ThermoFisher SCIENTIFIC

Transfection

Invitrogen transfection products

High-performance, lipid-based reagents and electroporation systems—delivered with over 30 years of expertise

invitrogen

Introduction

Transfection is a key step in cell biology, gene expression, gene knockdown, and genome editing experiments. Our high-quality products for lipid-based transfection and electroporation are useful across a wide variety of cell types and applications. Invitrogen™ Lipofectamine™ reagents are the most cited transfection reagents in the world, with more than 300,000 citations and over 30 years of performance leadership. Ever-evolving developments in research focused on hard-to-transfect cells and genome editing have paved the way for the increased popularity of electroporation systems and the specialized payload delivery reagents featured here.

Contents

What is transfection?

Transfection is the process of introducing foreign biological material, such as DNA, RNA, or protein, into eukaryotic cells *in vitro* or *in vivo*.

This process is commonly used for gene expression, RNA interference (RNAi), genome editing, protein expression for biologics production, and viral production for cell and gene therapy research.

Transfection methods

There are three types of transfection methods: chemical, physical, and biological. The selection of the best transfection method depends on the type of cells you are transfecting, delivery payload type and size, transfection efficiency, cell viability expectations, reaction scale, throughput, and budget.

Chemical methods

Cationic lipid–based transfection is the most popular chemical transfection method. Known for its ease of use, this method can be performed using high-performance reagents designed to work with a broad spectrum of cell types and payloads. This method is also compatible with high-throughput applications and generally more affordable per reaction than other methods. Cationic lipid–based transfection relies on the formation of a complex between the negatively charged delivery payload (e.g., DNA) and the transfection reagent (Figure 1). Other types of chemical methods are also available, such as methods that utilize calcium phosphate and cationic polymers.

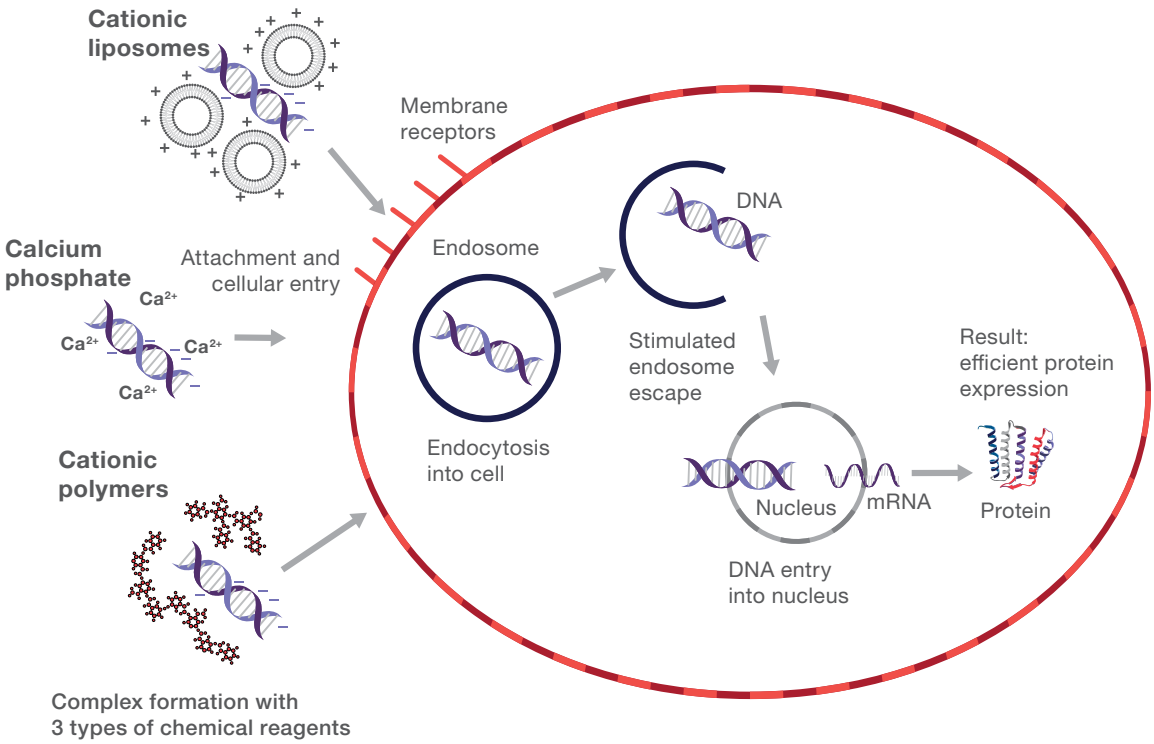


Figure 1. Mechanism of transfection using cationic lipids or other chemical reagents. The positive surface charge of the reagent–DNA complex enables attachment to the negatively charged cell surface and entry into the cell by endocytosis. Following escape from the endosome, the DNA payload is transcribed into mRNA in the nucleus and translated into protein in the cytoplasm. An mRNA payload would be translated directly in the cytoplasm.

Physical methods

Electroporation is the most widely used physical transfection method due to the possibility of acheiving high transfection efficiency, even with hard-to-transfect cell types, as well as its ease of use and biosafety. It is rapidly gaining popularity for cell therapy applications due to its effectiveness in immune cells. Electroporation uses an electrical pulse to create temporary pores that allow payloads to cross the cell membrane (Figure 2). Optimal performance can be achieved by adjusting electroporation parameters, such as the voltage, duration, pulse number, cell density, and payload amount. Other types of physical methods are also available, such as microinjection and sonoporation.

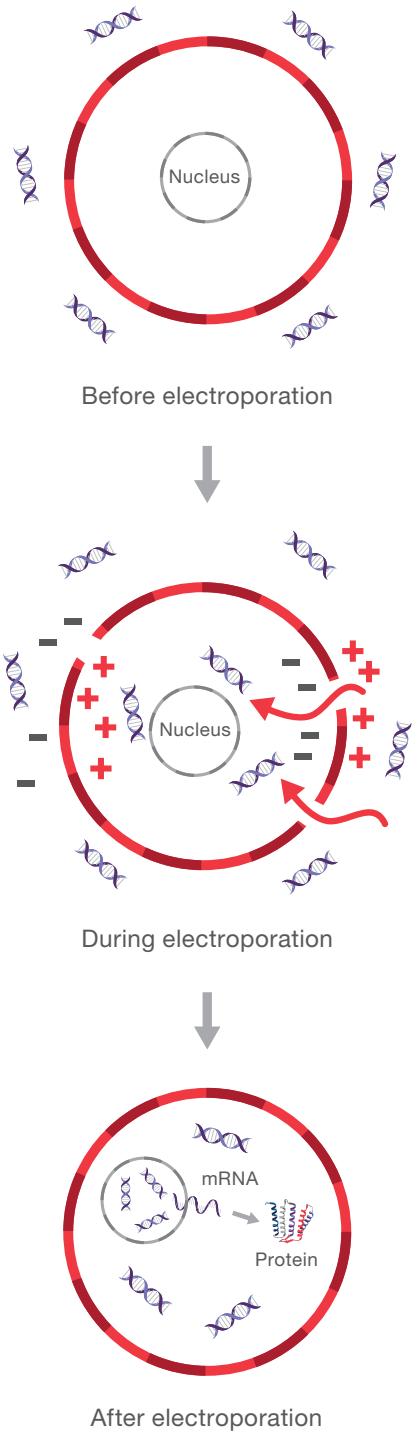


Figure 2. Mechanism of transfection using electroporation. Before electroporation, the cell membrane is intact, and the payload (e.g., DNA) is outside of the membrane. When an electric field is induced across the cell membrane, multiple pores form that allow the payload to enter the cell. After electroporation, the cell membrane recovers, and the payload is distributed in the cytoplasm and nucleus.

Biological methods

Virus-mediated transfection, also known as transduction, employs viral vectors like lentiviral vectors, adeno-associated virus (AAV), and retroviral vectors to deliver genes into all cells, including hard-to-transfect cell lines (Figure 3). Viral vectors are broadly used in popular applications, such as cell and gene therapies and vaccine development.

One of the main advantages of viral delivery is that the process can occur inside a living organism or in cell culture with gene delivery efficiencies approaching 100%.

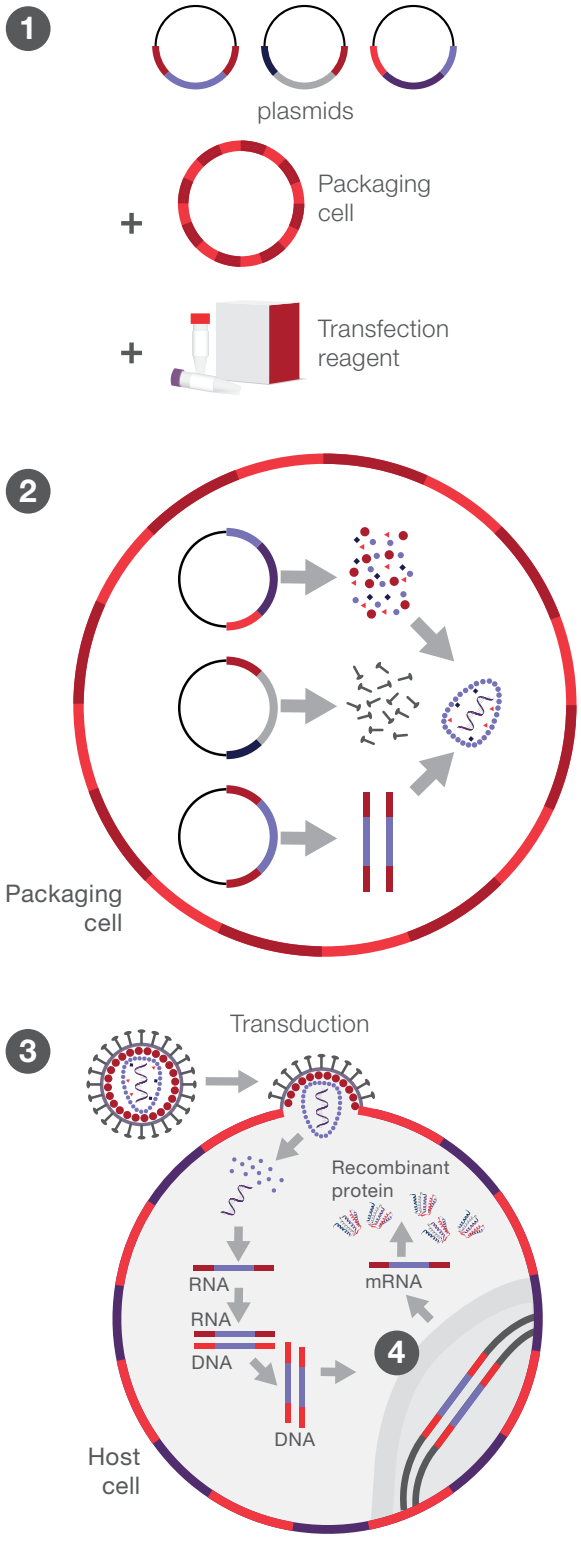


Figure 3. Preparation and use of a viral vector for recombinant protein expression in target cells. (1) Packaging cells (e.g., HEK293) are transfected with three or four plasmids encoding the gene of interest and necessary viral proteins. (2) The virus is assembled in the packaging cells, then harvested and purified. (3) The virus is used to transduce target cells, releasing the gene of interest. (4) In this example, RNA from a lentiviral vector is reversed-transcribed to DNA that integrates into the host genome for recombinant protein expression.

Selecting the right transfection method

An ideal transfection method should consistently provide high transfection efficiency and low cell toxicity, and should satisfy other criteria that are important to your goals (Table 1).

Table 1. Important factors for selecting the right transfection method.

Selection criteria	Cationic lipid–based Chemical method	Electroporation Physical method	Viral delivery Biological method
Efficiency: easy-to-transfect cells	+++	+++	+++
Efficiency: hard-to-transfect cells	++	+++	+++
Cell viability	+++	++	+++
Delivery of large payload (>7 kb)	++	+++	++
High-throughput suitability	+++	++	+++
Ease of use	+++	+++	+
Biosafety	+++	+++	+
Cost per reaction	+++	++	+

+++ excellent for most applications; ++ good for some applications; + our least recommended, but may be appropriate for some applications.

A checklist for successful transfection

Once you have chosen a transfection method, refer to this easy checklist and your chosen product's protocol to help ensure success.

- ❑ Grow cells in medium with supplements and maintain cells in log-phase growth
- ❑ Use contaminant-free cells with a low number of passages
- ❑ Culture cells with >90% viability without antibiotics prior to transfection
- ❑ Prepare endotoxin-free, high-quality plasmid DNA or other payload
- ❑ Transfect cells at 50–80% confluency (cell line–dependent; refer to product-specific protocol)
- ❑ Design proper positive and negative controls for use during transfection
- ❑ Optimize transfection conditions for your experiment, such as cell density, payload amount, payload-to-reagent ratio, or electroporation parameters
- ❑ Determine the best assay type and analysis time points after transfection

Scientists worldwide use Invitrogen transfection reagents

Invitrogen transfection reagents have been part of …

1980

Lipofectin™ transfection reagent

1st

Lipofectamine™ transfection reagent

Lipofectamine™ 2000 transfection reagent

2000

Lipofectamine™ RNAiMAX transfection reagent

Lipofectamine™ 3000, Invivofectamine™ 3.0, Lipofectamine™ MessengerMAX, Lipofectamine™ CRISPRMAX transfection reagents

2020

Lipofectamine™ Stem transfection reagent

… scientific breakthroughs for >30 years

1987: First commercially available cationic lipid transfection reagent

Philip L. Felgner and colleagues invented the first cationic lipid in the world that delivers both DNA and RNA into mammalian cells with high efficiency. This is commercialized under the name Lipofectin reagent [1].

2001: Discovery of RNA interference

Andrew Fire and colleagues showed that synthetic siRNAs can induce gene-specific inhibition of expression in cell lines from humans and mice [2]. These observations opened a path toward the use of siRNAs as a reverse genetic and therapeutic tool in mammalian cells.

2013: CRISPR-Cas9 editing of the human genome

Prashant Mali and George Church showed that RNA-guided editing could be used to engineer the genome of human cells [3].

2020: Enabling SARS-CoV-2 research

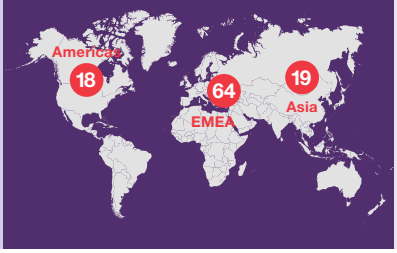
Alexandra C. Walls and colleagues showed structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein, which provided a blueprint for the design of vaccines and therapeutics [4].

Lipofectamine reagents are the most cited transfection reagents in global scientific journals

300,000

citations and counting …

Lipofectamine reagents are used by scientists from 101 countries



The most trusted transfection reagent family globally

Used by all of the top 20 universities globally

Used by all of the top 20 pharma companies globally

Enabling scientific discoveries

Invitrogen™ Lipofectamine™ transfection reagents are the most trusted and cited reagents in the scientific literature due to their superior transfection performance. With reagents for different payloads and cell types, there is a reagent suitable for most experiments.

1. Felgner PL et al. (1987) Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA* 84:7413–7417.

2. Fire A et al. (2001) Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. *Proc Natl Acad Sci USA* 98:9442–9747.

3. Mali P et al. (2013) RNA-guided human genome engineering via Cas9. *Science* 339:823–826.

4. Walls AC et al. (2020) Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181:281–292.

Lipid-mediated delivery of DNA

Lipofectamine 3000 Transfection Reagent with P3000 Reagent

Invitrogen™ Lipofectamine™ 3000 Transfection Reagent with P3000™ Reagent is our top recommendation for DNA delivery, offering the power to transfect your most difficult cells. The customizable, highly efficient, two-tube formulation (Figure 4) provides you with ultimate flexibility to tailor your transfection to the needs of specific cell types, including primary cells, stem cells, and cancer cell lines.

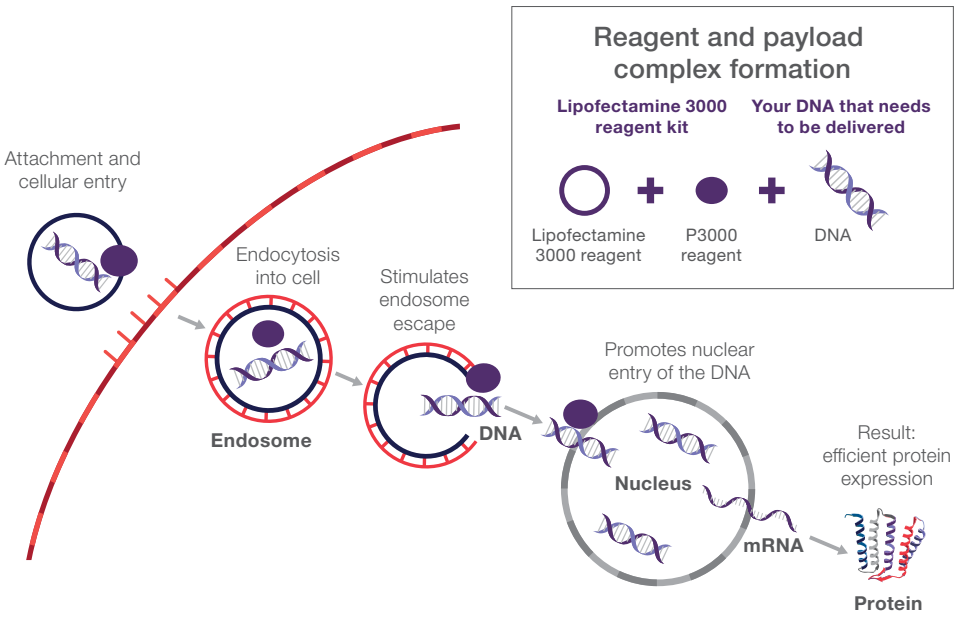


Figure 4. Complex formation and mechanism of transfection using Lipofectamine 3000 reagent with P3000 reagent.

When a lab needs one reagent to perform transfection across a wide variety of cell lines that are easy, moderately hard, or hard to transfect, Lipofectamine 3000 reagent with P3000 reagent for DNA delivery is the best choice (Figure 5).

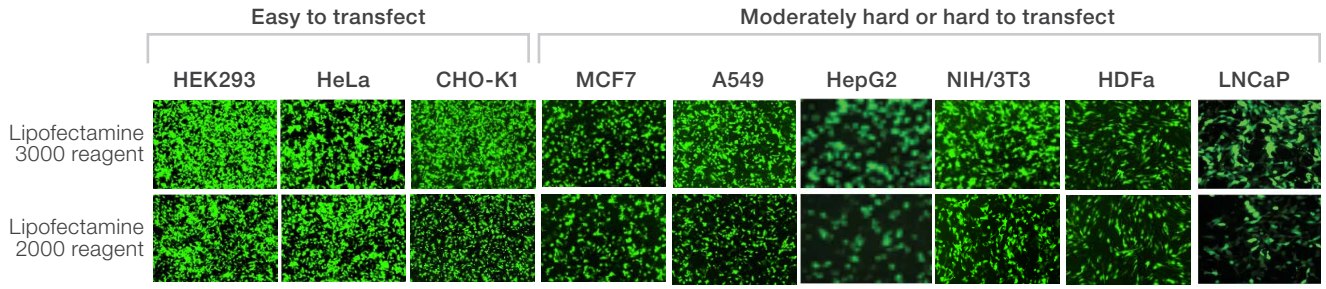


Figure 5. Lipofectamine 3000 or Lipofectamine 2000 reagent was used to transfect HEK293, HeLa, CHO-K1, MCF7, A549, HepG2, NIH/3T3, HDFa, and LNCaP cells in a 96-well format. GFP expression was analyzed 48 hours after transfection. Lipofectamine 3000 reagent provided higher transfection efficiency in all nine cell lines, especially cell lines that are more difficult to transfect.

Lipofectamine 2000 Transfection Reagent

Invitrogen™ Lipofectamine™ 2000 Transfection Reagent is one of the most referenced transfection reagents in scientific literature (Figure 6), and it is well suited for common and easy-to-transfect cell types. It is widely used in transfection experiments that require dependability, allowing researchers to focus on more important variables. This broad-spectrum versatility is derived from a simple protocol and well-known ability to work across several applications.

- Transfection conditions can be easily established for high-throughput applications involving automated or robotic systems
- It is the best choice for establishing stable cell lines and transfection of neuronal cells
- Effective in co-delivering both plasmid DNA and siRNA

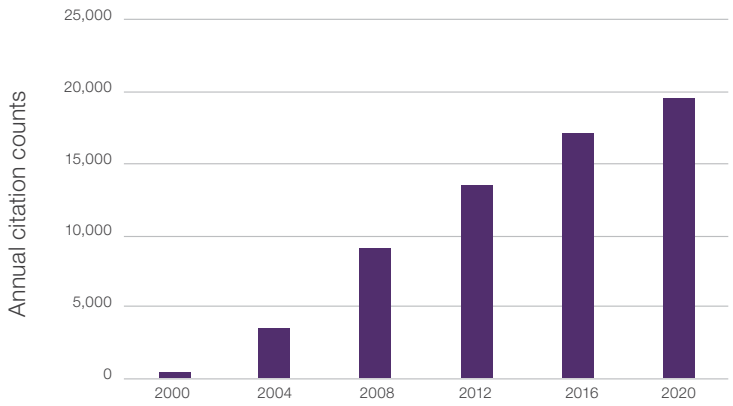


Figure 6. Number of citations for Lipofectamine 2000 reagent since the initial launch.
Source: Google Scholar™ search engine.

Find out more at **thermofisher.com/2000**

Lipofectamine LTX Reagent with PLUS Reagent

Invitrogen™ Lipofectamine™ LTX Reagent with PLUS™ Reagent combines a rapid, simple protocol with high efficiency and cell viability. Providing efficient, gentle DNA plasmid delivery, a simple streamlined protocol, and easy two-tube optimization, Lipofectamine LTX Reagent strikes the right balance between potency and gentleness.

It shines with exceptional transfection efficiency in CHO, primary fibroblast, and epithelial cells (MEF and HMEC cells).

Find out more at **thermofisher.com/ltx**

Lipid-mediated delivery of siRNA

Lipofectamine RNAiMAX Transfection Reagent

Invitrogen™ Lipofectamine™ RNAiMAX Transfection Reagent offers the highest transfection efficiencies on the widest variety of cell types for siRNA-mediated gene knockdown experiments, including high-throughput applications. A proprietary RNAi-specific cationic lipid formulation is designed for robust delivery of siRNA and miRNA into all cell types, especially when combined with Invitrogen™ *Silencer*™ Select siRNA. The simple, rapid protocol accommodates lower siRNA concentrations, thus enabling more effective gene knockdown with minimal nonspecific effects.

Find out more at **thermofisher.com/rnai**

Invivofectamine 3.0 Reagent for *in vivo* delivery

Invitrogen™ Invivofectamine™ 3.0 Reagent is a breakthrough reagent for *in vivo* siRNA delivery (Figure 7). Greatly improved performance and up to 85% knockdown can be achieved using microgram levels of siRNA. Creating complexes of Invivofectamine 3.0 Reagent and siRNA duplexes for delivery is easy: simply mix, incubate, and dilute.

- **Low toxicity**—Typical results of blood chemistry analysis at multiple time points show that the levels of various biomarkers in mice transfected using Invivofectamine 3.0 Reagent are not significantly different from those of untreated mice.
- **Highly effective knockdown**—Measuring Factor VII protein levels in serum using a chromogenic assay 24 hours after tail vein injection have repeatedly shown that the amount of protein knockdown is correlated with the amount of siRNA.

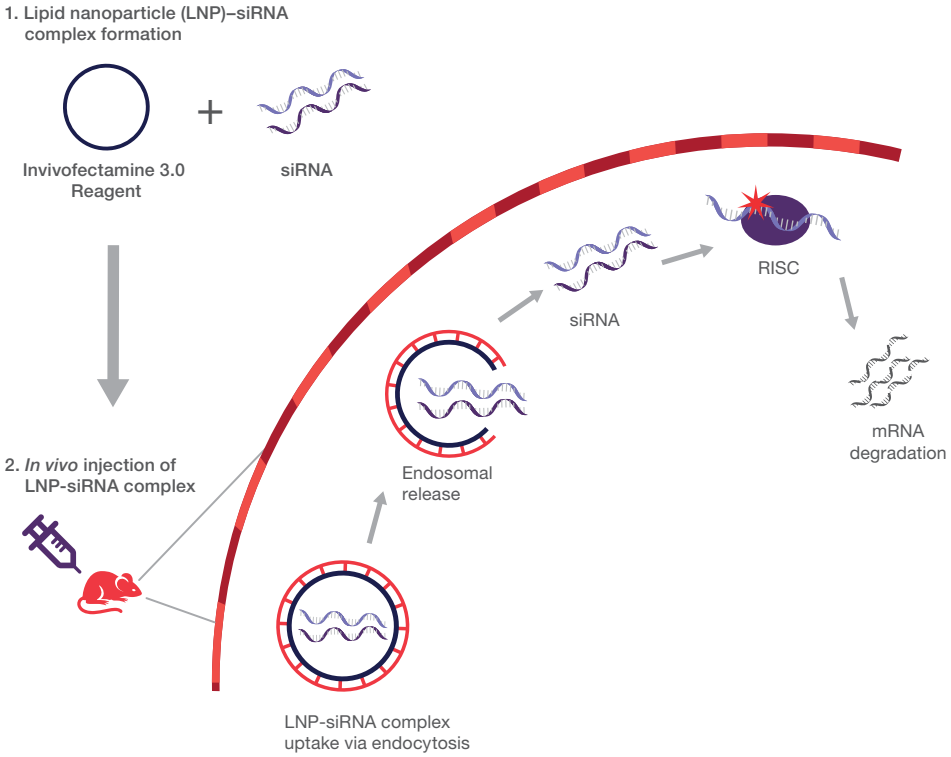


Figure 7. Overview of *in vivo* delivery of siRNA using Invivofectamine 3.0 Reagent.

Find out more at **thermofisher.com/invivofectamine**

Lipid-mediated delivery of mRNA

Lipofectamine MessengerMAX Transfection Reagent

Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent delivers amazing transfection efficiency for a broad spectrum of cell types, especially primary cells and neurons, enabling improved application outcomes and more biologically relevant research.

Transfection of mRNA with Lipofectamine MessengerMAX reagent results in faster protein expression because translation of mRNA occurs in the cytoplasm. Additionally, delivery of mRNA does not require nuclear entry (Figure 8), which eliminates the risk of genomic integration and enables high transfection efficiency in slowly dividing cells.

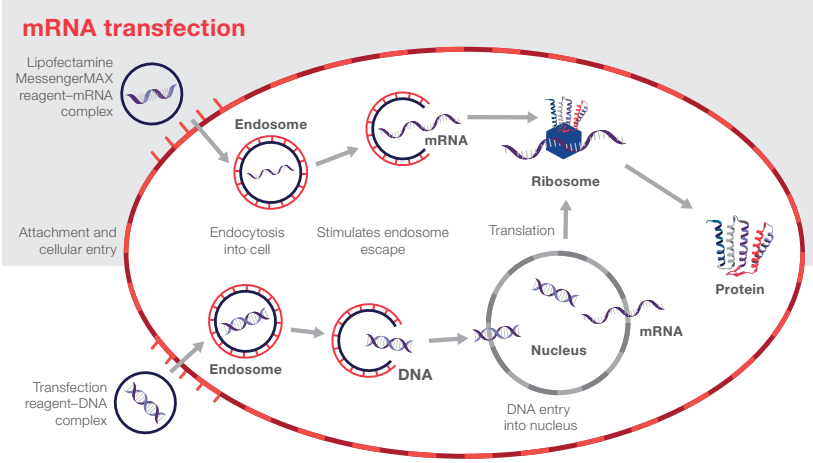


Figure 8. Comparison of mRNA transfection and DNA transfection. mRNA transfection bypasses nuclear entry, helping to improve protein expression in nondividing cells.

Lipofectamine MessengerMAX reagent is designed to transfect neurons and a broad spectrum of difficult-to-transfect primary cells with a larger amount of mRNA. This results in a more than two-fold improvement in transfection efficiency compared to other lipid-based reagents (Figure 9).

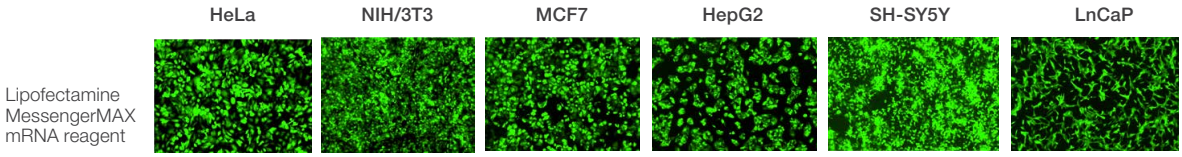


Figure 9. Lipofectamine MessengerMAX reagent was used to deliver GFP mRNA (100 ng/well) in a 96-well format. GFP expression was analyzed 48 hours after transfection. Lipofectamine MessengerMAX reagent provided high transfection efficiency in all six cell lines.

Find out more at **thermofisher.com/messengermax**

Lipid-mediated delivery of ribonucleoprotein for CRISPR-Cas9 genome editing

Lipofectamine CRISPRMAX Cas9 Transfection Reagent

Invitrogen™ Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent is the first optimized lipid nanoparticle transfection reagent for delivery of ribonucleoprotein (RNP) complexes for CRISPR-Cas9 genome editing. It is a high throughput–compatible, cost-effective alternative to electroporation.

Working together with Invitrogen™ TrueCut™ Cas9 Protein v2 and Invitrogen™ TrueGuide™ Synthetic gRNA, it provides:

- **Demonstrated cleavage efficiency**—tested in over 20 cell types, including iPSCs, mESCs, N2A, CHO, A549, HCT116, HeLa, HEK293, and several others
- **Low cell toxicity**—fewer cells needed to initiate your experiment
- **High-throughput compatibility**—an ideal delivery solution for 96-well formats
- **Cost savings**—whether cost per reaction or initial investment

Achieve high transfection efficiency in novel genome editing applications

Lipofectamine CRISPRMAX transfection reagent increases the likelihood of successful cleavage and recombination, especially when combined with TrueCut Cas9 Protein v2 and TrueGuide Synthetic gRNA (Figure 10). This ultimately maximizes the efficiency of performing genetic modifications.

Improve experimental outcomes using TrueCut Cas9 Protein v2

Unlike CRISPR plasmids or Cas9 mRNA, using Cas9 protein provides superior cleavage efficiency in primary cells and stem cells. It eliminates the need for transcription or translation of the payload, removes the risk of genomic integration, and is cell cycle–independent.

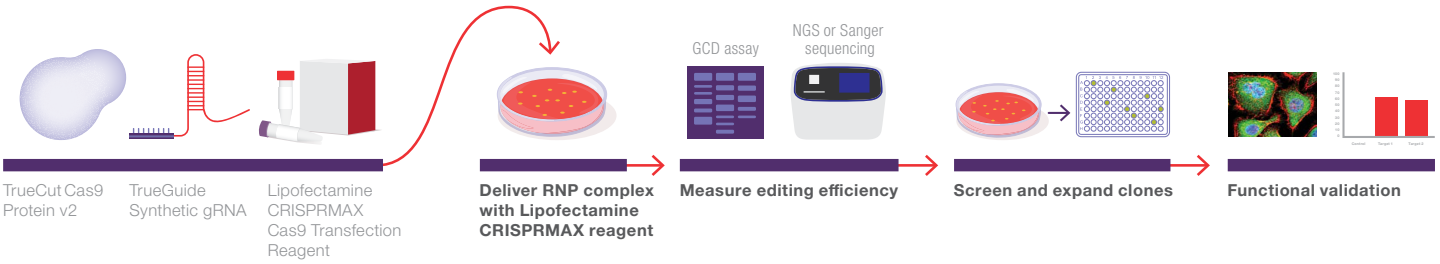


Figure 10. General workflow for assembly and delivery of RNP complexes for genome editing applications.

Find out more at **thermofisher.com/crisprmax**

Lipid-mediated delivery for stem cells

Lipofectamine Stem Transfection Reagent

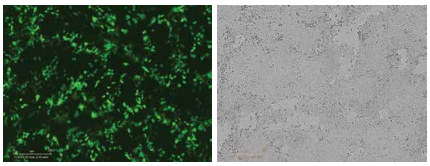
In research applications such as gene editing, gene expression, and directed differentiation, efficient delivery of DNA, RNA, or Cas9 protein complexes into stem cells has been difficult. Experiments involving large constructs have typically been carried out using electroporation, as reagent-based transfection yielded poor results.

Invitrogen™ Lipofectamine™ Stem Transfection Reagent has shown versatility for delivery of DNA, RNA, and Cas9 protein complexes into stem cells (Figure 11). It can be three times more efficient than existing transfection reagents in iPSCs, NSCs, and MSCs. It is flexible enough to transfect either adherent or suspension cells, and it is gentle enough to support continued cell proliferation without inducing differentiation.

A

iPSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	Essential 8 Medium
Extracellular matrix	Vitronectin
Cell density	50,000 cells/well

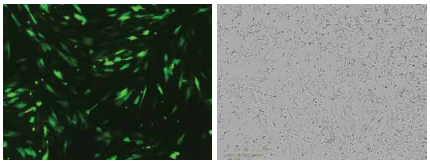


iPSCs, GFP plasmid
Transfection efficiency: 75%

B

iPSC-derived NSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	StemPro NSC SFM
Extracellular matrix	Geltrex matrix
Cell density	75,000 cells/well

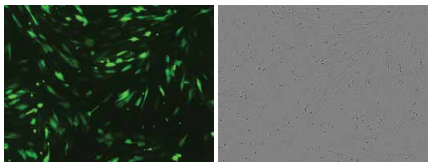


NSCs, GFP plasmid
Transfection efficiency: 60%

C

MSCs

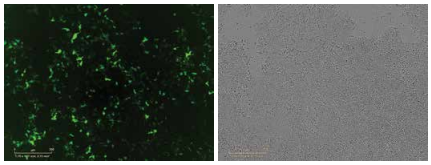
Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	MesenPRO RS Medium
Extracellular matrix	CTS CELLstart Substrate
Cell density	25,000 cells/well



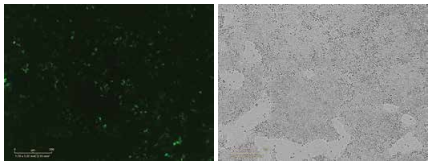
Adipose-derived MSCs,
GFP plasmid
Transfection efficiency: 47%

D

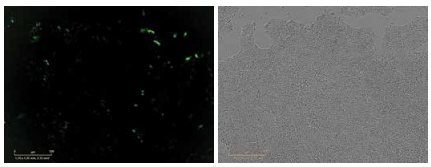
ESCs: transfection with large DNA constructs



H9 ESCs in StemFlex Medium
Lipofectamine Stem reagent
11 kb EF1α-GFP DNA plasmid
Transfection efficiency: 76%



H9 ESCs in mTeSR™1 Medium
Lipofectamine Stem reagent
11 kb EF1α-GFP DNA plasmid
Transfection efficiency: 44%



H9 ESCs in mTeSR1 Medium
Other supplier's reagent
11 kb EF1α-GFP DNA plasmid
Transfection efficiency: 5%

Figure 11. Transfection of human stem cells with small and large DNA plasmids using Lipofectamine Stem Transfection Reagent. High-efficiency transfection with small DNA plasmids is shown in **(A)** iPSCs, **(B)** NSCs, and **(C)** MSCs. **(D)** H9 ESCs were transfected with an 11 kb DNA plasmid using Lipofectamine Stem reagent (top, middle) with significantly higher efficiency than another leading supplier's reagent (bottom).

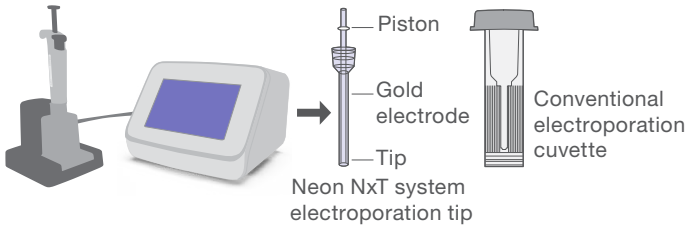
Find out more at **thermofisher.com/lipofectaminestem**

Neon NxT Electroporation System

Enabling better electroporation to achieve your ambitious scientific goals

The Invitrogen™ Neon™ NxT system's tip technology enables exceptional electroporation efficiency and cell viability by maximizing the distance between the two electrodes while minimizing their surface area. As a result, the sample experiences:

- A more uniform electric field
- Less ion formation
- Minimal pH change
- Negligible heat generation



Efficiently transfect hard-to-transfect cell types

Achieve **>90%** genome editing efficiency with primary human T cells

Saving sample | Saving time

- Unique design maximizes post-transfection cell viability
- Electroporation within the tip minimizes sample transfer loss
- Biosafety cabinet–compatible size minimizes contamination risk

- Shorter end-to-end processing time as compared to conventional electroporation
- Minimal hands-on training
- Protocol and customer support for timely success as you plan and execute your experiments

Flexibility

Customizable electroporation parameters

Deliver DNA, RNA, ribonucleoprotein (RNP), antibodies, and more

Transfect from 2 x 10^4 to 6 x 10^6 cells per reaction

Invitrogen™ TransfectionLab™ application
A cloud application that enables remote experiment design to enhance consistency and productivity

Simplicity

1 buffer kit for all cell types

3 simple steps with the Invitrogen™ Neon™ NxT pipette: aspirate, electroporate, and dispense

No more cuvette handling: with tedious capping/de-capping, aspirating/dispensing, and transferring from biosafety cabinet to instrument

Cell-specific protocols

150+
and counting

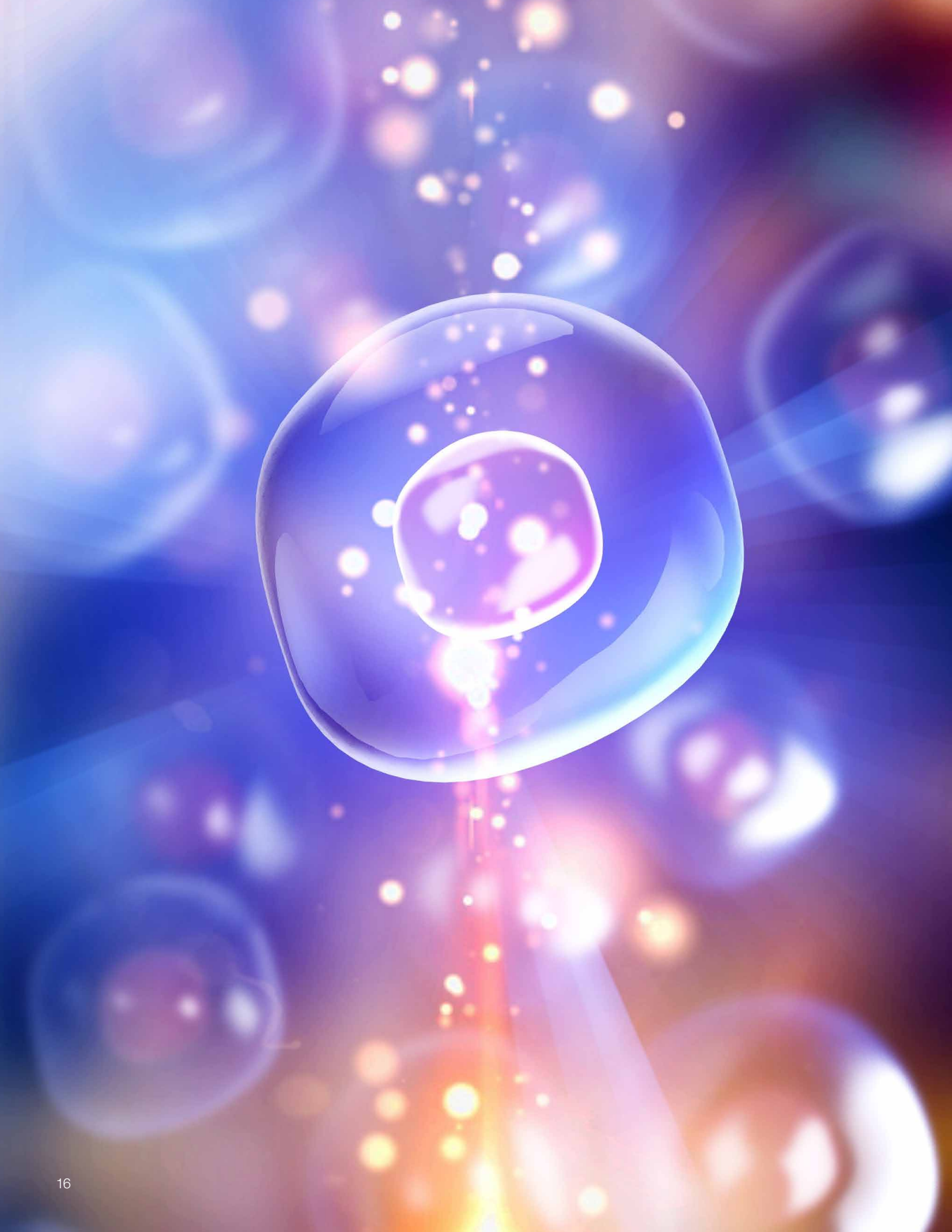
Peer-reviewed publications

11,200
and counting

Relied upon by scientists

Used in
thousands
of labs globally

Find out more at **thermofisher.com/neonnxt**



Product selection guide

Transfection products are available for DNA, siRNA, mRNA, and protein delivery, offering a range of options to best suit your transfection experiment. Use Table 2 to find the optimal solution for your needs.

Table 2. Transfection product selection guide.

Product	Neon NxT Electroporation System	Lipofectamine 3000 reagent	Lipofectamine 2000 reagent	Lipofectamine RNAiMAX reagent	Lipofectamine MessengerMAX reagent	Lipofectamine CRISPRMAX reagent	Lipofectamine Stem reagent	Invivofectamine 3.0 Reagent
Cell type	Workhorse	Workhorse	Workhorse	Workhorse	Workhorse	Workhorse		
	Hard-to-transfect	Hard-to-transfect		Hard-to-transfect	Hard-to-transfect	Hard-to-transfect		
	Primary	Primary		Primary	Primary	Primary		
	Stem	Stem		Stem	Stem		Stem	
			Neurons	Neurons	Neurons			
Payload	DNA	DNA	DNA				DNA	
	siRNA or miRNA			siRNA or miRNA			siRNA or miRNA	siRNA or miRNA
	mRNA				mRNA		mRNA	
	CRISPR-Cas9 RNP					CRISPR-Cas9 RNP	CRISPR-Cas9 RNP	
	Antibody							
		Cotransfection	Cotransfection					
Delivery	*In vitro*							*In vivo*

The TransfectionSelect product selection tool identifies your best solution for high transfection performance

Based on a few simple questions within your application and cell and payload types, the Invitrogen™ TransfectionSelect™ product selection tool recommends products, protocols, and citations that make it easy for you to choose quickly and wisely. Don't wonder if you'll get the efficiency you need. Use the TransfectionSelect product selection tool for increased certainty.

Find out more at **thermofisher.com/transfectionselect**

Related products

Gibco™ Opti-MEM™ I Reduced Serum Medium is a modified Eagle's minimum essential medium (MEM) that is recommended for dilution of nucleic acids and transfection reagents prior to complex formation. Transfection complexes formed in Opti-MEM medium are usually added directly to cells growing in culture medium without the need to remove complexes or change the medium after transfection.

Thermo Scientific™ Nunc™ treated cell culture plastics with Nunclon™ Delta surfaces undergo rigorous testing with Gibco™ media to help ensure consistent cell growth across multiple cell lines—it's a proven combination for happy cells and even happier scientists.

Find out more about how Nunc cell culture plastics and Gibco media are a proven combination at **thermofisher.com/bettertogether**

Ordering information

Product	Quantity	Cat. No.
Lipofectamine 3000 Transfection Reagent	1.5 mL	L3000015
Lipofectamine 2000 Transfection Reagent	1.5 mL	11668019
Lipofectamine LTX Reagent with PLUS Reagent	1 mL	15338100
Lipofectamine RNAiMAX Transfection Reagent	1.5 mL	13778150
Invivofectamine 3.0 Transfection Reagent Starter Pack	0.2 mL	IVF3001
Lipofectamine MessengerMAX Transfection Reagent	1.5 mL	LMRNA015
Lipofectamine CRISPRMAX Cas9 Transfection Reagent	1.5 mL	CMAX00015
Lipofectamine Stem Transfection Reagent	0.75 mL	STEM00008
Neon NxT Electroporation System Starter Pack	Combination pack	NEON1SK
Neon NxT Electroporation System 10 µL Kit	96 x 2 reactions	N1096
Neon NxT Electroporation System 100 µL Kit	96 x 2 reactions	N10096
TrueCut Cas9 Protein v2	500 µg	A36499
TrueGuide Modified Synthetic sgRNA	**thermofisher.com/crisprgrna**	
Opti-MEM I Reduced Serum Medium	100 mL	31985062
	500 mL	31985070
Nunc 6-Well Cell-Culture Treated Multidishes, Nunclon Delta Surface	Case of 75	140675
Nunc 24-Well Cell-Culture Treated Multidishes, Nunclon Delta Surface	Case of 75	142475
Nunc MicroWell 96-Well Microplate, Nunclon Delta Surface	Case of 50	167008
Nunc Edge 2.0 96-Well Microplate, Nunclon Delta Surface	Case of 50	167425

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