifuge tube, dilute 2.5 µg of plasmid DNA with Xfect Reaction Buffer to a final volume of 100 µl. Mix well by vortexing for 5 sec at high speed.
   NOTE: Do not use less than 2.5 µg of DNA per well of a 6-well plate

2. Add 0.3 µl of Xfect Polymer per 1 µg of plasmid DNA, which is 0.75 µl of Xfect Polymer to 2.5 µg of diluted plasmid DNA. Mix well by vortexing for 10 sec at high speed.
   NOTE: Always add your plasmid to the buffer *before* adding Xfect Polymer Always keep the ratio of Polymer:DNA the same. Use 0.3 µl of Xfect Polymer per 1 µg of plasmid DNA.

3. 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.

4. Spin down for 1 sec to collect the contents at the bottom of the tube.

5. 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.

6. Incubate the plate at 37°C for 4 hr.
   NOTE: 4 hr incubation with Xfect-DNA nanoparticles is sufficient for optimal transfection. If you have sensitive cells we recommend incubating for no more than 4 hr.

7. 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 or 72 hr post-transfection.

Table: Scaling Xfect transfections up or down

Culture Vessel	Surface Area/ Well	Number of adherent cells($1X10^5$)	Growth Medium	DNA	Final Dilution Volume (in Xfect Reaction Buffer)	Xfect Polymer Volume
24-well plate	2 cm^2	0. 5-0.8	250 µl	0.5 µg	100 µl	**Always use 0.3 µl of Xfect Polymer for every 1 µg of plasmid**
12-well plate	4 cm^2	0.8-1.5	500 µl	1µg	50 µl	
6-well plate	10 cm^2	2.2-3	1 ml	2.5 µg	100 µl	
10 cm dish	60 cm^2	20–40	10 ml	20–40 µg	600 µl	