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Viral Applications

MAdenoMag™

Transduction reagent

Enhance adenoviral infection and transduction efficiency

Protocol

Magnetofection Technology

This reagent needs to be used with a magnetic plate

OZBIOSCIENCES

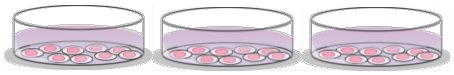
The art of delivery systems

AdenoMag Quick Protocol

To find the ideal conditions, **AdenoMag** must be tested at ratio **<u>1 to 4 µL/</u> 10^5 infectious adenoviral particles**. Adapt your MOI depending of your viral vector and the type of cells used.

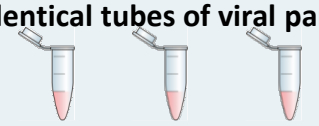
1

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



2

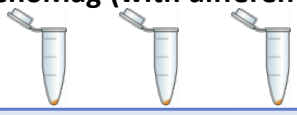
Prepare 3 identical tubes of viral particles (usually MOI 1)



96 well plate	24 well plate	6 well plate
For $1x10^4$ cells per well, dilute $1x10^4$ infectious units in 50µL serum-free medium or buffer* x3	For $1x10^5$ cells per well, dilute $1x10^5$ infectious units in 100µL serum-free medium or buffer* x3	For $5x10^5$ cells per well, dilute $5x10^5$ infectious units in 200µL serum-free medium or buffer* x3

3

Prepare 3 tubes of AdenoMag (with different amounts of magnetic beads)



96 well plate	24 well plate	6 well plate
0.1µL/0.2µL/0.4µL in an empty microtube	1µL/2µL/4µL in an empty microtube	5µL/10µL/20µL in an empty microtube

4

Mix each tube of viral particles (step 2) to each tube of AdenoMag (step 3)*

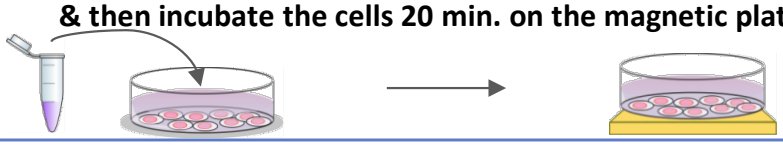
96 well plate			24 well plate			6 well plate		
Infectious units		*AdenoMag*	*Infectious units*		*AdenoMag*	*Infectious units*		*AdenoMag*
$1x10^4$	+	0.1µL	$1x10^5$	+	1µL	$5x10^5$	+	5µL
$1x10^4$	+	0.2µL	$1x10^5$	+	2µL	$5x10^5$	+	10µL
$1x10^4$	+	0.4µL	$1x10^5$	+	4µL	$5x10^5$	+	20µL

5

Incubate 15-20 min at room temperature or on ice

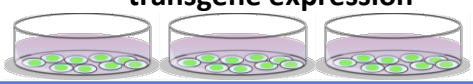
6

Distribute each mix drop by drop onto the cells to insure uniform distribution & then incubate the cells 20 min. on the magnetic plate



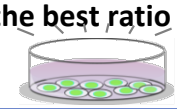
7

Remove the cells from the magnetic plate and incubate cells for 24 to 72h at 37°C until evaluation of transgene expression*



8

Choose the best ratio virus:AdenoMag



These conditions might require some further optimizations depending on your cells, virus types, MOI, 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.
- ✓ Adapt the MOI depending on the viral vector and the type of cells used. MOI can usually vary from 0.5 up to 100.
- ✓ Virus Preparation. Medium or buffer **without serum & supplement** must be used for the dilution of the virus and the preparation of the virus/AdenoMag complexes: DMEM or OptiMEM or salt-containing buffers such as HBS or PBS are recommended. Alternatively, you can directly use an aliquot of the culture supernatant from a producer cell line.
- ✓ For doses of AdenoMag less than 1µL, dilute the reagent exclusively with deionized water.
- ✓ We recommend respecting the order of addition; add the virus suspension into the AdenoMag tube.
- ✓ For most cell types, a medium change is not required after Magnetofection. However, it may be necessary for some cells that are sensitive to serum/supplement concentration or for transduction synchronization. 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.

IMPORTANT NOTES

- Do not freeze the magnetic nanoparticles!
- Polybrene or other additives must NOT be used in combination with AdenoMag
- 10 to 20 µL of Adenomag magnetic nanoparticles have been found to bind 10^6 **infectious** adenoviral particles.
- **The suggested volume of AdenoMag is related to infectious particles and not physical viral particles.** AdenoMag is designed to enhance infection efficiency, thus it is recommended to start with low MOI from 0.5 to 10 with several AdenoMag volumes.

AdenoMag Reagent | Specifications

Package content	AM70100: 100 µL of AdenoMag AM70200: 200 µL of AdenoMag AM71000: 1mL of AdenoMag KC30900: AdenoMag Starting Kit - 200 µL of AdenoMag reagent + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the AdenoMag reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled.
Product Descriptions	Adenomag is a magnetic nanoparticles formulation specifically dedicated to enhance Adenovirus and Adeno-Associated Virus (AAV) infection and transduction capacities.
Important notice	For research use only. Not for use in diagnostic procedures.

1. Cell Preparation

It is recommended to plate the cells the day prior transduction. Suspension cells should be prepared in the adequate vessel just before the infection (see below for specific protocol). The suitable cell density will depend on the growth rate and the cells condition. Best results are achieved if cells are at least 60-80 % confluent at the time of Magnetofection (refer to Table 1).

Culture vessel	Number of adherent cells (day of infection)	Final Transduction Volume*
96-well	0.05 – 0.15 x 10^5	150 µL
24-well	0.5 – 1 x 10^5	500 µL
6-well	2 – 5 x 10^5	2 mL

*Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the AdenoMag/virus mixture.

Table 1: Recommended cell number

2. Viral particles/AdenoMag complexes preparation

a. *AdenoMag*: Vortex the reagent and place the appropriate amounts (refer to Table 2) in an empty microtube.

Infectious adenoviral particles	AdenoMag Quantity (µL)
10^3	0.02
10^4	0.2
10^5	2
10^6	20
10^7	200

Table 2: Suggested AdenoMag volume depending on infection particles number

b. *Viral particles solution*: Add your virus suspension to the tube containing AdenoMag and mix immediately by pipetting up & down.
NOTE: Prefer adenovirus solutions made in serum-free medium or salt-containing buffers.

c. Incubate at room temperature for 15 to 20 minutes.

3. Transduction

a. Add the AdenoMag / Adenovirus complexes 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 minutes.

c. Remove the magnetic plate

d. 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 transduction or infection, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium.

1. Cell Preparation

Suspension cells should be prepared in the adequate vessel just before the infection. The suitable cell density will depend on the growth rate and the cells condition (refer to Table 3).

Culture vessel	Number of suspension cells (day of infection)	Final Transduction Volume*
96-well	0.5 – 1 x 10^5	150 µL
24-well	2 – 5 x 10^5	500 µL
6-well	1 – 2 x 10^6	2 mL

*Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the AdenoMag/virus mixture.

Table 3: Recommended suspension cell number

2. Viral particles/AdenoMag complexes preparation

a. *AdenoMag*: Vortex the reagent and place the appropriate amounts (see Table 2) in an empty microtube.

b. *Viral particles solution*: Add your virus suspension to the tube containing AdenoMag and mix immediately by pipetting up & down.
NOTE: Prefer adenovirus solutions made in serum-free medium or salt-containing buffers.

c. Incubate at room temperature for 15 to 20 minutes.

NOTES:

- If required, dilute the aliquot of your adenovirus preparation to be used for transduction with serum-free cell culture medium or another salt-containing buffer (HBS, PBS). Alternatively, you can directly use an aliquot of culture supernatant from a producer cell line.
- The ratios adenovirus / should be adjusted according to the viral titers and cell types used.

3. Transduction

a. While the AdenoMag / adenovirus mixtures incubate (step 2 above), prepare the cells to be transduced (as suggested in Table 3).

For example, dilute the cells to 5 x 10^5 - 1 x 10^6 / mL in culture medium and 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.

- Seed the cells on polylysine-coated plates and use the protocol for adherent cells,
  OR
- Briefly, centrifuge the cells (2 minutes) to pellet them and use the protocol for adherent cells
  OR
- Mix cell suspension with 20-30 µL of CombiMag reagent (Magnetofection) per 1 ml of cell suspension and incubate for 10 - 15 minutes. Then, distribute the cells to your tissue culture dish placed upon the magnetic plate and incubate for 15 more minutes.

b. Add the resulting mixture of AdenoMag / adenovirus to the cells while keeping the cell culture plate on the magnetic plate and incubate for 25 minutes.

c. Remove culture plate from magnetic plate.

d. Then, cultivate the cells as desired until evaluation of the transduction experiment.

Optimization Protocol

In order to get the best out of AdenoMag, several parameters can be optimized:

- AdenoMag dose & ratio to viral particles
- Cell density and incubation time

1. Start by optimizing the AdenoMag dose with a fixed MOI. This will vary the concentration of AdenoMag and the ratio AdenoMag / Virus. To this end, vary the amount of AdenoMag in the range suggested in the Table 4. For instance, for a MOI of 1: from 0.05 to 0.4µL of AdenoMag in a 96-well plate.

2. Next, use a fixed volume of reagent and vary the MOI.

3. Finally, you can optimize the cell number (density), kinetics of readout and also the incubation time for the magnetofection procedure

Culture Vessel	Adenoviral Infectious Particles	Suggested AdenoMag Quantity (µL)	Volume of AdenoMag for optimization	Final Transduction Volume*
96 well	0.05 – 0.15 x 10^5	0.1 – 0.3 µL	0.05 / 0.1 / 0.2 / 0.3 / 0.4 µL	150 µL
24 well	0.5 – 1 x 10^5	1 – 2 µL	0.5 / 1 / 1.5 / 2 / 3 µL	500 µL
6 well	2 – 5 x 10^5	4 – 10 µL	2 / 4 / 6 / 10 / 12 µL	2 mL

*Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the AdenoMag/adenovirus mixture

Table 4: Recommended optimization conditions for a MOI of 1

Additional products for your transduction experiments

- **Adenoblast** - Transduction enhancer
- **Mag4C-Ad** for adenovirus capture and concentration

Purchaser Notification

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