TransFast™ Transfection Reagent

INSTRUCTIONS FOR USE OF PRODUCTS E2431 AND E2432.

Quick PROTOCOL

Transfection Protocol for Adherent Cells

This is a general protocol. If you have optimized the transfection parameters for your cell line, use the empirically determined conditions for your experimental transfections. Volumes and amounts are given for transfections performed in 60mm plates (values for 100mm plates are given in parentheses).

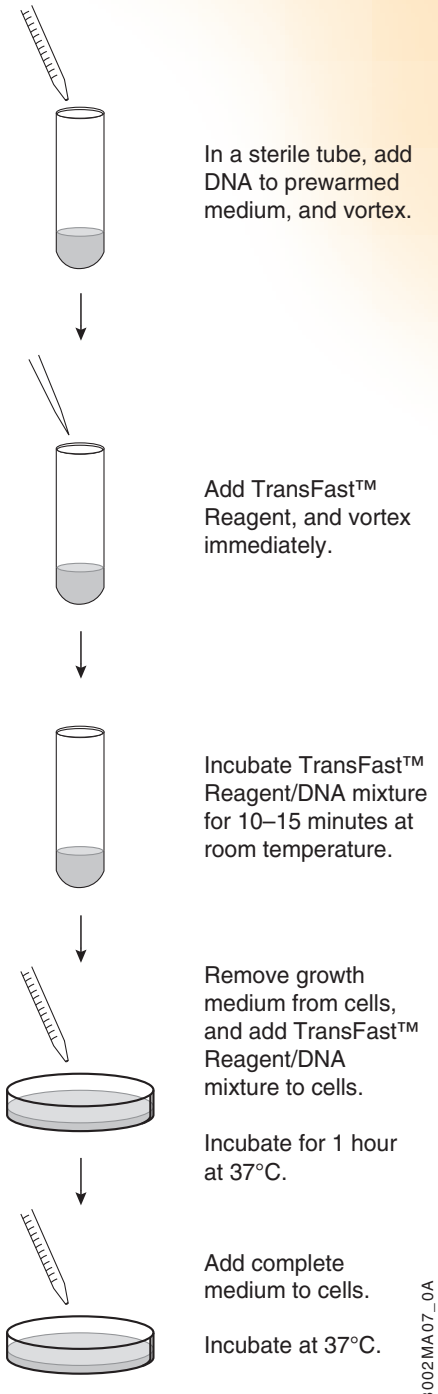
1. Add the appropriate amount of prewarmed (37°C) medium to a sterile tube (2ml total volume DNA, lipid and medium per 60mm plate, 6ml for a 100mm plate). Add 2.5–10µg of plasmid DNA to the medium (7.5–30µg for a 100mm plate), and vortex.
2. Add the amount of TransFast™ Reagent indicated in the table below for the desired ratio of reagent to DNA, and vortex immediately.

Charge Ratio of TransFast™ Reagent to DNA	Volume of Transfast™ Reagent per µg of DNA*
1:1	3.0µl
2:1	6.0µl
3:1	9.0µl

*Volumes given are for use with TransFast™ Reagent suspended in 400µl/vial.

3. Incubate the TransFast™ Reagent/DNA mixture for 10–15 minutes at room temperature.
4. Remove the growth medium from the cells.
5. Briefly vortex the TransFast™ Reagent/DNA mixture. Add 2ml (or 6ml) of the TransFast™ Reagent/DNA mixture to each plate, and immediately return the cells to the incubator for 1 hour. During the incubation warm an appropriate volume of complete medium (containing serum) to 37°C.
6. At the end of the incubation period, gently overlay the cells with 4ml (or 12ml) of complete medium. Do not remove the transfection medium containing the TransFast™ Reagent. Return cells to the incubator, and continue incubation for the appropriate length of time for the analysis.
7. Check the transfection efficiency using the appropriate assay. For transient transfection, cells are typically harvested 48 hours after transfection.

See additional protocol information in Technical Bulletin #TB260, available online at: **www.promega.com**



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Transfection Protocol for Suspension Cells

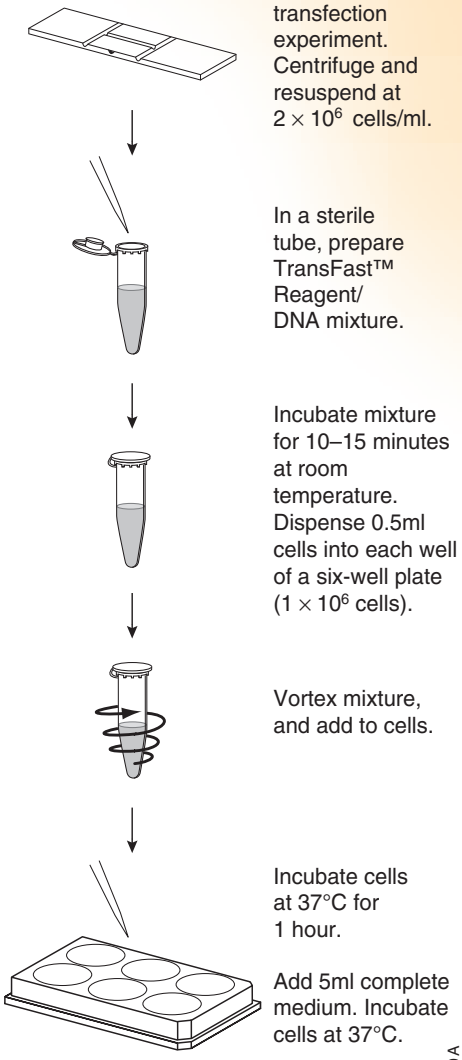
1. Determine cell density using a hemacytometer, and concentrate enough cells to complete the transfection experiment. Centrifuge the cells for 5 minutes at 300 × *g* in a swinging bucket rotor. Suspend the cell pellet to a concentration of 2×10^6 cells/ml in serum-free medium.
2. To a sterile tube, add the indicated amount of medium (prewarmed to 37°C) and DNA to a total volume of 0.5ml, and vortex. Add the indicated amount of TransFast™ Reagent from the table below, and vortex immediately.

	Amount of DNA			
	1µg	2µg	3µg	4µg
Medium to a final volume of:	0.5ml	0.5ml	0.5ml	0.5ml
DNA	1µg	2µg	3µg	4µg
TransFast™ Reagent*	3µl	6µl	9µl	12µl

*Volumes given are for use with TransFast™ Reagent suspended in 400µl/vial.

3. Incubate the TransFast™ Reagent/DNA mixture for 10–15 minutes at room temperature.
4. Dispense 0.5ml of cells (1×10^6 cells) to each well of a 6 well plate.
5. Briefly vortex the TransFast™ Reagent/DNA mixture, and add to the cells (0.5ml/well). Incubate cells at 37°C for 1 hour. During the incubation prewarm complete medium to 37°C.
6. After incubation, add 5ml of prewarmed complete medium to each well. Continue incubation for appropriate length of time before analysis. Forty-eight hours is a typical incubation period for both transient transfections and many reporter assays.

See additional protocol information in Technical Bulletin #TB260, available online at: **www.promega.com**



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