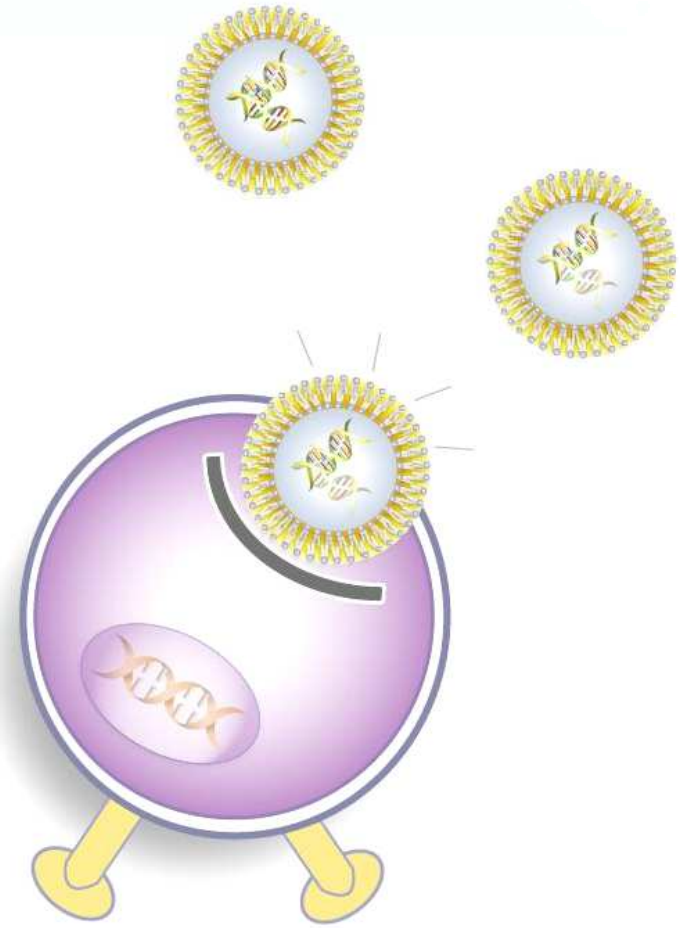
EcoTransfect™

INSTRUCTION MANUAL



OZBIOSCIENCES
The art of delivery systems
www.ozbiosciences.com

EcoTransfect ™

Instruction Manual

EcoTransfect™

The perfect solution for routine experiments in most common cell lines

The best quality / price ratio transfection reagent: Economical & Efficient

Cost of One Transfection (with 1µg of DNA) under 0.35 € (or USD)

List of EcoTransfect™ Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
ET-10500	EcoTransfect ™	500	250
ET-11000	EcoTransfect ™	1000	500
ET-13000	EcoTransfect ™	3 X 1000	1500

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (telephone, fax, mail, e-mail) or directly through our website. For all other supplementary information, do not hesitate to contact our dedicated technical support: tech@ozbiosciences.com.

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1. Technology

1.1. Description

Congratulations on your purchase of the ***EcoTransfect™*** transfection reagent!
EcoTransfect™ is an economic and powerful reagent dedicated to the transfection of the most commonly used cell lines. This lipid -based transfection reagent is issued from OZ Biosciences innovative Tee-Technology. ***EcoTransfect™*** was specifically developed to achieve good transfection efficiency in most popular cell lines for everyday experiments. Indeed, many transfection experiments are simply made to check biological activity of DNA constructs, insert (new clones), transcriptionally activated PCR fragments, mRNA or antisense oligonucleotides as well as producing stable transfection, to named a few. In this context, we have designed the best quality/price ratio transfection reagent since ***EcoTransfect™*** is the most economical transfection reagent on the market with a transfection cost under 0.35 € (or $) per transfection (with 1µg of DNA).

EcoTransfect™ is the perfect solution to quickly analyze the biological activity of your nucleic acids, to perform routine transfection assays and to accomplish high through put screening. ***EcoTransfect™*** main features are:

- Efficient and Reliable
- Simple, Ready-to-use and Rapid
- Non-toxic
- Compatible with serum-containing culture media
- Economical

The Tee-Technology

The cationic lipids (lipoplexes) and polymers (polyplexes) are the most employed non-viral gene delivery systems. The Tee-Technology (Triggered Endosomal Escape) combines and exploits the properties of both entities to achieve very efficient DNA delivery into cells. EcoTransfect™ formulation contains a chimera lipopolyamines and neutral lipids. The nucleic acids/EcoTransfect complexes formed (called lipoplexes) bind to cell surface and are taken up by endocytosis. Inside the endosomes, the lipids hydrophobic property acts in synergy with the endosomes buffering capacity of the polyamine residues. It allows an efficient release of large DNA amounts into cells.

Principal Tee-Technology advantages are:

- Compaction of DNA in nanoparticles efficiently internalized by cells
- Protection of nucleic acids against nucleases degradation
- Efficient membrane destabilization and DNA delivery
- High efficiency

1.2. Kit Contents

OZ Biosciences offers three sizes of EcoTransfect™ reagents. Kit contents vary according to their size.

- One tube containing 500 µL of EcoTransfect™ good for 250 transfections with 1 µg of DNA
- One tube containing 1 mL of EcoTransfect™ good for 500 transfections with 1 µg of DNA
- 3 tubes containing 1 mL of EcoTransfect™ good for 1500 transfections with 1 µg of DNA

Stability and Storage

Storage: +4°C. Upon receipt and for long-term use, store all reagent tubes in the fridge. EcoTransfect™ kits are stable for at least one year at the recommended storage temperature.

- **DO NOT FREEZE THE EcoTransfect™ FORMULATION!**
- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF EcoTransfect™ REAGENT!**

Shipping condition: Room Temperature.

2. Applications

2.1. Application Areas

EcoTransfect™ has been developed for very efficient transfections of commonly used cells lines. The EcoTransfect™ formulation is compatible with serum-containing culture media and serum free culture media. ***EcoTransfect***™ is suitable for plasmid DNA, linear DNA, transcriptionally activated PCR fragments, mRNA, antisense oligonucleotides and for producing stably transfected cells. This product is very stable, ready-to-use and intended for research purpose only.

2.2. Cell Types

EcoTransfect™ reagent can be used with numerous cell types. If a particular cell type is not listed in Table 1, this does not imply that EcoTransfect™ is not going to work. An updated list of cells successfully transfected is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

Table 1: Example of cells successfully transfected with EcoTransfect™ reagent.

Cell Lines	*Cell Type*	*Species*	*% Transfected Cells*
293, 293T	Kidney	Human	70-75%
CHO, CHO-K1	Ovary (epithelial like)	Chinese Hamster	80 -85 %
COS-1	Kidney	Green Monkey	75-80 %
COS-7	Kidney	Green Monkey	75-80 %
HEK293	Kidney	Human	65-75 %
HeLa	Cervix carcinoma	Human	45-50 %
NIH-3T3	Fibroblasts	Mouse	40-50 %
3T6	Embryonic Fibroblasts	Mouse	35-40 %
A549	Non-small cell lung carcinoma	Human	30-35 %
B16-F10	Melanoma	Mouse	35-45 %
BEAS2B	Bronchial Epithelial	Human	50 – 55 %
BHK-21	Kidney	Hamster	55 – 60 %
CV-1	Fibroblast-like (Kidney)	Monkey	25-35%
FRT	Fisher Rat Thyroid	Rat	25-30 %
MDCK	Kidney	Dog	30-35 %
N2A	Neuroblastoma	Mouse	50-55 %
U87	Glioma	Human	45-50%
Vero	Kidney	Green Monkey	25-35%

3. General Protocols

3.1. General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of cell lines. Optimal conditions may vary depending on the nucleic acid, cell types, size of cell culture dishes and presence or absence of serum. Therefore, the amounts and ratio of the individual components (DNA and EcoTransfect™) may have to be adjusted to achieve best results. Consequently, we suggest you to optimize

the various transfection parameters (components concentration, cell number, incubation time...) as described in section **3.4)** Optimization Protocol. The following recommendations can be used as guidelines to quickly achieve very good transfection. As working concentration, we recommend to use **2µL of EcoTransfect™ / 1µg of DNA.** You can use your routine culture medium for the transfection, except during the preparation of the EcoTransfect™ / DNA complexes (see **3.3** below).

- **Cells** should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. The cell proliferating rate is a critical parameter and the optimal confluency has to be adjusted according to the cells used.
- **Nucleic acids** should be as pure as possible. Endotoxins levels must be very low since they might interfere with transfection. We suggest avoiding long incubation time of the DNA solution in buffers or serum free medium before the addition of EcoTransfect reagent to circumvent any degradation or surface adsorption.
- **Antibiotics**. The exclusion of antibiotics from the media during transfection has been reported to enhance gene expression levels. This effect is cell type dependent and usually small. We did not observe any significant difference in the presence or in the absence of antibiotics by using the EcoTransfect™ reagent.
- **Materials**. Glass, polypropylene and polystyrene tubes can be used to prepare the DNA and transfection reagent solutions.

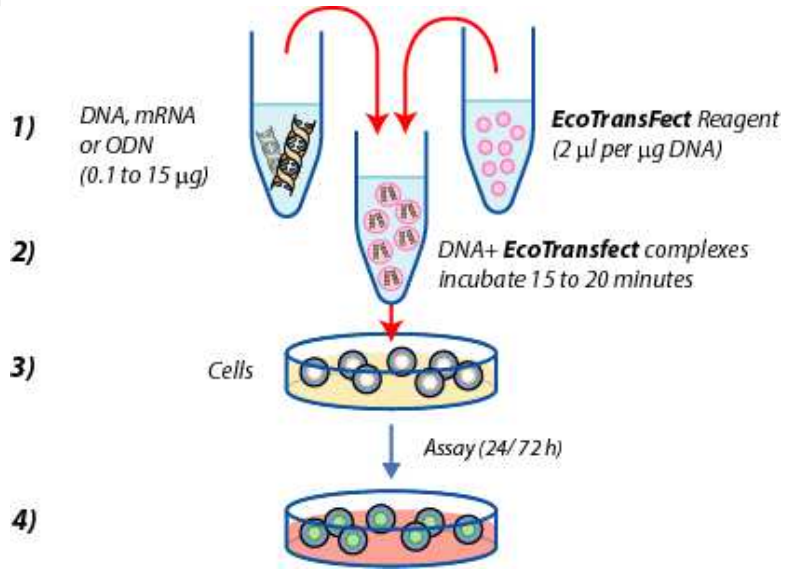
3.2. Cells Preparation

Adherent cells. It is recommended to seed or plate the cells the day prior transfection. The suitable cell density will depend on the growth rate and the conditions of the cells. Cells should not be less than 60 % confluent (percentage of growth surface covered with cells) at the time of transfection (see the suggested cell number in the table 2). The correct choice of optimal plating density also depends on the planned time between transfection and transgene analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Table 2: Cell number, DNA amount, EcoTransfect™ volume and transfection conditions suggested.

Tissue Culture Dish	Adherent Cell Number	DNA Quantity (µg)	EcoTransfect Volume (µl)	Dilution Volume (µl)	Transfection Volume
96 well	0.05 – 0.2 x 10^5	0.2	0.4	2 x 50	200 µl
24 well	0.5 – 1 x 10^5	1	2	2 x 50	500 µl
12 well	1 – 2 x 10^5	2	4	2 x 50	1 mL
6 well	2 – 5 x 10^5	3	6	2 x 100	2 mL
60 mm dish	5 – 10 x 10^5	5	10	2 x 150	4 mL
90 - 100 mm	10 – 30 x 10^5	10	20	2 x 250	8 mL
T-75 flask	20 – 50 x 10^5	15	30	2 x 350	10 -12 mL

3.3. Rapid Protocol



The DNA and EcoTransfect solutions should have an ambient temperature and be gently vortexed prior to use. The rapid protocol is as simple as follows: Use 2 µL of EcoTransfect per µg of DNA.

1) . **DNA solution**. Dilute 0.1 to 15 µg of DNA in 25 to 350 µl (see Table 2) of culture medium without serum and antibiotics.
. **EcoTransfect solution**. Dilute 0.2 to 30 µl of EcoTransfect in 25 to 350 µl (see Table 2) of culture medium without serum and antibiotics.

- *Do not use serum-containing media for this step!*
- *Prevent the EcoTransfect and DNA stock solutions to come into contact with any plastic surface. First, add serum-free culture medium to the tube and then drop the EcoTransfect and DNA stock solution directly into the medium. Contact of EcoTransfect and DNA with the tube surface (plastic or glass) would result in materials lost by adsorption.*

2) Combine the two solutions, mix gently by carefully pipetting up and down and incubate the mixture for 15 - 20 minutes at room temperature. EcoTransfect / DNA ratio of 2/1 can be used as a starting point and ratio might need to be optimized, see below section 3.4.

- *The diluted solution should be combined within 5 minutes.*
- *Do not vortex or centrifuge!*

3) Add the complexes to the cells growing in serum-containing culture medium and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

4) Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to 72 hours following transfection.

- *For some cells, 24 hours post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells. **
- *If some cytotoxicity is observed in the case of sensitive cells, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium. **

*Note: EcoTransfect / DNA complexes are prepared in medium without serum because serum interferes with vector assembly. According to the protocol, the serum free complexes solution is added to the cells that are covered with complete medium. Therefore, the addition of the transfection cocktail will result in the dilution of supplements such as serum, antibiotics or other additives of your standard culture medium. Although a medium change after transfection is not required for most cell types, it may be necessary for cells that are sensitive to serum/supplement concentration. Alternatively, the cells may be kept in serum-free medium during the first 4 hours of transfection follow by a medium change.

Stable transfection. The same protocol can be used to produce stably transduced cells except that 48 hours post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48 hours before exposing the transduced cells to selection media. For producing stably transfected cells, we recommend maintaining the complexes at least 48hours in contact with cells; in this case do not replace the culture medium, if required just add fresh culture medium to the cells. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.

3.4. Optimization Protocol

Although high transfection efficiencies can be achieved in most popular cell lines with the rapid protocol, some optimization may be needed in order to obtain maximum efficiency in particular cells. For best results, we recommend optimization of the transfection protocol for each combination of plasmid and cell line used. We advise you to optimize your transfection conditions in order to get the best out of EcoTransfect™. Several parameters can be optimized:

- Ratio of EcoTransfect™ to nucleic acid
- Quantity of nucleic acid
- Cell density
- Culture medium composition (+/- serum)

OZ Biosciences' team has investigated numerous factors; we recommend that you optimize one parameter at a time and start from the experimental procedure described above (sections 3.1 to 3.3). The two most critical variables are the ratio of EcoTransfect™ reagent to DNA and the quantity of DNA.

1) EcoTransfect™ / DNA ratio:

This is a main optimization parameter. EcoTransfect™ has to be used in excess compare to DNA but the optimal ratio will depend on the cell line and the vessel used. It is particularly true for 96 well plates because of adsorption processes. For optimization, first maintain a fixed quantity of DNA (according to the size of your culture dish or cell number) and then vary the ratio of EcoTransfect™ reagent to DNA over the suggested range in the table 3. You can test ratios from 1 to 5 µl of EcoTransfect™ reagent per 1 µg DNA.

Table 3: Suggested range of EcoTransfect™ for optimization.

Tissue Culture Dish	DNA Quantity (µg)	EcoTransfect™ Volume (µL)	EcoTransfect™ Volume (µL) proposed interval
96 well	0.2	0.2 - 1	0.2 – 0.4 – 0.6 – 0.8 – 1
24 well	1	1 – 5	1 – 2 – 3 – 4 - 5
12 well	2	2 - 10	2 – 4 – 6 – 8 – 10
6 well	3	3 - 15	3 – 6 – 9 – 12 – 15
60 mm dish	5	5 - 25	5 – 10 – 15 – 20 – 25
90 - 100 mm dish	10	10 - 50	10 – 20 – 30 – 40 – 50
T-75 flask	15	15 - 75	15 – 30 – 45 – 60 – 75

2) Quantity of DNA:

In order to obtain the highest transfection efficiency, the amount of DNA used can be increased. However a high amount of the complexes can result in over expression or lysis of the cell. These effects vary with the number of cells so, it is important to always keep the number of cells and the incubation time constant during your optimization procedure. Thus, after optimization of the EcoTransfect™ / DNA ratio proceed to adjust the best amount of DNA required by maintaining a fixed ratio of EcoTransfect™ reagent to DNA, and vary the DNA quantity over the suggested range (table 4)

Table 4: Suggested range of DNA amounts for optimization.

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.1 – 0.8	200 µl
24 well	0.5 – 2	500 µl
12 well	1 – 4	1 mL
6 well	2 – 8	2 mL
60 mm dish	3 – 12	4 mL
90 - 100 mm dish	5 – 30	8 mL
T-75 flask	8 – 40	10 -12 mL

Following these two steps process, culture medium compositions, cell number, incubation times can also be optimized.

3) Cell number:
The cell proliferating rate is a critical parameter and the optimal confluency has to be adjusted according to the cells used. Thus, the next step is to use the optimize ratio and DNA amount obtained previously and varied the cell number to be assayed.
For stable transfection, cells can be seeded with lower density. 48 to 72 hours post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection.

4) Effect of serum /Transfection volume:
Almost all cell lines transfected with EcoTransfect™ showed excellent results if serum is present during the transfection. Some cell lines may behave differently and transfection efficiency can be increased without serum or under reduced serum condition. **Remember that presence of serum during complex formation is strictly prohibited, as the serum will inhibit their formation.** Transfection efficiency is attained when the initial 3-4 hours of incubation is done. Consequently, the cells may be kept in serum-free medium during the first 4 hours of transfection, then replace it by a culture medium containing serum or just add serum to the wells according to your standard culture condition after this period.

5) Incubation time:
The optimal time range between transfection and assay for gene activity varies with cells, promoter activity, expression product, etc. The transfection efficiency can be monitored after 24 - 72 hours by analyzing the gene product. Reporter genes such as GFP, -galactosidase, secreted alkaline phosphatase or luciferase can be used to quantitatively measured gene expression. For example, percentage of cells expressing the -galactosidase transgene can be visualized by histochemical staining with X-Gal (see related product).

4. Appendix

4.1 Quality Controls

To assure the performance of each lot of EcoTransfect™ produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
Purity	Silica Gel TLC assays. Every compound shall have a single spots.
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
Biological Activity	Transfection efficacies on CHO and COS-7 cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot

4.2. Troubleshooting

Our dedicated and specialized technical support group will be pleased to answer any of your requests and to help you with your transfection experiments. tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

5. Related Products

Please visit our website (www.ozbiosciences.com) for information and details about OZ Biosciences other related products such as:

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
In vivo Magnetofection
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3DfectIN *(for hydrogels culture)*
3Dfect *(for scaffolds culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit -Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g G-418, Sulfate 1g
X-Gal powder 1g

Please, feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list.

Purchaser Notification

Limited License

The purchase of the EcoTransfect™ reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the EcoTransfect™ reagent. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

Buyers may end this License at any time by returning all EcoTransfect™ reagent material and documentation to OZ Biosciences, or by destroying all EcoTransfect™ components. Purchasers are advised to contact OZ Biosciences with the notification that an EcoTransfect™ kit is being returned in order to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

This document covers entirely the terms of the EcoTransfect™ reagent research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The EcoTransfect™ reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

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