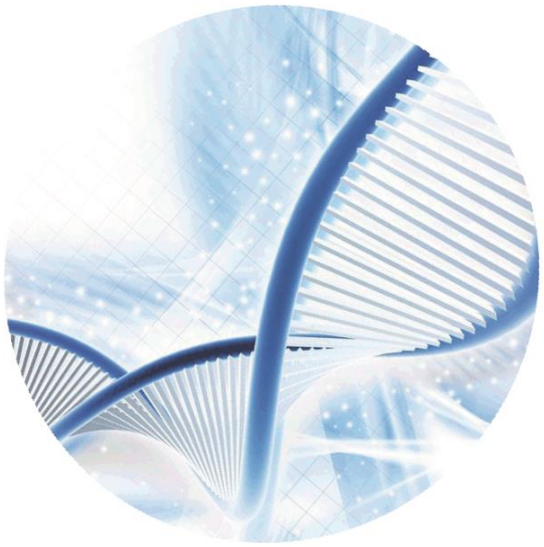


Transfection reagent

Magnetofectamine O2 Kit

Improve Transfection Efficiency

For primary and hard-to-transfect cells

Protocol

Magnetofection Technology

This reagent needs to be used with a magnetic plate

OZBIOSCIENCES

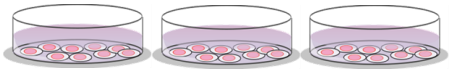
The art of delivery systems

Magnetofectamine™ O2 Quick Protocol

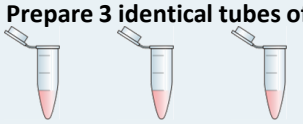
MTX Reagent + CombiMag + MTXBoost (100X)

To find the ideal conditions, Magnetofectamine O2 must be tested at ratios **2 µL/µg**, **3 µL/µg** and **3.5 µL/µg** (µL of MTX/µg of DNA); CombiMag must be used at 1µL /µg of DNA and MTXBoost must be diluted 100x. For the DNA quantity, we suggest **0.125 µg** per well in 96-well, **0.5 µg** per well in 24-well and **2 µg** per well in 6-well.

1 **Seed cells to be at 70% confluent the day of transfection***



2 **Prepare 3 identical tubes of DNA**



96 well plate	24 well plate	6 well plate
0.125µg in 25µL of serum-free medium or buffer* x 3	0.5µg in 50µL of serum-free medium or buffer* x 3	2µg in 100µL of serum-free medium or buffer* x 3

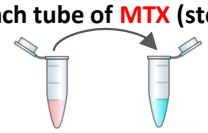
3 **Prepare 3 tubes of MTX (with 3 different amounts of reagent)**

96 well plate	24 well plate	6 well plate
0.25µL/0.375µL/0.45µL in 25µL of serum-free medium or buffer*	1 µL/1.5µL/1.75µL in 50µL of serum-free medium or buffer*	4µL/6µL/7µL in 100µL of serum-free medium or buffer*

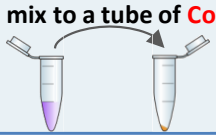
4 **Prepare 3 identical tubes of CombiMag™**

96 well plate	24 well plate	6 well plate
0.125µL in an empty microtube x 3	0.5µL in an empty microtube x 3	2µL in an empty microtube x 3

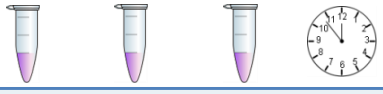
5 **Mix each tube of DNA (step 2) to each tube of MTX (step 3) and immediately proceed to next step**



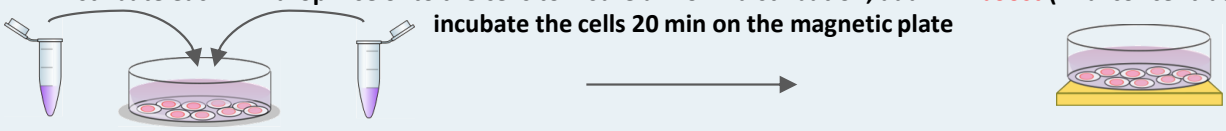
6 **add each mix to a tube of CombiMag (step 4)**



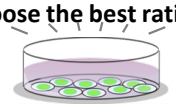
7 **Incubate 20 min at room temperature**



8 **Distribute each mix dropwise onto the cells to insure uniform distribution, add MTXboost (final concentration 1X) & incubate the cells 20 min on the magnetic plate**



9 **Remove the cells from the magnetic plate, incubate cells for 24 to 72h at 37°C until evaluation of transgene expression and choose the best ratio DNA:Magnetofectamine O2**



These conditions might require some further optimizations depending on your cells, DNA, RNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ **Medium or buffer without serum & supplement** must be used for the DNA/MTX/CombiMag complexes preparation. Culture medium such as MEM, DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.
- ✓ Dilute reagents with deionized water for doses less than 1µL.
- ✓ For most cell types, a medium change is not required after Magnetofection. However, it may be necessary for cells that are sensitive to serum/supplement concentration. This can be done immediately after the 20min incubation on the magnetic plate while keeping the cells onto the magnetic device, or 4 to 6h post-Magnetofection. Alternatively, the cells may be kept in serum-free medium during Magnetofection (up to 4h). In this case, a medium change will be required after Magnetofection.
- ✓ Do not freeze the magnetic nanoparticles CombiMag

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

Package content	MTX2-0750: 750 µL of MTX reagent + 250 µL of CombiMag + 3mL of MTX Boost 100X MTX2-1000: 750 µL of MTX reagent + 250 µL of CombiMag + 3mL of MTX Boost 100X + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Upon reception, store the MTX Reagent and MTX Boost 100X at -20°C and CombiMag at +4°C
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	Magnetofectamine O2 is a magnetic nanoparticles formulation specifically designed to achieve high transfection efficiency of primary cells, hard-to-transfect cells and cell lines.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cell Preparation

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 (refer to Table 1). 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.

Tissue Culture Dish	Adherent Cell Number	DNA Quantity (µg)	MTX reagent volume (µL)	Lipoplexes volume (µL)	CombiMag Volume (µL)	MTX Boost 1X (µL)
96 well	0.02 – 0.2 x 10^5	0.125	0.375	2 x 25	0.25	2
24 well	0.5 – 0.8 x 10^5	0.5	1.5	2 x 50	0.5	5
6 well	2 – 10 x 10^5	2	6	2 x 100	2	20

Table 1: Suggested cell number, DNA amount and transfection reagent volumes per well

2. DNA/Magnetofectamine O2 complexes preparation

a. *DNA*: Dilute the indicated quantity of DNA (refer to Table 1) in 25 to 100 µL of culture medium without serum and supplement.

b. *MTX Reagent*: Dilute the indicated quantities of MTX reagent in 25 to 100 µL of culture medium without serum and supplement (see Table 1).

c. *CombiMag*: Vortex the CombiMag and place the appropriate amounts in an empty microtube (refer to table 1).

d. Add the DNA solution to the MTX solution, mix gently by carefully pipetting up and down and incubate the mixture at room temperature for 5 min. Do not vortex.

e. Add the mix to CombiMag reagent and gently mix by pipetting up & down and incubate 20 min at room temperature.

3. Transfection

a. Add the complexes (DNA/MTX/CombiMag) onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Add MTX boost 100X at 1X final directly onto the cells.

c. Place the cell culture plate on the magnetic plate during 30 min.

d. Remove the magnetic plate.

e. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression (from 24h up to 7 days).

NOTE: in case of cells very sensitive to transfection, the medium can be changed after 3-4h or 24h incubation with fresh medium.

IMPORTANT OBSERVATION FOR PROTEIN PRODUCTION OVER 24H

In case of protein production experiment over 24h, we recommend using two times more amounts of DNA per well to yield maximal levels of protein.

Protocol | DNA or shRNA vectors transfection in suspension cells

1. Cell Preparation

The day before transfection split the cells at a density of 2 to 5 x 10^5 cells / mL, so they are in excellent condition on the day of transfection. Incubate overnight in complete culture medium (refer to Table 2).

Tissue Culture Dish	Cell density
96 well	0.5 – 1 x 10^5
24 well	2 – 4 x 10^5
6 well	8 – 16 x 10^5

Table 2: Suggested range of suspension cell densities

2. DNA/MTX complexes preparation

a. *DNA solution*. Dilute the indicated quantity of DNA in 25 to 1500 µL of culture medium without serum (refer to Table 3).

Tissue Culture Dish	DNA Quantity (µg)	MTX Volume (µL)	Dilution Volume (µL)[1]	CombiMag Volume (µL)	Total culture medium Volume
96 well	0.25	0.75	2 x 25	0.25	100 µL
24 well	1	3	2 x 50	1	500 µL
6 well	4	12	2 x 250	4	2 mL

[1] Volumes of dilution medium in step 3a and 3b

Table 3: Suggested DNA amount, MTX volume and transfection conditions

b. *MTX solution*. Vortex the reagent and dilute the indicated quantities of MTX in 25 to 250 µL of culture medium without serum (refer to table 3).

c. *CombiMag reagent*. Vortex the CombiMag and place the appropriate amounts in an empty microtube (see Table 3).

d. Combine the DNA/MTX solution with the MTX solution, mix gently by carefully pipetting up and down and incubate at room temperature for 5 min.

3. Transfection

a. While the complexes are incubating, prepare your cells in serum-free medium (or serum-containing medium) and transfer the appropriate volume to the culture dish according to Table 2. In 24-well plates for instance, plate 2x10^5 suspension cells just before transfection in 250 µL of serum free medium. Generally, serum-free condition leads to higher transfection efficiency.

b. Perform one of the following three options to sediment the cells at the bottom of the culture dish in order to promote the contact with the magnetic nanoparticles.

1. Seed the cells on polylysine-coated plates and use the protocol for adherent cells

OR

2. Briefly, centrifuge the cells (2 min) to pellet them and use the protocol for adherent cell

OR

3. Mix cell suspension with 30 µL of *CombiMag* reagent per mL of cell suspension.
    - Incubate for 10 - 15 min.
    - Distribute cells to your tissue culture dish placed upon the magnetic plate (volume of culture medium containing cells depends on the culture dish size; see suggested transfection volume in table above as indication).
    - Incubate for 15 min

OR

4. Incubate the cells in serum free medium during 2h prior Magnetofection. The absence of serum allows some cells to adhere onto the plastic dish surface.

c. Next, place the cells onto the magnetic plate and add the complexes directly onto the cells dropwise.

d. Incubate for 30 min and remove the magnetic plate.

e. Incubate 3 to 6 h (4h is commonly used) in serum-free medium at 37°C under 5% CO2.

f. If transfection is performed in serum free medium, add serum to adjust its concentration

g. Incubate the cells at 37°C in a CO2 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 72h following transfection.

NOTE: depending on the cell type, 24h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.

IMPORTANT OBSERVATIONS

- Note that transfections are optimum when performed in the absence of serum. However, transfection can also be achieved directly in the presence of serum.
- For some cells, 24h post-transfection replace the old medium with fresh medium or just add fresh growth culture medium to the cells.
- GeneBlaster™ Topaz (catalog # GB20013) can be used to boost the gene expression level in some cell lines.

Protocol | stable transfection

The same protocol can be used to produce stably transduced cells except that 48h post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48h before exposing the transduced cells to selection media. For suspension cells, we suggest exposing the cells to selection media at least 72h post-transfection.

Protocol | Co-transfection

For co-transfection of several plasmids DNA, mix the same amount of each plasmid and transfect as described above. For example, if you have two DNA plasmids, mix 0.5 µg of each plasmid, complex the 1 µg of DNA with 3 µL of MTX and 1 µL of CombiMag.

Option for co-transfection

Transfections can be realized sequentially instead of simultaneously. So, cells can be transfected with one plasmid DNA first and 4h to 24h later can be transfected with the other plasmid DNA. Follow the procedure as detailed above for DNA transfection. A medium changed can be also performed between the two transfections.

Protocol | siRNA

1. Cell Preparation

The day prior transfection, prepare the cells as described in Table 1. Generally, siRNA transfection requires lower cell density than DNA transfection. The correct choice of optimal density depends on the planned time between transfection and gene knockdown analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

2. siRNA / MTX / CombiMag complexes preparation.

The siRNA,MTX and CombiMag solutions should have an ambient temperature, be gently vortexed prior to use and be combined within 5 min.

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM) in 50 or 100 µL (see Table 4) of PBS or culture medium without serum. We advise starting with a final siRNA concentration of 50nM.

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50µL		50 µL		100 µL	
*Amount of siRNA (1 µM stock)**						
Final siRNA concentration	(µL)	(ng)	(µL)	(ng)	(µL)	(ng)
10 nM	2	27	5	67.5	20	270
20nM	4	54	10	135	40	540
50 nM	10	135	25	337.5	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

Table 4: Suggested dilution procedure and amount of siRNA to test

b. MTX preparation. Dilute the MTX reagent in 50 or 100 µL (see Table 5) of PBS or culture medium without serum.

Culture vessel	96-well	24-well	6-well
Dilution serum-free medium	50 µL	50 µL	100 µL
Final transfection Volume	200 µL	500 µL	2 mL
Final siRNA concentration	*Amount of MTX (µL)*		
10nM	0.15	0.5	2
20nM	0.25	1	4
≥ 50 nM	0.5	2	8

Table 5: Recommended amount of MTX per nM of siRNA used

c. Combine the two solutions, mix gently by pipetting up and down and incubate the mixture for 5-10 min at room temperature. Do not vortex.

d. Prepare a tube containing 1 µL of CombiMag per µg siRNA.

e. Add MTX / siRNA complexes to the CombiMag solution and incubate 20 min at room temperature in an empty microtube.

3. Transfection

a. Add the mixture drop by drop directly onto the cells. The total transfection volume per well is indicated in table 1 (culture medium + complexes solution).

b. Place the cell culture plate upon the magnetic plate for 15-20 min and remove the magnetic plate.

c. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene silencing. We recommend 24h for RNA analysis and 48h to 72h for protein knockdown analyses.

NOTES:

- Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection
- For some cells, 24h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.
- If cells are very sensitive to transfection, the medium can be changed after 3-4h or 24h incubation.

IMPORTANT OBSERVATIONS

- Ensure to avoid the presence of serum when preparing the transfection reagent/siRNA complexes. Use a medium well pH (some old medium can turn pink or purple instead of being orange or red) which could influence complexes formation and siRNA stability.

- Avoid incubating your diluted siRNA too long in your serum-free medium; prepare first your transfection reagent, dilute your siRNA and quickly transfer the diluted siRNA into the MTX tube.

- Start with 50nM siRNA and test four amounts of MTX with a fixed ratio of CombiMag.

- The gene silencing is highly dependent on your protein half-life and consequently it will be good to analyze your protein expression by western at 48h, 72h and 96h.

- Treating your cells twice with 25nM siRNA instead of once with 50nM can enhance significantly siRNA effects. Basically, on day one, incubate your cells with 25nm siRNA /MTX / CombiMag. On day two, change your medium and repeat the treatment with 25nm siRNA / MTX / CombiMag.

Protocol Optimization

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 6.
- Finally, culture medium composition (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

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

Table 6: Suggested range of DNA amounts for optimization (per well)

NOTES

Additional products for primary and hard-to-transfect cells experiments

- **SilenceMag** for siRNA applications
- **NeuroMag** for transfections of neurons
- ***In vivo* PolyMag** for *in vivo* applications

Purchaser Notification

Limited License
The purchase of the Magnetofectamine O2 kit 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 Magnetofectamine O2 kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all Magnetofectamine O2 kit reagents and documentation to OZ Biosciences, or by destroying all Magnetofectamine O2 components. Purchasers are advised to contact OZ Biosciences with the notification that a Magnetofectamine O2 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). This document covers entirely the terms of the Magnetofectamine O2 kit 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.

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the use of the kit components by following proper research laboratory practices.

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