Protocol-at-a-Glance

Xfect™ mESC Transfection Reagent Protocol-at-a-Glance

(PT5004-2)

This protocol is provided for transfection with Xfect mESC Transfection Reagent (Cat. Nos. 631320 & 631321). Use this procedure to transfect DNA into mouse pluripotent stem cells in a 6-well format. Transfections can be carried out entirely in the presence of serum.

A. Notes

1. Storage & Handling
   - Thaw Xfect mESC Polymer (100 µg/µl) at room temperature just prior to use. Once thawed, store Xfect mESC 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 mESC Polymer is closed tightly and return to the supplied foil pouch containing desiccant.
2. **Mock transfections:** Use a plasmid that does not contain your gene of interest. You must include a source of nucleic acids to assemble with the Xfect mESC Polymer.

B. Transfection Protocol

1. 5 hr prior to the transfection, plate 5 x 10^5–1 x 10^6 mouse pluripotent stem cells in 1 ml of complete growth medium on a 0.2% gelatin-coated plate.

   NOTE: To prevent ES cells from differentiating, we strongly suggest using 0.2% gelatin-coated plates to perform your transfections. Use a 10X dilution of 2% gelatin from Sigma, Cat. No. G1393, to coat the plate. Allow the coated plate to dry overnight before plating the ES cells.

2. Just before you begin, thoroughly vortex the Xfect mESC Polymer.
3. For each transfection sample, prepare two microcentrifuge tubes:

Tube 1 (Plasmid DNA)		
____	µl	(5 µg) Plasmid DNA†
____	µl	Xfect Reaction Buffer
100	µl	Total Volume

Tube 2 (Polymer)		
2.5	µl	Xfect mESC Polymer (always use 0.5 µl of Xfect mESC Polymer per 1 µg of plasmid DNA)
97.5	µl	Xfect Reaction Buffer
100	µl	Total Volume

NOTES:

- *These quantities are per well of a 6-well plate.* Please see Table I on page 2 for other formats.
- It is crucial that the Xfect mESC Polymer does not remain in aqueous solution for longer than 30 min at room temperature.

†5 µg of plasmid DNA works best for most cell lines. However, the first time you use Xfect mESC, 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.

4. Vortex each tube well to mix.
5. Add the polymer solution to the DNA solution and vortex well at a medium speed for 10 sec.
6. Incubate the samples for 10 min at room temperature to allow nanoparticle complexes to form.
7. Add the entire 200 µl of nanoparticle complex solution (Step B.5) dropwise to cultured cells from Step B.1. Rock the plate gently back and forth to mix.

   NOTE: 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 3 hr.
9. Remove nanoparticle complexes from cells by aspiration and replace with 2 ml fresh complete growth medium containing 4 x 10^5 MEF cells. Return the plate to the 37°C incubator.
10. Change medium daily. Peak expression is generally reached 48 hr post-transfection.

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Table I. Scaling Xfect mESC Transfections Up or Down

Culture Vessel	Surface Area/ Well	Growth Medium*	DNA	DNA Dilution Volume (in Xfect Reaction Buffer)	Xfect mESC Polymer Volume	Polymer Dilution Volume (in Xfect Reaction Buffer)
24-well plate	2 cm^2	250 µl	1–2.5 µg	25 µl	Always use 0.5 µl of Xfect mESC Polymer for every 1 µg of plasmid	25 µl
12-well plate	4 cm^2	500 µl	2.5–5 µg	50 µl		50 µl
6-well plate	10 cm^2	1 ml	5–10 µg	100 µl		100 µl
10 cm dish	60 cm^2	10 ml	30–50 µg	600 µl		600 µl

* This is the volume required for transfection. After 3 hr transfection (Step B.7), replace with twice the volume of complete growth medium containing MEF cells.

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