Xfect™ Protein Transfection Protocol

PT5166-2

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I. Required Materials

- Xfect Protein Transfection Reagent
- Xfect Protein Buffer
- Deionized H_2O (sterile)
- Beta-Galactosidase Control
- X-gal staining kit or reagents (for the control; use any standard kit or protocol)

II. Protocol

A. Preparation of the Xfect Protein Transfection Reagent Stock Solution

1. Add Deionized H_2O directly to the vial of lyophilized Xfect Protein Transfection Reagent, and mix gently. Be sure to add the amount of H_2O appropriate for the reagent supplied with your kit:
    - *If you are using the **30 rxn kit***, add 225 µl deionized H_2O to one vial of Xfect Protein Transfection Reagent; this 1X stock will provide enough reagent for 15 rxns.
    - *If you are using the **100 rxn kit***, add 750 µl deionized H_2O to one vial of Xfect Protein Transfection Reagent; this 1X stock will provide enough reagent for 50 rxns.
2. Store small aliquots of the 1X stock solution at –20°C for up to 6 months. Avoid multiple freeze-thaw cycles.

B. Transfection of Cells with a Protein of Interest

The following protocol describes the transfection of protein into one well of a 6-well plate. See Table 1 (at the end of this section) for information on scaling the transfections up or down.

1. Seed **adherent cells** on a 6-well plate the day before performing the transfection so that they will be 60–80% confluent at the time of transfection.

   Seed **non-adherent cells** on a 6-well plate at 1.5 x 10^6 cells/well on the day the transfection is performed. See Step 4 for details.
2. For each transfection reaction, prepare two microcentrifuge tubes:
    a. In Tube 1, dilute 15 µl of the 1X Xfect Protein Transfection Reagent stock solution (see Section A) in 85 µl Deionized H_2O. Gently vortex or pipette up and down to mix.

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

United States/Canada 800.662.2566
Asia Pacific +1.650.919.7300
Europe +33.(0)1.3904.6880
Japan +81.(0)77.543.6116

b. In Tube 2, pipette 2–5 µg of your protein of interest, then add enough Xfect Protein Buffer to bring the volume up to 100 µl. Gently pipette up and down to mix.

3. Add the contents of Tube 2 to Tube 1, gently vortex or pipette to mix, and incubate at room temperature for 30 min.

4. During the 30 min incubation in Step 3, prepare the cells as follows:

   For **adherent cells** (6-well format):

   a. Aspirate the medium and wash the cells once with warm PBS.

   b. Remove the PBS and add 400 µl of serum-free medium to each well containing cells. Rock the plate gently to ensure the liquid covers the bottom of the wells.

   For **non-adherent cells** (6-well format):

   a. Dispense 1.5 x 10^6 cells into a sterile microcentrifuge tube. Pellet the cells by centrifugation at 500 x g for 5 min.

   b. Remove the medium and wash the cells with warm PBS. Pellet the cells again and discard the PBS.

   c. Resuspend the cells in 400 µl serum-free medium, and transfer the cells into the well of a 6-well plate. Rock the plate gently to ensure the liquid covers the bottom of the well. *If you wish to perform the transfection in a microcentrifuge tube, leave the cells in the tube; do not transfer them to a 6-well plate.*

5. Add the 200 µl transfection reagent/protein mixture from Step 3 to the cells in serum-free medium (from Step 4).

6. Incubate at 37°C for 60 minutes.

7. Proceed with any detection assay that is appropriate for your protein.

To scale the transfection reactions up or down, use the amounts recommended in Table 1, below.

Table 1. Reaction Component Amounts Recommended for Scaling Transfections Up or Down.

	Tube 1		Tube 2		
Culture Vessel	1X Xfect Protein Transfection Reagent Stock[1] (See Section II.A)	Deionized Water	Your Protein[1]	Xfect Protein Buffer	Serum-Free Medium[2]
96-well plate	0.75 µl	4.25 µl	0.1–0.25 µg	to 5 µl	40 µl
24-well plate	3 µl	17 µl	0.4–1.0 µg	to 20 µl	110 µl
12-well plate	6 µl	34 µl	0.8–2.0 µg	to 40 µl	220 µl
6-well plate	15 µl	85 µl	2.0–5.0 µg	to 100 µl	400 µl
10 cm dish	90 µl	510 µl	12–30 µg	to 600 µl	2400 µl

[1]Start with the recommended amounts of protein and transfection reagent listed in the table. If further optimization is required for your specific protein, we recommend increasing the ratio of transfection reagent to protein. For example, when transfecting 1 µg of protein, use 3 µl, 5 µl, 7.5 µl and 15 µl of Xfect to determine your optimal transfection conditions.

[2]Depending on the brand of tissue culture plastic ware you use, it may be necessary to increase the volume of serum-free medium up to 15% to ensure complete coverage of the cells.

C. Control Reaction: Transfection of Cells with the Beta-Galactosidase Control

1. Plate the cells as described in Section B, Step 1 on the previous page.
2. Dilute 15 µl of the Xfect Protein Transfection Reagent stock (see Section A) in 85 µl Deionized H_2O. Mix.
3. In a separate tube, dilute 2 µl of the Beta-Galactosidase Control in 98 µl Xfect Protein Buffer.
4. Add 100 µl of the diluted Beta-Galactosidase Control to the tube containing 100 µl of diluted transfection reagent. Gently vortex or pipette to mix, and incubate at room temperature for 30 min.
5. During the 30 min incubation in Step 4, prepare the cells as follows:

   For **adherent cells** (6-well format):

   a. Aspirate the medium and wash the cells once with warm PBS.

   b. Remove the PBS and add 400 µl of serum-free medium to the well. Rock the plate gently to ensure the liquid covers the bottom of the well.

   For **non-adherent cells** (6-well format):

   a. Dispense 1.5 x 10^6 cells into a sterile microcentrifuge tube. Pellet the cells by centrifugation at 500 x g for 5 min.

   b. Remove the medium and wash the cells with warm PBS. Pellet the cells again and discard the PBS.

   c. Resuspend the cells in 400 µl serum-free medium, and transfer the cells into the well of a 6-well plate. Rock the plate gently to ensure the liquid covers the bottom of the well. *If you wish to perform the transfection in a microcentrifuge tube, leave the cells in the tube; do not transfer them to a 6-well plate.*
6. Add the 200 µl transfection reagent/Beta-Galactosidase mixture from Step 4 to the cells in serum-free medium (from Step 5).
7. Incubate at 37°C for 60 minutes.
8. *For adherent cells*, remove the liquid from the wells and add complete medium; *for non-adherent cells*, add two volumes of complete medium to the liquid in the wells. Incubate at 37°C for 2 hrs.
9. Stain the cells with X-gal to determine the efficiency of Beta-Galactosidase transfection using our Beta-Galactosidase Staining Kit (Cat. No. 631780).

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: 650.424.1064
web: www.clontech.com	web: www.clontech.com
e-mail: orders@clontech.com	e-mail: tech@clontech.com