

Magnetofectamine O2 Kit

Improve transfection efficiency for primary and hard-to-transfect cells

To find the ideal conditions, MTX must be tested at ratios of **2 $\mu\text{L}/\mu\text{g}$, 3 $\mu\text{L}/\mu\text{g}$, 3.5 $\mu\text{L}/\mu\text{g}$ and 4 $\mu\text{L}/\mu\text{g}$** (μL of MTX O2 / μg of DNA), CombiMag at ratio 1:1 and MTXBoost at 1X final. 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. Prepare 4 wells: seed the cells to be at 70% confluent on the day of transfection



2. Prepare 3 identical tubes of DNA in serum-free medium



96-well plate

0.125 μg in 25 μL of medium **x3**

24-well plate

0.5 μg in 50 μL of medium **x3**

6-well plate

2 μg in 100 μL of medium **x3**

3. Prepare 3 tubes of MTX with 3 different ratios and 3 identical tubes of CombiMag



96-well plate

MTX 0.25 μL /0.375 μL /0.45 μL
in 25 μL of serum-free medium

24-well plate

1 μL /1.5 μL /1.75 μL
in 50 μL of serum-free medium

6-well plate

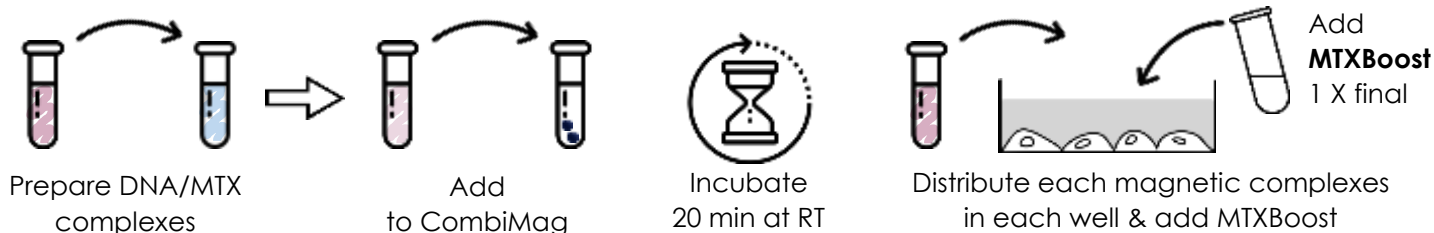
4 μL /6 μL /7 μL
in 100 μL of serum-free medium

CM 0.125 μL in 3 empty tubes

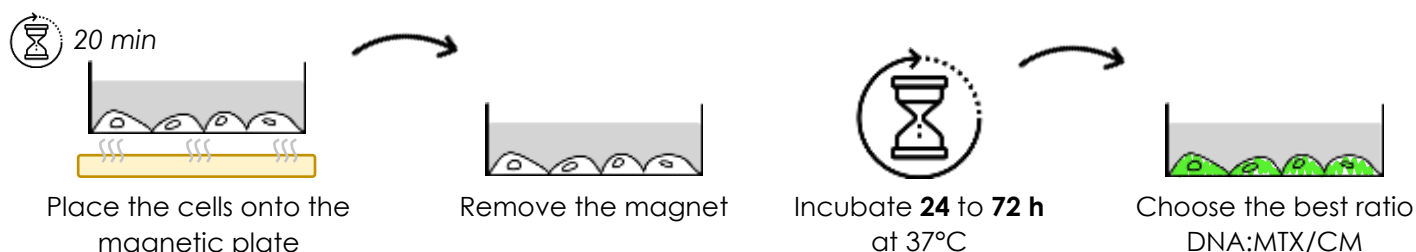
0.5 μL in 3 empty tubes

2 μL in 3 empty tubes

4. Mix each DNA suspension to each tube of MTX and add complexes to CombiMag



5. Incubate the cells 20 min on the magnetic plate and follow transgene expression



Contact us regarding protocols for DNA transfection in suspension cells, optimization procedures or for gene silencing with siRNA at: tech@ozbiosciences.com or visit our website: www.ozbiosciences.com.

Before you begin

Magnetofectamine O2™ is a Magnetic nanoparticle formulation specifically designed to achieve high transfection efficiency of microglial cell lines and primary cells.

Protocol | General considerations

- Allow reagents to reach room temperature (RT).
- Use medium w/o any supplement for preparation of complexes, avoid using RPMI.
- For some sensitive cells, medium change can be performed after 20 min magnetofection while cells are still on the magnetic plate, or 4 to 6 h after.

⚠ For doses of MTX or CombiMag less than 1 µL, dilute the reagents with deionized water only.

⚠ **Do not freeze CombiMag magnetic nanoparticles.**

1. Cells preparation:

One day before transfection prepare the cells according to table 1 (60-80% confluence).

Cell culture format	Surface area per well	Adherent cell nb. / well	Transfection volume
96-well	1 cm ²	0.2-0.4 x 1.10 ⁵	200 µL
24-well	2 cm ²	0.5-0.8 x 1.10 ⁵	500 µL
6-well	10 cm ²	2-4 x 1.10 ⁵	2 mL

Table 1: Suggested cell number per well.

2. Preparation of DNA/MTX complexes:

Refer to table 2 for reagents and dilutions volumes.

a. **DNA:** Dilute the indicated quantity of DNA in **25 to 100 µL** of culture medium w/o supplement.

b. **MTX reagent:** Dilute the indicated volumes of MTX reagent in **25 to 100 µL** of culture medium w/o supplement.

c. **CombiMag:** Vortex CombiMag tube and add the appropriate volume in an empty microtube.

d. Gently mix DNA and MTX solutions by carefully pipetting up and down and incubate 5 min at RT.
⚠ Do not vortex or centrifuge.

e. Add DNA/MTX complexes to CombiMag reagent, gently mix by pipetting up and down and incubate at 5 min at RT.

3. Transfection | Magnetofection:

a. Add the magnetic complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Add MTX Boost (1X final) to the cells.

c. Place the cell culture plate on the magnetic plate for **30 min** (RT or 37°C).

d. Remove the magnetic plate and cultivate the cells at **37°C** in a CO₂ incubator under standard conditions until evaluation of transgene expression.

Cell culture format	DNA quantity	MTX reagent volume (µL)	Dilution volume	CombiMag volume (µL)	MTXBoost volume (µL)
96-well	0.125 µg	0.25 to 0.45	2x25 µL	0.125	2.5
24-well	0.5 µg	1 to 1.75	2x50 µL	0.5	5
6-well	2 µg	4 to 7	2x100 µL	2	20

Table 2: Recommended DNA amounts and transfection reagents volumes. For other formats, adjust conditions accordingly.

Protocol | Suspension cells | Co-transfection | siRNA transfection | Optimization |

Contact us regarding protocols for suspension cells, co-transfection, gene silencing, optimization procedure, or any technical question:

✉ - tech@ozbiosciences.com.

Use, handling and storage

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.

Shipping conditions: Room Temperature

Storage conditions: MTX reagent & MTX Boost: -20°C, CombiMag: 4°C.

Shelf life: 1 year from the date of purchase

Kit contents

MTX2-0750 : 750 µL of MTX reagent + 250 µL CombiMag + 3 mL MTX Boost 100X

MTX2-1000 : 750 µL of MTX reagent + 250 µL CombiMag + 3 mL MTX Boost 100X

Certificate of analysis on demand.

Related Products

Ref	Description
#PP00200	PolyMag Premium transfection reagent
#PL00020	pVectOZ transfection plasmids – positive controls

Purchaser Notification | Conditions of Sale

This product is sold in accordance with our general conditions of sale that you can find on our website: <https://ozbiosciences.com/content/3-terms-and-conditions>.