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Lipofectamine™ 2000 Transfection Reagent

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PRODUCT DESCRIPTION

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Lipofectamine™ 2000 Transfection Reagent is a proprietary formulation for the transfection of nucleic acids (DNA and RNA) into eukaryotic cells and provides the following advantages:

- Highest transfection efficiency in many cell types and formats (*e.g.* 96-well). Refer to the Cell Lines Database for a list of cell types successfully transfected. Detailed in-house transfection protocols are also available at this site where available.
- DNA-Lipofectamine™ 2000 complexes can be added directly to cells in culture medium, in the presence or absence of serum.

Lipofectamine™ 2000 may be used in the following applications:

- Transient and stable transfection of adherent and suspension cells
- High throughput transfections
- Delivery of Stealth RNAi and siRNA into cells For information on transfecting mammalian cells with short interfering RNAs (siRNA) for use in RNA interference (RNAi) studies, visit our RNAi Central web page at www.invitrogen.com\rnai. Cell line-specific protocols are available under the Protocols tab.

Lipofectamine™ 2000 gives superior transfection efficiency for the following cell lines:

293F	293H	BE(2)C (w/o serum)
CHO-K1	CHO-S (adherent)	COS-1 (w/o serum)
COS7-L	Primary Human Fibroblasts (w/o serum)	
HT-29 (w/o serum)	HT-1080	MDCK
MRC-5 (w/o serum)	PC12	SK-BR3
Vero	HepG2	NIH 3T3

Lipofectamine™ 2000 CD is a 100% syntheticversion of Lipofectamine™ 2000. Use it in the same way as Lipofectamine™ 2000, but be sure to use animal origin-free reagents.

SHIPPING CONDITIONS

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This kit is shipped on wet ice.

STORAGE CONDITIONS

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Lipofectamine™ 2000 Transfection Reagent should be stored at 4^0 C.

STABILITY

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- The stability of Lipofectamine™ 2000 Transfection Reagent is guaranteed for 6 months when it has been stored as recommended.
- Lipofectamine™ 2000 Reagent should not be frozen. The performance may be affected.

QC SPECIFICATIONS

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Lipofectamine™ 2000 is tested for the absence of microbial contamination using blood agar plates, Sabaraud dextrose agar plates, and fluid thioglycolate medium, and functionally by transfection of CHO-K1 cells with a reporter plasmid.

PROTOCOL AND APPLICATION NOTES

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General protocol notes

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- It is not necessary to remove complexes or change/add medium after transfection, but complexes may be removed after 4-6 hours.
- DMEM or RPMI 1940 can be used instead of Opti-MEM when making Lipofectamine™ 2000 – DNA complexes. However, the efficiency of complex formation may not be as high as with Opti-MEM. Cells in PBS can be transfected using Lipofectamine™ 2000.
- As long as the cells are healthy in the PBS, the transfection is likely to work.
- For a general plasmid DNA transfection protocol, please refer to the product insert: http://www.invitrogen.com/content/sfs/manuals/Lipofectamine™2000_man.pdf
- A general protocol for transfecting Stealth RNAi or siRNA into mammalian cells can be found at the following site: http://www.invitrogen.com/content/sfs/manuals/stealth_sirna_tsf_lf2k_man.pdf

Surface Areas of Tissue Culture Vessels

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Culture Vessel	96-well	48-well	24-well	12-well	6-well	35-mm	60-mm	100-mm	150-mm	T25	T75
Surface Area (cm^2)	0.3	0.7	2	4	10	10	20	60	140	25	75
Ratio to 24-well plate	0.2	0.4	1	2	5	5	10	30	70	12.5	37.5

Tubes recommended for use with Lipofectamine™ 2000

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It is best to use polypropylene tubes when pre-mixing Lipofectamine 2000 ™ and DNA. Polystyrene may not work as well.

Optimizing plasmid DNA transfections with Lipofectamine™ 2000

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The conditions that could be optimized include Lipofectamine™ 2000 amount, DNA concentration, and cell number. Keeping two variables constant, vary the third.

- For example: to optimize the amount of Lipofectamine™ 2000 for transfection in a 24-well plate, start with cells at >90% confluency and use a fixed amount of DNA (0.8-1.2 µg). With cell number and DNA concentration held constant, vary the amount of Lipofectamine™ 2000 to determine the optimal concentration (usually 1.5-3 µl). In the same way, the cell number and amount of DNA can also be optimized.
- It is recommended to use a range of 0.5 to 5 µl of Lipofectamine™ 2000 per µg of DNA. It is possible to minimize the effect of transfection on cell growth and viability by increasing the number of cells plated per well or by decreasing either Lipofectamine™ 2000 amount or DNA concentration. With careful optimization, this can be achieved with little impact on the level of transgene expression.
- Transfection efficiency is typically measured as the percentage of cells translating and accumulating the protein of interest for detection in the total population. If the levels of translation or protein accumulation are low, a lower transfection efficiency may be obtained. A transfection control such as our BLOCK-iT Fluorescent Oligo (catalog #

2013) is a more accurate measure of the efficiency of DNA delivery since its detection is independent of expression in the cell.

- The following citation discusses the effect of variables such as cell density, liposome and DNA concentrations, liposome-DNA complexing time, and media components (serum and antibiotics) on transfection with Lipofectamine™ 2000. In addition, it also looks at high throughput transfections, siRNA transfections, and transfection of primary neurons.
- Advanced transfection with Lipofectamine™ 2000 reagent: primary neurons, siRNA, and high-throughput applications - Methods, Volume 33, Issue 2, June 2004, Pages 95-103 Brian Dalby, Sharon Cates, Adam Harris, Elise C. Ohki, Mary L. Tilkins, Paul J. Price and Valentina C. Ciccarone
- Though most cells transfect well in the presence or absence of serum, there are a few such as HeLa cells and Normal Human Fibroblasts that give better transfection efficiency in the absence of serum.

We suggest Oligofectamine for **Hela and Huvec** cell lines (Po-Tsan Ku --in-house verified data 3/06).

Cell lines successfully transfected with Lipofectamine™ 2000:

293F	293H, 293	BE(2)C (w/o serum)
CHO-K1	CHO-S (adherent)	CHO-S (suspension in CD CHO media)
COS-1 (w/o serum)	COS7-L(w/o serum)	Primary Human Fibroblasts
HT-29 (w/o serum)	HT-1080	MDCK
MRC-5 (w/o serum)	PC12	SK-BR3
Vero	CHO	CHO-DG44
MCF7	MDA-MB-361	HCT 116
H1299	RKO	Hep3B, HepG2
HeLa	Rzneo	HOS
C3H/10T1/2	NIH3T3	Jurkat
K562	HUVECS	LoVo
A549		

Some cell lines for which transfection protocols are available (at the cell lines database)
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Cell type	**Transfection Efficiency (%)**	**Cells per well (24-well plate)**	**Lipofectamine™ 2000 μl per well in 24-well plate**
293H	99	2×10^5	2
293F	99	2×10^5	2
BE(2)C	77	2×10^5	2.5
BHK21	-	1.0×10^5	3.0
CHO-K1	-	1.2×10^5	2.5
CHO-S(adherent)	96	1.5×10^5	2.5
Cos 1	-	8×10^4	3.0
COS7L	99	8×10^4	2.5
CV-1	70	8×10^4	1.5
HeLa	94	8×10^4	1.5
HT-29	-	1.5×10^5	3.0
HT1080	81	8×10^4	1.5
HUVEC	<2%	8.0×10^4	2.0
MDCK	43	6×10^4	4
MRC-5	Not measured	1.5×10^5	2.5
Murine Embryonic Stem Cells, D3 (6-well plates)	75	$1X10^6$ (6-well)	8-12(6-well)
NIH3T3	Not measured	1.5×10^5	2
PC12	85	$2.5 X 10^5$	2

Primary Human Fibroblasts	48	8 x 10^4	2
Primary Human Keratinocytes	-	8 x 104	2
RKO (field test) (6-well plates)	40 - 60	See protocol	See protocol
SKBR3	49	1.5 x 10^5	2
Vero	86	8 x 10^4	2
Rat Hepatocytes	50	1.25 x 10^5	1.5
Rat E18 Cortical Neurons	20-25	2 x 10^5	4

Co-transfection of siRNA and plasmid DNA
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- Plasmid and siRNA co-transfection are possible. Co-transfections have been tested with Lipofectamine™ 2000 in GripTite™ cells (293 derived cells) plated at 1.8 x 10^5 cells/well in a 24-well format (0.5ml medium, no antibiotics). 200ng of two different reporter plasmids were co-transfected with 10pmol of siRNA following the standard Lipofectamine™ 2000 protocol, with 2ul of Lipofectamine™ 2000 per well. The total volume of the transfection mixes was 100ul, and it was added to the medium already in the wells.

Transfection of fluorescently labeled oligos with Lipofectamine™ 2000
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Fluorescently labeled oligos that depend on hairpin structures for quenching (like LUX primers) may fluoresce upon mixing with Lipofectamine™ 2000. In such cases Oligofectamine is suggested. Oligofectamine is very different chemically, and therefore not expected to exhibit the same strength of interaction.

Transfection of cells in 96-well plates (also see Focus 21.3 page58)
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Use the 24-well plate protocol with the following modifications:

- Plate 2-6 x 10^4 cells per well in 100 µl of the appropriate complete growth medium without antibiotics and with serum if cells are normally cultured in the presence of serum.
- For each well of cells, dilute 240 to 320 ng of DNA into 25 µl medium without serum (e.g., OptiMEM® I Medium) in 96-well, sterile micro titer plates.
- For each well of cells, dilute 0.8-1 µl of Lipofectamine™ 2000 into 25 µl OptiMEM® Medium and incubate for 5 min at room temperature. Once the Lipofectamine™ 2000 is diluted, combine it with the DNA within 30 min. Longer incubation times may result in decreased activity. This dilution can be prepared in bulk for multiple wells.
- Add 25 µl of the diluted Lipofectamine™ 2000 (from step 3) to each well containing diluted DNA (from step 2), mix gently, and incubate at room temperature for 20 min to allow DNA- Lipofectamine™ 2000 complexes to form.
- Add the DNA- Lipofectamine™ 2000 complexes (50 µl) directly to each well of the plates containing cells and mix gently.

Optimal transfection conditions for transfections in 96-well plates:

Cell Line	Seeding Density (Cells per well)	DNA per Well	Lipofectamine™ 2000 (µl)
CHO-S	2 x 10^4	240 ng	1 µl
COS-7L	2.5 x 10^4	320 ng	1 µl
293H, 293F	5 x 10^4	320 ng	1 µl

Alternate rapid protocol for 96-well transfections without pre-plating cells (also see Focus 21.3, page58):

- This protocol is designed as a rapid alternative that does not require plating cells the day before transfection. Instead, a suspension of cells is added directly to complexes prepared in 96-well plates. This protocol has been used successfully with the cells and conditions outlined below. Use poly-lysine coated plates (D or L) for best results.
- Dilute ~320 ng of each DNA to be tested into 25 µl medium without serum (e.g., OptiMEM® I Medium). Prepare the dilutions directly in 96-well cell culture plates.
- For each well, dilute 0.4-0.8 µl of Lipofectamine™ 2000 into 25 µl OptiMEM® I Medium and incubate for 5 min at room temperature. Prepare this dilution in bulk for multiple wells. Once the Lipofectamine™ 2000 is diluted, combine it with the DNA within 30 min. Longer incubation times may result in decreased activity.
- Add 25 µl of the diluted Lipofectamine™ 2000 (from step 2) to each well containing diluted DNA (from step 1), mix gently, and incubate at room temperature for 20 min to allow DNA-Lipofectamine™ 2000 complexes to form.
- Prepare a cell suspension so that the appropriate number of cells per well is contained in 100 µl of growth medium. Use approximately twice the cell density, depending on cell type, than with the standard protocol.
- Add 100 µl of the cell suspension (from step 4) to each of the wells containing the DNA-Lipofectamine™ 2000 complexes (from step 3) and mix gently.
- Incubate at 37°C in a CO_2 incubator until ready to assay (24-48 h post transfection). It is not necessary to remove the complexes or change the medium. Cells will adhere as usual in the presence of the complexes.

Transfection conditions for rapid 96-well protocol:

Cell Line	**Cells per well**	**DNA per well (100 µl suspension)**	**Lipofectamine™ 2000 per well**
CHO-S	5×10^4	320 ng	0.8 µl
COS-7L	6×10^4	320 ng	0.6-0.8 µl
293H, 293F	1.2×10^5	320 ng	0.4 µl

Transient transfection of suspension cells

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The following protocol was optimized with Jurkat and K562 cells, but can be used as a guideline for other types of suspension lines.

- For each transfection, add a cell suspension containing 4-8 x 10^5 cells in 500 µl of growth medium with serum but without antibiotics, to a well of a 24 well plate. For transfection of larger number of cells, scale up all the reagents (cells, media, DNA, Lipofectamine™ 2000 and plate size) proportionately to the number of cells transfected.
- For each well, dilute 0.8 - 1.2 µg of DNA into 50 µl of medium without serum (e.g., Opti-MEM). This can be prepared in bulk for multiple wells.
- For each well, dilute ~2 µl of Lipofectamine™ 2000 into 50 µl OptiMEM® I Medium and incubate for 5 min at room temperature. Once the Lipofectamine™ 2000 is diluted, combine it with the DNA within 30 min. Longer incubation times may result in decreased activity. This dilution can be prepared in bulk for multiple wells.
- Combine the diluted DNA from step 2 with the diluted Lipofectamine™ 2000 from step 3. Incubate at room temperature for 20 min to allow DNA-Lipofectamine™2000 complexes to form.
- Add the DNA-Lipofectamine™ 2000 complexes from step 4 (100 µl) directly to each well containing cells (from step 1) and mix gently by rocking the plate back and forth.
- Incubate for 4 h at 37°C in a CO_2 incubator.
- In Jurkat cells, addition of PHA-L (Phytohemagglutinin L) and PMA (phorbol myristate acetate) at final concentrations of 1 µg/ml and 50 ng/ml, respectively, enhances CMV promoter activity and gene expression. In K562 cells, PMA alone is sufficient to enhance promoter activity. PMA and PHA are added after the 4-h incubation.
- Assay the cells at 24-48 h post-transfection for the appropriate activity. It is not necessary to remove the complexes or change the medium.

Note: Jurkat cells are difficult to transfect and have low expression following transfection.
The use of PHA-L and PMA did not affect the expression level of a beta-gal reporter (by ONPG assay) in either Jurkat cells or K562 cells in our hands. They are widely used in transfecting these cell types, and their use is most likely historical. Their effectiveness in transfections with currently available lipid reagents has probably not been tested before.

Protocol for protein transfection using Lipofectamine™ 2000

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We do not promote LF2K for protein transfections; however, one time R&D tested this application.
The following protocol can be given to customers upon request.

We have delivered -galactosidase protein to rat primary hippocampal neurons using either Lipofectamine™ 2000 or Lipofectin. Similar protocols were used for each lipid. In most cell lines Lipofectamine™ 2000 is more efficient, but in the case of neurons Lipofectin worked better than Lipofectamine™ 2000.

Protocol:
Prepare the following dilutions in separate tubes:
50 l opti-MEM + 2 l transfection reagent
25 l opti-MEM + 25 l -gal protein (25 ug)
Incubate at room temperature for 5 min.
Combine transfection reagent and protein dilutions
Add mixture (~100 l) to cells growing in a 24-well plate
Leave overnight, fix and stain cells with X-gal

PRODUCT DOCUMENTATION

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Brochures	**Cell Lines**	**Citations**
COA	**FAQ**	**Licensing**
Manuals	**MSDS**	

REFERENCES

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- Krista Evans, et al..PGreen Lantern-1, A Superior Green Fluorescent Protein Mammalian Cell Transfection Reporter, Focus 18(2): 40.
- Valentina Ciccarone, et al. Lipofectamine™ 2000 Reagent for Rapid, Efficient Transfection of Eukaryotic Cells - Focus 21(2):54.
- Jean-Pierre Pichet and Valentina Ciccarone. Transfection of Mammalian Cells in 96-Well Plates with Lipofectamine™ 2000 Reagent. Focus 21(3):58.
- Cationic Lipid Reagent Selection. Focus 21(3):61.
- Linda Roy, et al. High Transfection Efficiency of Cloned Cell Lines. Focus 21(3):62.
- Achieve the highest transfection efficiencies and higher expression levels (with Lipofectamine™ 2000). Expressions 8(3):18.

PRODUCT NAME AND CATALOG NUMBERS

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Name	Size	Part Number	Catalog Nnumber
Lipofectamine™ 2000 Reagent	0.75 ml	52758	11668-027 (11668027)
Lipofectamine™ 2000 Reagent	1.5 ml	52887	11668-019 (11668019)
Lipofectamine™ 2000 CD Reagent	1.0 ml	52888	12566-014 (12566014)

ASSOCIATED PRODUCTS

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OPTI-MEM I Reduced Serum Medium (catalog # 31985-062)

Antibiotics
GIBCO® Cell Culture Products
Technical Support: Nikita Warner

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