In vitro DNA Transfection using Thermo Scientific TurboFect™ Transfection Reagent

Protocol

This protocol is for the *In vitro* DNA Transfection using Thermo Scientific TurboFect™ Transfection Reagent.

Reagents to be Supplied by the User:

Serum free DMEM, RPMI or other growth medium. The presence of antibiotics in the medium has no effect on transfection efficiency. The protocol below is provided for 24-well plate. Quantities and volumes should be scaled up or down according to the number of cells/or wells to be transfected (see Table below for guidelines).

1. In each well, seed ~ 5×10^4 adherent cells or ~ 5×10^5 suspension cells 24 hours prior to transfection.

Note

- The recommended confluency for adherent cells on the day of transfection is 50-70%.
- Suspension cells should be in logarithmic growth phase at the time of transfection.

2. Dilute 1 µg of DNA in 100 µL of serum-free DMEM or other growth medium.
3. Add 2 µL of TurboFect™ to the diluted DNA and mix the solution by pipetting.
4. Incubate 15-20 minutes at room temperature.

Note

- Prepare immediately prior to transfection.
- We recommend starting with 1 µg of DNA and 2 µL of TurboFect per well in a 24-well plate (see Table below).
- Subsequent optimization may further increase transfection efficiency depending on the cell line and transgene used.

5. Dilute 1 µg of DNA in 100 µL of serum-free DMEM or other growth medium.
6. Gently rock the plate to achieve even distribution of the complexes.
7. Incubate at 37 °C in a CO_2 incubator.
8. Analyze transgene expression 24-48 hours. For stable transfection cells should be grown in selective medium for 10-15 days. Plates can be centrifuged for 2-5 minutes at 200 × g to facilitate sedimentation of cells to the bottom of the plate.

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Table. Scale-up /down ratios for transfection with TurboFect™ Transfection Reagent.

Tissue Culture Vessel	Growth Area, cm^2/well	Media, mL	Adherent cells to seed the day before transfection*	DNA, µg** (µL***)	Volume of TurboFect (µL)**	
					Recommended	Range
96-well plate	0.3	0.2	$0.5\text{-}1.20 \times 10^4$	0.2 (20)	0.4	0.3-0.6
48-well plate	0.7	0.5	$1.0\text{-}3.0 \times 10^4$	0.5 (50)	1.0	0.5-1.4
24-well plate	2.0	1.0	$2.0\text{-}6.0 \times 10^4$	1.0 (100)	2.0	1.0-2.8
12-well plate	4.0	2.0	$0.4\text{-}1.2 \times 10^5$	2.0 (200)	4.0	2.0-6.0
6-well plate	9.5	4.0	$0.8\text{-}2.4 \times 10^5$	4.0 (400)	6.0	4.0-8.0
60 mm plate	20.0	6.0	$2.0\text{-}6.3 \times 10^5$	6.0 (600)	12.0	8.0-16.0

*These numbers were determined using HeLa cells. Actual value depends on the cell type.
**Amount of DNA and TurboFect™ Transfection Reagent used may require optimization.
***The volume of the DNA solution should represent 1/10 of the total volume of the culture medium.

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