Transfection reagent

# CombiMag™

Improve Transfection Efficiency of any reagents

# Protocol

Magnetofection Technology
This reagent needs to be used with a magnetic plate

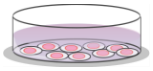
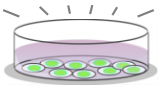
OZBIOSCIENCES
The art of delivery systems

# CombiMag Quick Protocol

To find the ideal conditions, CombiMag must be tested at ratio **1 µL/µg** DNA, 2 strategies can be used with CombiMag:

**1)** is to prepare a standard complex of DNA and a commercial transfection reagent according to the instructions of the manufacturer, followed by mixing with CombiMag (as shown below).

**2)** is to first mix DNA and CombiMag followed by immediate mixing with the transfection reagent. In this case, the manufacturer's instructions are used except that instead of DNA alone, a mixture of DNA and CombiMag is added to the transfection reagent.

| | |
|---|---|
| 1 | **Seed cells to be at 70% confluent the day of transfection***  |
| 2 | **Prepare 1 tube with 1µL of CombiMag per µg of DNA to be transfected**  |
| 3 | **Prepare your DNA/transfection reagent complexes according to the reagent's manufacturer instructions and immediately proceed to the next step***  Use serum-free medium or buffer* |
| 4 | **Mix the DNA/Transfectant complexes (step 3) to CombiMag (step 2)***  Use serum-free medium or buffer* |
| 5 | **Incubate 15 to 30 min at room temperature**  |
| 6 | **Distribute each mix drop by drop onto the cells to insure uniform distribution & then place the cells 20 min. on the magnetic plate**  |
| 7 | **Remove the cells from the magnetic plate and incubate cells for 24 to 72h at 37°C until evaluation of transgene expression***  |

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

- ✓ Depending on the transfection reagent used, the mixing order of components may influence the final efficiency of Magnetofection. We recommend starting with the classic protocol and change the order of addition of reagents based on the results.

- ✓ It is suggested to use 1 or 2 µL of CombiMag per µg of DNA in initial experiments. However, depending on the cell line to be transfected and the commercial transfection reagent used, the optimal composition may be found above or below this ratio.

- ✓ For cell lines, 24h before transfection seed the cells 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/CombiMag/Transfection reagent 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.

- ✓ During complex formation, prepare the DNA / transfection reagent complexes according to the reagent's manufacturer instructions, but omit the usual final incubation step after mixing DNA & reagent and immediately proceed to next step.

- ✓ For doses of CombiMag less than 1µL, dilute the reagent with deionized water.

- ✓ 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 6 h post-Magnetofection.

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

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Any questions?

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| | |
|---|---|
| Package content | **CM20100**: 100 µL of CombiMag reagent<br>**CM20200**: 200 µL of CombiMag reagent<br>**CM21000**: 1 mL of CombiMag reagent<br>**KC30200**: 100 µL of CombiMag reagent + 100µL of PolyMag Neo reagent + 100µL PolyMag reagent + Super Magnetic Plate<br>**MTX2-1000**: 250 µL of CombiMag reagent + 750 µL of MTX reagent + 3 mL of MTX Boost 100X |
| Shipping conditions | Room Temperature |
| Storage conditions | Store the CombiMag transfection reagent at +4°C upon reception |
| Shelf life | 1 year from the date of purchase when properly stored and handled |
| Product description | CombiMag Transfection Reagent is the only existing reagent based on the use of magnetic nanoparticles, for improving your transfection reagent efficiency. It has been designed to be employed in association with any commercial transfection reagent and can be used with all types of nucleic acids. |
| Important notice | For research use only. Not for use in diagnostic procedures |

## 1. Cell Preparation

It is recommended to seed the cells the day prior transfection. The suitable cell density will depend on the growth rate and the cells conditions. Cells should be 60-80% confluent at the time of Magnetofection (see the suggested cell number in the table 1). For suspension cells, use the specific protocol given below. Immediately preceding transfection, the medium can be replaced with fresh medium (optionally without serum) if necessary.

| Tissue Culture Dish | Cell Number | DNA Quantity (µg) | Transfection Volume |
|---|---|---|---|
| 96 well | 0.5 – 2 x $10^4$ | 0.1 – 0.5 | 200 µL |
| 24 well | 0.5 – 1 x $10^5$ | 0.5 - 2 | 500 µL |
| 6 well | 2 – 4 x $10^5$ | 2 - 6 | 2 mL |

Table1: Suggested adherent cell number and transfection volume

## 2. DNA/CombiMag complexes preparation

a. Add 1 µL of CombiMag per µg of DNA to be transfected to a microtube.

b. Prepare the DNA / transfection reagent complexes according to the reagent's manufacturer instructions and immediately proceed to next step.

c. Add the DNA / transfection reagent complex solution into the *CombiMag* solution and, mix gently by carefully pipetting up and down. Do not vortex or centrifuge.

d. Incubate 20 min at room temperature.

## 3. Transfection

a. Add the resulting mixture of DNA / transfection reagent / CombiMag onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

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

c. Remove the magnetic plate and 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).

**NOTES:**

- For some cells (primary cells such as neurons), a medium change at this step significantly improves the transfection efficiency and greatly minimizes potential cytotoxicity.
- In case of cells very sensitive to transfection, the medium can be changed right after the Magnetofection procedure: keep cells onto the magnetic plate and replace the transfection medium with fresh pre-warmed complete culture medium.

## 1. Cell Preparation

Suspension cells should be prepared in the adequate vessel just before transfection. The suitable cell density will depend on the growth rate and the cells condition (refer to Table 2).

| Culture vessel | Number of suspension cells (day of infection) | Transfection Volume |
|---|---|---|
| 96-well | 0.5 – 1 x $10^5$ | 200 µL |
| 24-well | 2 – 5 x $10^5$ | 500 µL |
| 6-well | 1 – 2 x $10^6$ | 2 mL |

Table 2: Recommended suspension cell number and transfection volume

## 2. DNA/CombiMag complexes preparation

a. Add 1 µL of CombiMag per µg of DNA to be transfected to a microtube.

b. Prepare the DNA / transfection reagent complexes according to the reagent's manufacturer instructions and immediately proceed to next step.

c. Add the DNA / transfection reagent complex solution into the *CombiMag* solution and mix by vigorous pipetting.

d. Incubate 20 min at room temperature.

## 3. Transfection

a. While DNA / transfection reagent / CombiMag complexes are incubating, dilute the cells to be transfected to 5 x $10^5$ - 1 x $10^6$ / mL in medium (with or without serum- or supplement; depending on cell type and sensitivity of cells towards serum-free conditions) and perform one of the following four 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 cells

   **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 2 h prior Magnetofection. The absence of serum allows some cells to adhere onto the plastic dish surface.

b. Add the resulting mixture of DNA / transfection reagent / CombiMag to the cells while keeping the cell culture plate on the magnetic plate.

c. Incubate for 15-20 min.

d. Remove the magnetic plate and 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).

## Protocol | stable transfection

This protocol can be used to produce stably transfected cells except that 48 h post transfection fresh medium containing the appropriate antibiotics are transferred to cells for selection. It is important to wait at least 48 h before exposing the transfected cells to selection media.

## Protocol Optimization

Several parameters can be optimized:

- Nucleic acid dose
- Ratio of *CombiMag* / Nucleic Acid
- Cell density
- Incubation time

1. Start by optimizing the ratio *CombiMag* / DNA or *CombiMag* / transfection reagent. To this end, use a fixed amount of DNA. Vary the amount of *CombiMag* from 0.25 to 5µL / µg of DNA.
2. Thereafter, change the nucleic acid dose with a fixed ratio of *CombiMag* / transfection reagent that has been previously optimized. For this purpose, you can perform a serial dilution of a preformed magnetic vector complex.
3. After having identified the correct quantity of *CombiMag*, nucleic acid, transfection reagent (commercial), you could pursue the process by optimizing the cell number as well as the incubation times for the complex formation and for the magnetic field application.

## Specific kits with CombiMag

- **Magnetofectamine O2 Starting Kit (contains CombiMag + MTX transfection reagent + MTX Boost)** for primary and hard-to-transfect cells.
  Increased transfection efficiency and minimized toxicity.

## Additional Magnetofection reagents for primary and hard-to-transfect cells experiments

- **SilenceMag** for siRNA transfection applications
- **NeuroMag** dedicated to neurons transfection
- **PolyMag/PolyMag Neo** polymer complex for all nucleic acids transfection
- **ViroMag/ViroMag RL/ AdenoMag** for enhancing viral transduction efficiency



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Rev. 06/2019