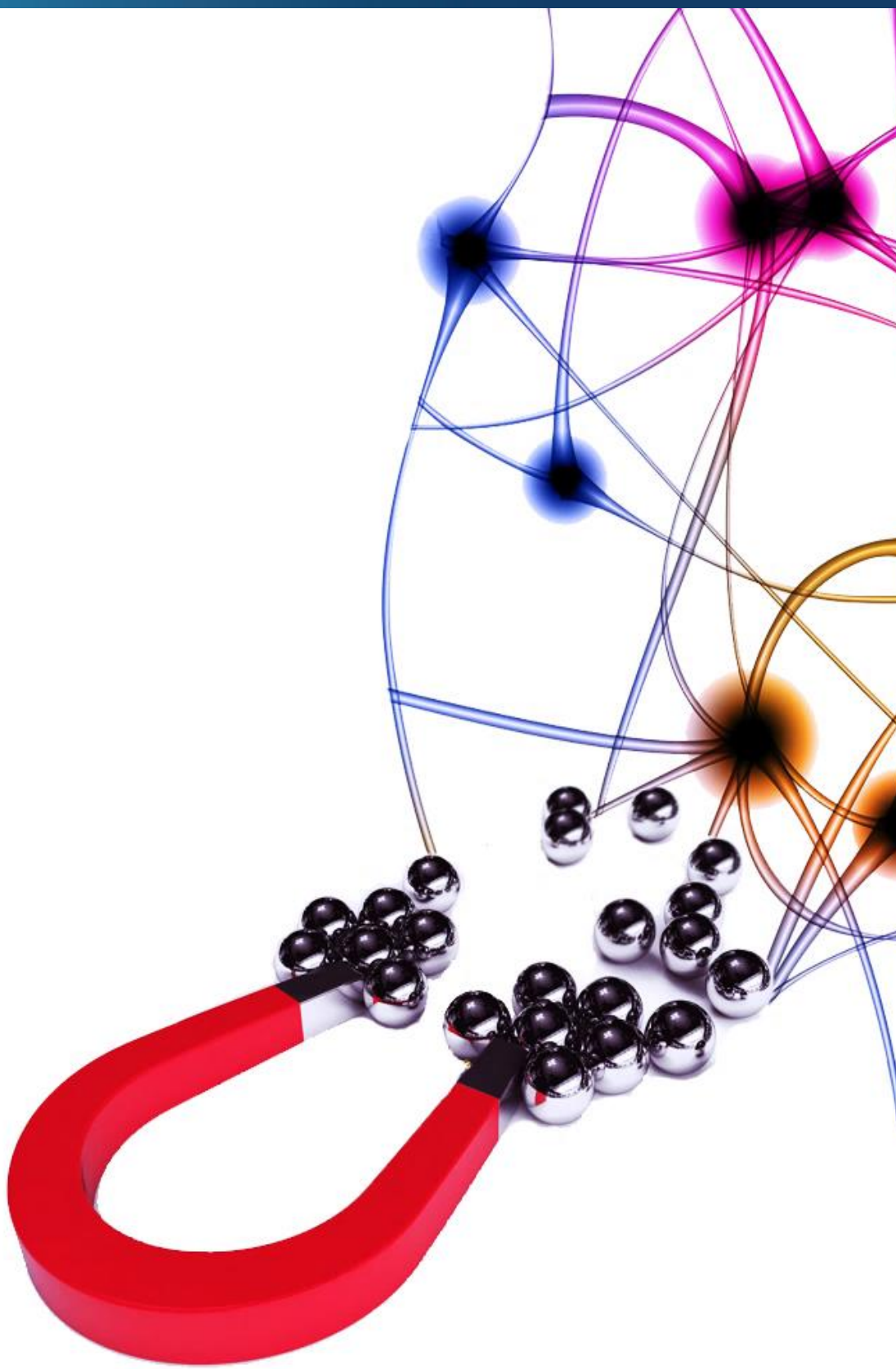
Magnetofectamine O2 Kit

INSTRUCTION MANUAL



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The art of delivery systems

Magnetofectamine™ O2

Instruction Manual

Magnetofectamine™ O2 is the newest version of Magnetofectamine transfection Kit taking advantages of the latest development in transfection reagents.

Magnetofectamine™ O2 is ideal for transfecting Primary cells as well as cell lines.

List of Magnetofectamine™ O2 kits

Catalog Number	Description	Volume (µL)	MTXBoost 100X Volume	Number of transfections/µg DNA
MTX2-750	Magnetofectamine™ O2[1]	250 µL + 750 µL	3 mL	250
MTX2-1000	Magnetofectamine™ O2 Starting Kit [2]	250 µL + 750 µL + 1 magnetic plate	3 mL	250

[1] Contains 1 vial of each reagent (250µL CombiMag + 750µL MTX reagent + 3 mL MTXBoost)

[2] Contains 1 vial of each reagent and a Magnetic plate (250µL of CombiMag + 750µL MTX reagent + 3 mL MTXBoost + Super Magnetic plate MF10000)

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

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1. Technology

Magnetofectamine O2 is the association of the powerful Magnetofection reagent COMBIMAG, with MTX reagent- a biodegradable lipid-based transfection reagent- and MTXBoost, an enhancer of efficiency. **Magnetofectamine O2 kit** takes advantages of the latest development in transfection reagents and offers an increase in efficiency and viability when compared to the first generation of the Magnetofectamine Kit. It is especially suited for hard-to-transfect and primary cells. MTXBoost lowers the susceptibility of cells to detect foreign nucleic acids enhancing the dose delivered to nucleus.

Magnetofection™ is a highly efficient gene delivery method that exploits magnetic force exerted upon gene vectors associated with magnetic particles to drive the nucleic acids towards and into- the target cells. In this manner, the complete applied nucleic acid dose gets concentrated onto the cells within a few minutes so that 100% of the cells get in contact with a significant vector dose.

Magnetofectamine O2 transfection reagent principal advantages:

- Improved transfection efficiency
- Biodegradable transfection reagent leading to minimized toxicity
- High level of nucleic acid compaction
- Ideal for hard-to-transfect cells
- Easy and straightforward protocol
- Compatible with serum & any culture medium.

Kit Contents, Stability and Storage

Contents

• 1 vial of CombiMag (250µL), 1 vial of MTX REAGENT (750µL) & 1 vial of MTXBoost 100X (3 mL) good for up to 250 transfections with 1µg DNA.
• Starting kit: same as above **and a super magnetic plate**.

Stability, Storage and Shipping
Stability: Magnetofectamine O2 Kit components are stable for at least 18 months at the recommended storage temperature.

Storage: Upon reception and for long-term use, store the MTX REAGENT and MTXBoost at -20°C and CombiMag at +4°C.

Shipping condition: Room Temperature

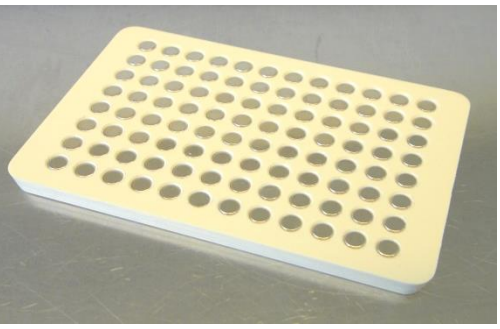
Magnetofectamine O2 transfection reagents are stable for several days at room temperature or at +4°C without losing activity. The numbers of freeze and thaw cycles (MTX REAGENT and MTXBoost) do not affect the efficiency of the reagents.

2. Applications

Magnetofectamine™ O2 is the second generation of our Magnetofectamine Kit. It can be used with various kinds of nucleic acids (DNA, mRNA, siRNA...) on any cell types, from cell lines to primary or hard-to-transfect cells and primary neurons. The combination of Magnetofection with lipid-based reagent enables using smaller amounts of nucleic acids and increasing the overall efficiency of transfection while gaining reduction in toxicity.
This transfection kit is serum compatible and is used for transient as well as stable transfection. This product is very stable, ready-to-use and intended for research purpose only.

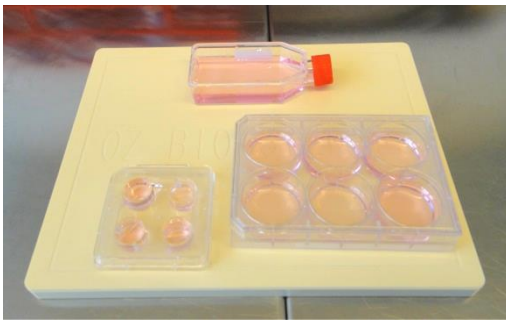
3. Magnetofection™ Apparatus

Apart from suitable magnetic nanoparticles, Magnetofection™ requires appropriate magnetic fields. A magnetic plate especially designed for Magnetofection is provided to exert these specific magnetic fields. Its special geometry not only produces strong magnetic fields it is applicable for any plate formats (T-75 flasks, 60 & 100 mm dishes, 6-, 12- and 24-well plates). Super Magnetic Plate suits for all cell culture supports and Mega Magnetic Plate is designed to hold up to 4 culture dishes at one time. The magnetic plate design allows producing a heterogeneous magnetic field that magnetizes the nanoparticles in solution, forms a very strong gradient and covers all the surface of the plate.



Magnetic plate 96 magnets

Super Magnetic Plate



Mega Magnetic Plate

4. General Protocol

4.1. General Considerations / Important Guidelines

The instructions given below represent standard protocol. Optimal conditions may vary depending on the plasmid, cell type, clone, size of cell culture dishes and conditions of culture. As a starting point, use **2 or 3µL MTX REAGENT/ 1µL CombiMag per µg of DNA.** Refer to the optimization procedure to find optimal transfection conditions.

- **Cells** should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. Use regularly passaged cells lines or freshly prepared primary cells at confluence between 60 and 80% (visual confluence). Do not use cells that have been cultured for too long (> 2 months).
- **Nucleic Acids** should be as pure as possible and free of contaminants. We suggest avoiding long storage of the diluted nucleic acid solution before the addition of Magnetofectamine O2 reagents to circumvent any degradation or surface adsorption. We recommend using pVectOZ-GFP plasmid for an efficient transfection control.
- **Culture Medium**. The exclusion of antibiotics from the media during transfection has been reported to enhance gene expression levels. We did not observe a significant effect of the presence or absence of antibiotics with the Magnetofectamine O2 transfection reagent.

4.2. Cells Preparation

Cell culture prior to transfection: one day before transfection prepare the cells according to the table below.

Adherent cells. It is recommended to plate the cells the day prior transfection* in classical culture medium. Cells should be 60-80 % confluent at the time of transfection (see the suggested cell number in the Table 1). The correct choice of optimal plating density also depends on the planned time between transfection and protein expression analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Table 1: Cell number, DNA amount, MTX REAGENT and COMBIMAG volumes and transfection conditions suggested (per well).

Tissue Culture Dish format	Surface area per well[1]	Cell Number	DNA Quantity (µg)	MTX REAGENT Volume (µL)[2]	Lipoplexes Volume	COMBIMAG Volume (µL)[2]
96 wells	0.3 cm^2	0.02 – 0.2 x 1.10^5	0.25	0.5-0.75	2x25	0.25
48 wells	1 cm^2	0.2 – 0.4 x 1.10^5	0.3	0.6-0.9	2x25	0.3
24 wells	2 cm^2	0.5 – 0.8 x 1.10^5	0.5	1.0-1.5	2x50	0.5
12 wells	4 cm^2	1 – 4 x 1.10^5	2	4-6	2x50	2
6 wells	10 cm^2	2 – 10 x 1.10^5	3	6-9	2x100	3
60 mm dish	20 cm^2	5 – 20 x 1.10^5	6	12-18	2x150	6
100 mm dish	60 cm^2	10 – 60 x 1.10^5	10	20-30	2x250	9

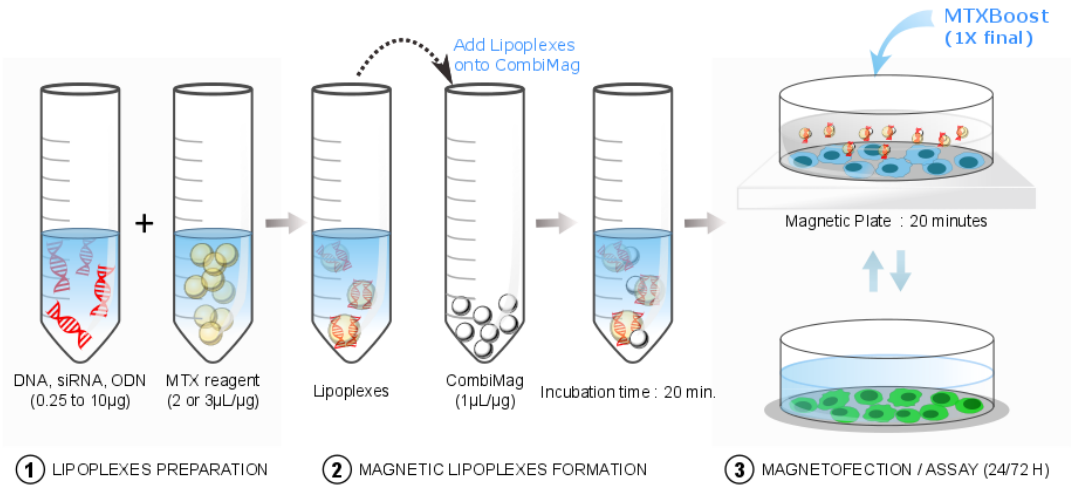
[1] Surfaces area may vary depending on the manufacturer.
[2] For low volumes, to ensure a correct pipetting, we recommend preparing dilution of Magnetofectamine O2 reagents in sterile culture-grade H2O.
* Some primary cells require being prepared 48H before transfection; change half of the culture medium 24H transfection.

Suspension cells. For fast growing cells, split the cells the day before transfection at a density of 2 to 5 x 1.10^5 cells/mL so they are in excellent condition on the day of transfection.
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 suspension cells, we suggest exposing cells to selection medium at least 72 hours post-transfection

4.3. DNA Transfection Protocol

Use the following procedure to transfect DNA using the **Magnetofectamine O2** kit. The Table 2 shows optimized transfection conditions according to different cell culture formats (all amounts are given on per-well basis).
Use this rapid protocol: **3 µL of MTX REAGENT/1µL CombiMag per µg of DNA** and **1X MTXBoost**.

Note: We suggest beginning with the recommended ratios and optimize it, if required.



1) **Reagents preparation.** Allow reagents to reach room temperature before beginning.
   a. *Nucleic acid solution.* Dilute **0.25** to **10 µg** of nucleic acid in **25** to **250 µL** of culture medium without any supplement (SVF, antibiotics, growth factors...) or PBS (refer to table 1).
   b. *MTX REAGENT solution.* Dilute **0. 5** to **30 µL** of MTX REAGENT in **25** to **250 µL** of culture medium without any supplement (SVF, antibiotics, growth factors...) or PBS (refer to table 1).
   c. *COMBIMAG solution.* Add **1 µL** of CombiMag per **µg** nucleic acid to a new microtube.

2) **Lipoplexes formation.** Combine the nucleic acid and MTX REAGENT solutions. Mix gently by carefully pipetting up and down and incubate the mixture for 5 minutes at room temperature (RT). Do not vortex or centrifuge!

   Note: The diluted solutions should be combined within 5 minutes.

3) **Magnetic Lipoplexes formation.** Add the lipoplexes to the CombiMag reagent. Mix gently by carefully pipetting up and down and incubate the mixture 20 minutes at RT.

4) **Transfection**.
   a. Add the complexes in a dropwise manner onto the cells growing in complete culture medium and homogenize by rocking the plate back and forth to ensure a uniform distribution of the mixture.
   b. Add MTXBoost 100X (**1X final**) directly onto the cells.

5) **Magnetofection**. Place the cells upon the specific magnetic plate for 20-30 min

6) Remove the magnetic plate and cultivate cells under standard conditions for 2h.

   Note: At this step, a medium change may optionally be performed. Keep the magnetic plate beneath the cell culture dish and replace medium with fresh medium and then remove the magnetic plate.

7) **Assay.** Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the protein expression. We recommend performing assay from 24 to 72h.

Reverse transfection. Prepare the complexes as described above, then transfer them into an empty culture dish or well and finally and directly add the cells at twice the recommended cell density.

Other protocols for optimization or co-transfection are also available on our website at www.ozbiosciences.com or by contacting our technical support department (tech@ozbiosciences.com).

OZ Biosciences offers plasmids coding for CAT (#PL00010), **GFP** (#PL00020), **LacZ** (#PL00030), **LUC** (#PL00040) **and SEAP** (#PL00050) **as transfection controls**. These control plasmids are recommended to set up optimization procedure.

4.4. Optimization protocol for transfection

To achieve the highest efficiency, optimize the transfection conditions as follows:

- Vary the MTX REAGENT (µL) / Nucleic Acid (µg) ratio from 2/1 to 4/1.
  We recommend trying 2.0, 3.0, 3.5 and 4 µL MTX REAGENT per µg of nucleic acid.
- Once the optimal MTX REAGENT ratio is found, adjust the nucleic acid quantity according to Table 2.
- Finally, culture medium composition (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

Table 2: Suggested range of DNA amounts for optimization (per well).

Tissue Culture Dish format	Nucleic acid quantity (µg)
96 well	0.05 to 0.25
48 well	0.25 to 0.5
24 well	0.3 to 1
6 well	2 to 10

5. Appendix

Our dedicated and specialized technical support team will be pleased to answer any of your requests at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com.

5.1 Quality Controls

To assure the performance of each lot of Magnetofectamine O2 reagent produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each component.

Specification	Standard Quality Controls
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 15 days.
Biological Activity	Transfection efficacies on BV2 cells. Every lot shall have an acceptance specification of > 85% of the activity of the reference lot.

5.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **Optimization of Magnetofectamine O2/ Nucleic Acid (NA) ratio.** See section 3.4. 2- **NA amount.** Use different quantities of NA with the optimized ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency should range from 60 to 80% but most favorable cell density may vary according to the cell subtype; preferably mid-log growth phase. 4- **Nucleic Acid quality.** NA should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins. 5- **Type of promoter**. Ensure that DNA promoter can be recognized by the cells to be transfected. Use pVectOZ plasmids as controls for transfection. 6- **Cell condition.** 1) Cells in culture for a long time (> 8 weeks) may become resistant to transfection. Use freshly thawed cells that have been passaged at least once. 2) The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. 7- **Medium used for preparing the complexes**. It is critical to use serum-free medium or buffer (HBS, PBS) during the complexes preparation. 8- **Culture medium composition.** 1) In some cases, transfection efficiency can be increased in absence of serum. Transfect these cells in serum-free medium during the first 4h. 2) The presence of antibiotics might affect cell health and transfection efficiency. 9- **Incubation time and transfection volume.** 1) The optimal time range between transfection and assay varies with cells, promoter, expression product, etc. The transfection efficiency can be monitored after 18h depending on the readout and the cell. 2) To increase transfection efficiency, transfection volume suggested can be reduced for the first 24 hours. 10- **Old transfection reagent / NA complexes.** The transfection reagent / NA complexes must be freshly prepared each time to avoid aggregation. 11- **Transfection reagent temperature.** Reagents should be at ambient temperature and be vortexed prior to use.
Cellular toxicity	1- **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials. 2- **Protein expression is toxic.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a control plasmid. 3- **NA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 4- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes added to the cells by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously.

6. Related Products

MAGNETOFECTION TECHNOLOGY

Super Magnetic Plate *(standard size for all cell culture support)*
+ **Transfection reagents**:
PolyMag Neo - *for all nucleic acids*
CombiMag – *Transfection enhancer for all nucleic acids*
NeuroMag - *dedicated for neurons*
SilenceMag - *for siRNA application*
ViroMag - *for viral application / transduction enhancer with low vector doses*

PLASMIDS PVECTOZ

pVectOZ-LacZ, Luc, CAT, GFP, SEAP

ASSAY KITS

Bradford – Protein Assay Kit / FluoProdige – Fluorescent Protein Quantification Assay Kit
MTT cell proliferation kit – Cellular Senescence Kit
β-Galactosidase assay kits (CPRG/ONPG)

BIOCHEMICALS

D-Luciferin, K^+ and Na^+
X-Gal powder 1g / G-418, Sulfate

Our dedicated and specialized technical support group will be pleased to answer any of your request and to assist you in your experiments. Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list.

7. Purchaser Notification

Limited License

The purchase of the Magnetofectamine O2 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). Reagents are intended **for in-house research only** by the buyer. Such use is limited to 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 Magnetofectamine O2 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 Magnetofectamine O2 reagent material and documentation to OZ Biosciences, or by destroying all Magnetofectamine O2 reagent components. Purchasers are advised to contact OZ Biosciences with the notification that a Magnetofectamine O2 reagent kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

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Product Use Limitations

The Magnetofectamine O2 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.

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