I. Introduction

A. Summary

This Protocol-At-A-Glance is provided for transfection of cells with RNA using the **Xfect RNA Transfection Reagent** (Cat. No. 631450). It describes transfection of mammalian cells with RNA (mRNA, sgRNA, microRNA, or shRNA) in a 12-well plate format. For formats other than 12-well plates, see Tables I and II for appropriate reaction volumes. Transfections can be carried out entirely in the presence of serum.

The protocol is divided into two sections:

- Section II describes transfection of cells with RNA only, without the cotransfection of DNA.
- Section III describes cotransfection of cells with both RNA and DNA.

NOTE: When transfecting cells with mRNA, expression may be increased by using serum-free medium during transfection, since serum may contain RNases that can reduce the amount of full-length mRNA.

B. General Considerations

Storage & handling

- Store the Xfect RNA Transfection Polymer at –20°C. Do not thaw until ready to use. Once thawed, store at 4°C for up to 12 months.

  NOTE: The Xfect RNA Transfection Polymer is a milky suspension and should be vortexed briefly prior to use to ensure that it is fully resuspended.

- 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.

Xfect Polymer

The protocol for cotransfection with DNA (Section III) requires the use of the Xfect Polymer (not included; sold as part of the DNA Xfect Transfection Reagent, Cat. Nos. 631317, 631318).

II. Protocol: Transfection of cells with RNA

Use this protocol to transfect cells with RNA only. For cotransfection with both RNA and plasmid DNA, please follow the protocol in Section III.

1. Prepare cells for transfection (12-well plate format).
   - **Adherent cells:** One day prior to transfection, plate cells in 1 ml of complete growth medium so that the cells will be 80–90% confluent at the time of transfection (e.g., for HeLa cells, seed 1 x 10^5 cells).
   - **Suspension cells:** Just prior to preparing complexes (Step 3), plate 6–8 x 10^5 cells in 1 ml of complete growth medium.
2. Thoroughly vortex the Xfect RNA Transfection Polymer.
3. For each sample, prepare two microcentrifuge tubes:

Tube 1 (RNA)		Tube 2 (Transfection Polymer)	
x µl	small RNA (50 pmol) or mRNA (0.8–1.2 µg)	5 µl	Xfect RNA Transfection Polymer
x µl	Xfect Reaction Buffer (as needed)	45 µl	Xfect Reaction Buffer
50 µl	Total Volume	50 µl	Total Volume

NOTE: These quantities are per well of a 12-well plate. Please see Table I for other formats.

Table I. Reaction Volumes for Different Culture Vessel Formats
(Transfections with RNA only; see Table II for cotransfections with plasmid DNA.)

Culture Vessel	Surface Area per Well	Growth Medium	Tube 1 (RNA)		Tube 2 (Transfection Polymer)	
			Small RNA / mRNA	Xfect Reaction Buffer (to total volume)	Xfect RNA Transfection Polymer	Xfect Reaction Buffer
96-well plate	0.3 cm^2	100 µl	5 pmol / 0.1 ug	5 µl	0.5 µl	5 µl
24-well plate	2 cm^2	500 µl	25 pmol / 0.3–0.6 ug	25 µl	2.5 µl	25 µl
12-well plate	4 cm^2	1 ml	50 pmol / 0.8–1.2 ug	50 µl	5 µl	50 µl
6-well plate	10 cm^2	2 ml	100 pmol / 1.5–2.0 ug	100 µl	10 µl	100 µl
60-mm dish	20 cm^2	4 ml	200 pmol / 2–4 ug	200 µl	20 µl	200 µl
10-cm dish	60 cm^2	10 ml	500 pmol / 4–8 ug	500 µl	50 µl	500 µl

4. Mix Tube 1 and Tube 2 (from Step 3) well by vortexing.
5. Transfer the contents of Tube 2 into Tube 1. Mix well by vortexing for 5 sec at medium speed.
6. Incubate the samples for 10 min at room temperature to allow nanoparticle complexes to form.
7. Add the entire 100 µl of nanoparticle complex solution (from Step 6) drop-wise to the cell culture medium from Step 1. 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. However, in order to yield the highest expression level from a transfection with mRNA, the use of serum-free media during transfection might protect the mRNA from digestion by RNases.

8. Incubate the plate at 37°C in a CO_2 incubator for 4 hr.
9. Remove the transfection complexes by aspirating the media from the cells. (If using suspension cells, first pellet the cells by centrifugation at 500 x g for 5 min.) Add 1 ml of fresh media.
10. Incubate the plate at 37°C in a CO_2 incubator until the time of analysis (24–48 hr post-transfection).

III. Protocol: Cotransfection of Cells with Small RNA and Plasmid DNA

For some applications (e.g., transfections with sgRNA or microRNA) it might be necessary to cotransfect cells with both small RNAs and plasmid DNA at the same time. In these cases, it will be necessary to also purchase the DNA Xfect Transfection Reagent (Cat. Nos. 631317, 631318), which includes the Xfect Polymer. When cotransfecting cells with both plasmid DNA and your RNA, you must also add a small amount of the Xfect Polymer to the reaction mix, as described below.

NOTE: Store the Xfect Polymer with desiccant at –20°C. Do not thaw until ready to use. Once thawed, store 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 the tube to the supplied foil pouch containing desiccant.

1. Prepare cells for transfection (in a 12-well plate format).
   - **Adherent cells:** One day prior to the transfection, plate cells in 1 ml of complete growth medium so that the cells will be 80–90% confluent at the time of transfection (e.g., for HeLa cells, seed 1 x 10^5 cells).
   - **Suspension cells:** Just prior to preparing complexes (Step 3), plate 6–8 x 10^5 cells in 1 ml of complete growth medium.
2. Thoroughly vortex the Xfect RNA Transfection Polymer and Xfect Polymer.
3. For each sample, prepare two microcentrifuge tubes:

Tube 1 (small RNA and plasmid DNA)	
x µl	small RNA (50 pmol)
x µl	plasmid DNA (1.25 µg)
x µl	Xfect Reaction Buffer (as needed)
60 µl	Total Volume

Tube 2 (Transfection Polymers)	
0.5 µl	Xfect Polymer
54.5 µl	Xfect Reaction Buffer
Combine, vortex, and then add:	
5 µl	Xfect RNA Transfection Polymer
60 µl	Total Volume

NOTES:
- Always add Xfect Polymer to the buffer and vortex **before** adding the Xfect RNA Transfection Polymer.
- These quantities are per well of a 12-well plate. Please see Table II on the following page for other formats.

Table II. Reaction Volumes for Different Culture Vessel Formats
(Cotransfection with small RNA and plasmid DNA)

Culture Vessel	Surface Area per Well	Growth Medium	Tube 1 (Small RNA and Plasmid DNA)			Tube 2 (Transfection Polymers)		
			Small RNA	Plasmid DNA	Xfect Reaction Buffer (to total volume)	Xfect Polymer	Xfect Reaction Buffer (to total volume)	Xfect RNA Transfection Polymer (add last)
96-well plate	0.3 cm^2	100 µl	5 pmol	0.2–0.3 µg	6 µl	**Use 0.3 µl Xfect Polymer for every 1 µg plasmid DNA.**	6 µl	0.5 µl
24-well plate	2 cm^2	500 µl	25 pmol	1–1.5 µg	30 µl		30 µl	2.5 µl
12-well plate	4 cm^2	1 ml	50 pmol	2–3 µg	60 µl		60 µl	5 µl
6-well plate	10 cm^2	2 ml	100 pmol	2.5–7.5 µg	120 µl		120 µl	10 µl
60-mm dish	20 cm^2	4 ml	200 pmol	7.5–12.5 µg	240 µl		240 µl	20 µl
10-cm dish	60 cm^2	10 ml	500 pmol	20–30 µg	600 µl		600 µl	50 µl

4. Mix Tube 1 and Tube 2 (from Step 3) well by vortexing.
5. Transfer the contents of Tube 2 into Tube 1. Mix well by vortexing for 5 sec at medium speed.
6. Incubate the samples for 10 min at room temperature to allow nanoparticle complexes to form.
7. Add the entire 120 µl of nanoparticle complex solution (from Step 6) drop-wise to the cell culture medium from Step 1. 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 in a CO_2 incubator for 4 hr.
9. Remove the transfection complexes by aspirating the media from the cells. (If using suspension cells, first pellet the cells by centrifugation at 500 x g for 5 min.) Add 1 ml of fresh media.
10. Incubate the plate at 37°C in a CO_2 incubator until the time of analysis (24–48 hr post-transfection).

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