I. Introduction

This protocol is provided for transfection with **Xfect Single Shots (Midi)** (Cat. Nos. 631366, 631446), single-transfection aliquots of lyophilized Xfect Transfection Reagent supplied in individual tubes. Use this procedure to transfect DNA into mammalian cells **in a 6-well format**. The amount of reagent in each tube is optimized for a single well of a 6-well plate. Transfections can be carried out entirely in the presence of serum.

II. General Considerations

A. Storage and Handling

- Store Xfect Single Shots at –20°C in the supplied foil pouch containing the desiccant sachet.
- Make sure to return any unused Xfect Single Shots to the supplied foil pouch containing the desiccant sachet, and store at –20°C.

B. 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.

III. Transfection Protocol

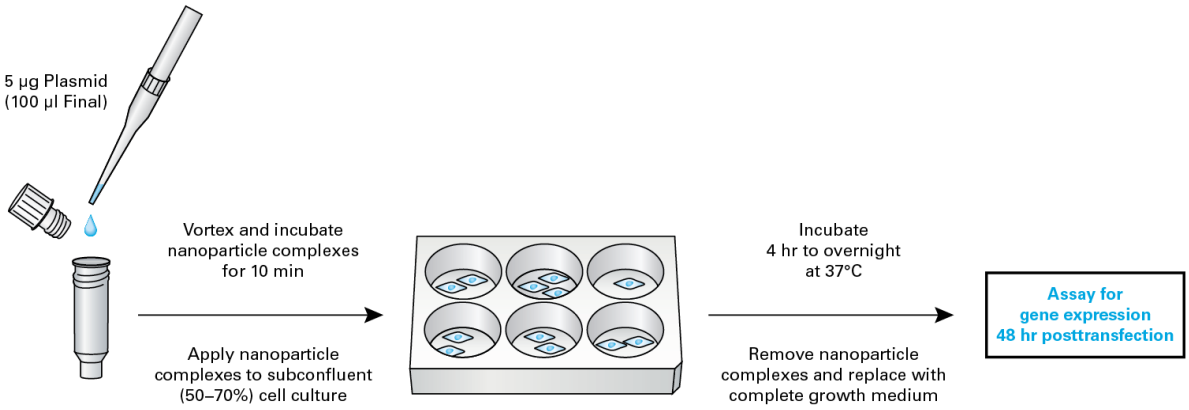

Figure 1. The Xfect Single Shots (Midi) transfection protocol.

1. Prepare cells for transfection **in a 6-well plate** as follows:
   - **Adherent cells:** One day prior to the transfection, plate cells in 1–2 ml of complete growth medium so that the cells will be 50–70% confluent at the time of transfection.
   - **Suspension cells:** Just before performing the transfection, plate 5 x 10^5–1.25 x 10^6 cells in 1 ml of growth medium.

2. In a sterile microfuge tube, dilute 5 µg of your plasmid DNA with sterile water to a final volume of 100 µl. Mix thoroughly by vortexing.

NOTES:

- **Always dilute your DNA in water prior to mixing with Xfect—do not add water and DNA separately to the Xfect Single Shot.**
- **Do not use less than 2.5 µg of DNA per well of a 6-well plate**—5 µg of plasmid DNA works best for most cell lines.

3. Add the 100 µl of diluted DNA to a tube of Xfect Single Shots, replace the cap, and vortex well at a high speed for 10 sec. The pellet should dissolve completely.

4. Incubate the samples for 10 min at room temperature to allow nanoparticle complexes to form. After the 10 min incubation, centrifuge the tube for 2 sec to collect the contents at the bottom of the tube.

NOTE: Sample tubes can be inserted into 1.5 ml microfuge tubes for centrifugation.

5. Add the entire 100 µl of nanoparticle complex solution dropwise to the cell culture prepared in Step 1. Rock the plate gently back and forth to mix.

NOTE: It is normal for the medium to change color slightly upon addition of nanoparticle complex solution.

6. Incubate the cells at 37°C from 4 hr to overnight.

NOTE: A 4 hr incubation with Xfect-DNA nanoparticles is sufficient for optimal transfection. Incubation may be continued overnight for convenience but does not generally increase transfection efficiency. If you have sensitive cells, we recommend incubating for no more than 4 hr.

7. Remove the nanoparticle complexes from the cells by aspiration, replace with 2 ml of fresh complete growth medium, and return the cells to the 37°C incubator until the time of analysis. Peak expression is typically reached 48 hr posttransfection.

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