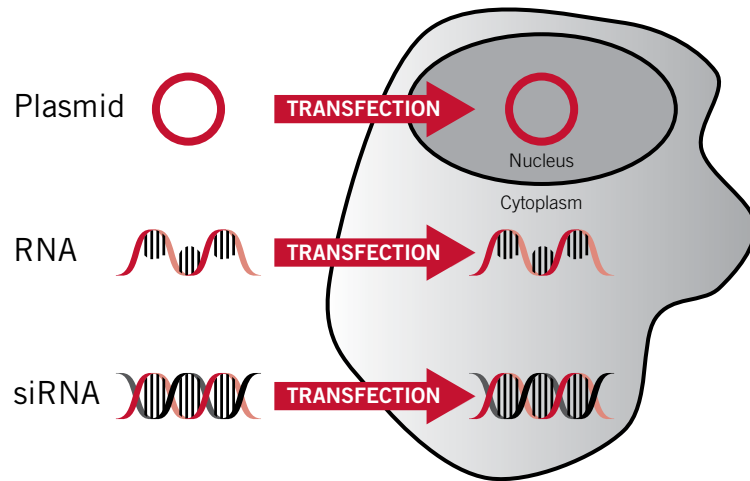


WHAT IS TRANSFECTION?

Traditionally, transfection has been the introduction of foreign DNA by non-viral means into cultured eukaryotic cells. Currently, the term transfection applies to the introduction of any naked nucleic acid molecule, not just DNA, into cultured eukaryotic cells.



Optimizing Transfection Performance – General Suggestions For All Reagents, Cells and Nucleic Acids

1. Ratio of transfection reagent to nucleic acid

For each cell type, simultaneously transfect different amounts of nucleic acid using various amounts of transfection reagent.

2. Transfection complex formation time

Test different transfection complex formation times (reagent + nucleic acid incubation time) for each reagent and cell type being transfected.

3. Cell density (confluency) at time of transfection

Transfect each cell type at various densities ranging from 50% to 90% and monitor transfection performance.

4. Post-transfection incubation time (time after addition of transfection complexes to cells)

Harvest cells at different times post-transfection to determine the optimal assay time based on the experimental goals.

Tissue Culture Vessel Surface Areas and Recommended Media and Nucleic Acid Amounts per Vessel

Culture Vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	60 mm dish	100 mm dish	T75 flask	T150 flask
Surface Area ^a (cm ²)	0.35	1.0	1.9	3.8	9.6	21.3	59	75	150
Growth Media ^b (ml)	0.09	0.26	0.5	1.0	2.5	5.6	15.5	20	40
Serum-free Media ^b (μl)	9.0	26	50	100	250	560	1,550	2,000	4,000
DNA or large RNA ^b (μg)	0.09	0.26	0.5	1.0	2.5	5.6	15.5	20	40
siRNA ^c	16.3 ng	47 ng	90 ng	180 ng	450 ng	1 μg	2.8 μg	3.6 μg	7.2 μg

a Surface areas are based on Greiner tissue culture plates and Falcon 60 mm and 100 mm dishes and T75 and T150 flasks. All growth media volumes are per one well of a plate or one dish of the indicated size.

b Growth media volume is scaled based on the surface area of the culture vessel. When scaling transfections between vessels, all components (growth media, cells, transfection reagent, nucleic acid, and serum-free media for complex formation) should be scaled according to surface area. See our reagent specific protocols for more details.

c siRNA amounts are based on transfections using half the indicated volume of growth media per well. See our siRNA transfection reagent protocols for more details.

Assessing Transfection Performance Using Reporters

Green Fluorescent Protein (GFP)—An inherently fluorescent protein used to determine transfection efficiency (percentage of transfected cells in the population) either visually by fluorescence microscopy or quantitatively by flow cytometry.

b-galactosidase (b-gal)—An *E. coli* enzyme encoded by the *lacZ* gene used to determine transfection efficiency. Cells can be stained with X-gal, and cells expressing b-gal will turn blue. Transfection efficiency is determined by counting the blue stained cells. b-gal activity can also be assayed in cell lysates using colorimetric or chemiluminescent substrates.

Luciferase—An enzyme found in many organisms, most popular the firefly, catalyzes the production of light in the reaction between luciferin and ATP. Luciferase assays are highly sensitive making this enzyme an excellent reporter for determining relative transfection efficiencies between samples. Most firefly based systems require cell lysis.

Secreted Alkaline Phosphatase (SEAP)—A modified enzyme from human placenta that is secreted from mammalian cells. Cells transfected with this reporter do not need to be lysed to assay for activity; the media from the transfected sample is harvested and assayed using a variety of methods enabling repeated assays of the same transfected well over time.