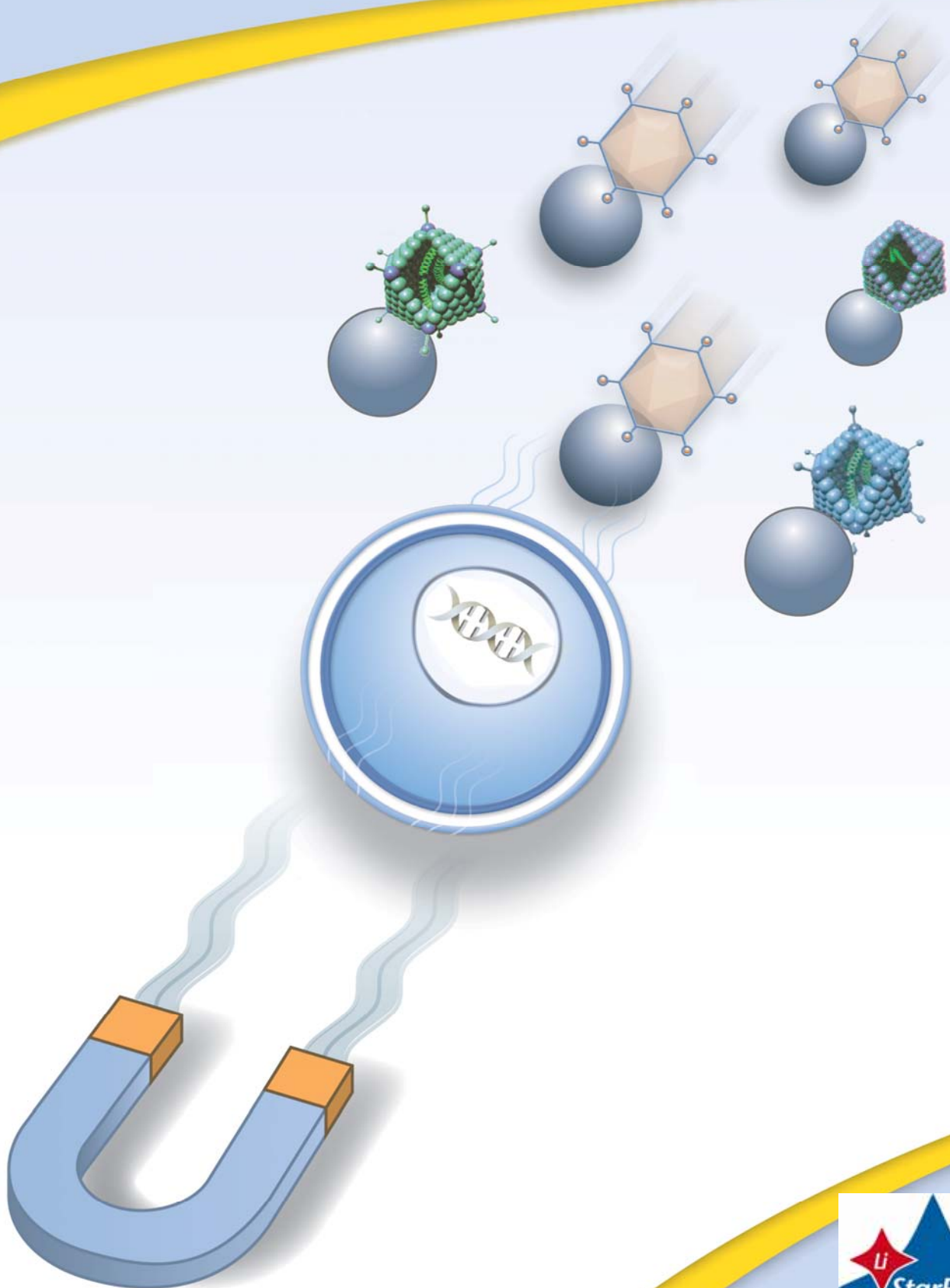


Magnetofection™ - AdenoMag

INSTRUCTION MANUAL



AdenoMag is an enhanced reagent based on the Magnetofection™ technology specifically designed for Adenovirus & Adeno-Associated Virus.

List of AdenoMag Kits

| Catalog Number | Description | Volume (µL) | Number of transductions / 24 well plates* | Number of transductions / 96 well plates** |
|----------------|--|-------------|---|--|
| AM70100 | AdenoMag 100 | 100 | 50-100 | 500-1000 |
| AM70200 | AdenoMag 200 | 200 | 100-200 | 1000-2000 |
| AM71000 | AdenoMag 1000 | 1000 | 500-1000 | 5000-10000 |
| KC30900 | AdenoMag Starting Kit ¹ | 200 | 100-200 | 1000-2000 |
| KC30996 | AdenoMag Starting Kit ² | 200 | 100-200 | 1000-2000 |
| KC30600 | ViroMag Triple Starting Kit ³ | 3 x 100 | 150-300 | 1500-3000 |
| KC30696 | ViroMag Triple Starting Kit ⁴ | 3 x 100 | 150-300 | 1500-3000 |
| KM30500 | ViroMag Selection Kit ⁵ | 3 x 100 | 150-300 | 1500-3000 |
| MF10096 | Magnetic Plate with 96-magnets | - | | |
| MF10000 | Super Magnetic Plate | - | | |
| MF14000 | Mega Magnetic Plate | - | | |

¹ Contains 1 vial of AdenoMag AM70200 and a Super Magnetic Plate MF10000

² Contains 1 vial of AdenoMag AM70200 and a Magnetic Plate with 96-magnets MF10096

³ Contains 1 vial of ViroMag VM40100, 1 vial of ViroMag R/L RL40100, 1 vial of AdenoMag AM70100 and a Super Magnetic Plate MF10000

⁴ Contains 1 ViroMag vial VM40100, 1 ViroMag R/L vial RL40100, 1 AdenoMag vial AM70100 and a Magnetic Plate with 96-magnets MF10096

⁵ Contains 1 vial of ViroMag VM40100, 1 vial ViroMag R/L RL40100 and 1 vial of AdenoMag AM70100

* Based on MOI of 1 for 10⁵ cells/well

** Based on MOI of 1 for 10⁴ cells/well

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

1. Technology

1.1. Description

Congratulations on your purchase of the **AdenoMag** reagent!

AdenoMag is an optimized specific nanoparticles formulation issued from our Magnetofection™ technology, specially designed to be used in association with all Adenoviral and Adenoviral-Associated Virus (AAV) vectors. This reagent allows scientists to increase transduction efficiency, infect non permissive cells, concentrate virus onto cells or in culture medium and accelerate infection process or synchronize adsorption, without modification of the adenoviruses, just by associating **AdenoMag** reagents to the viral vectors. Moreover, AdenoMag leads to higher Adenoviral and AAV infection efficiency with lower multiplicity of infection (MOI) or number of plaque forming units (PFU) per mL. **AdenoMag** is the only reagent available offering a solution for such applications.

Magnetofection™ is a novel, simple and highly efficient viral and non-viral gene delivery method. It exploits magnetic force exerted upon gene vectors associated with magnetic particles to drive the nucleic acids or virus towards and into the target cells. In this manner, the complete applied nucleic acid and viral dose gets concentrated onto cells within a few minutes so that 100% of the cells get in contact with a significant vector dose. **AdenoMag** is an exclusive and specific reagent dedicated to Adenoviral and AAV applications. This reagent demonstrates an exceptionally high efficiency to promote, control and assist viral transductions.

AdenoMag is dedicated to Adenovirus and AAV and presents unique properties allowing to:

1. Increase transduction efficiency in terms of percentage of transduced cells and transgene expression
2. Concentrate viruses onto cells very rapidly
3. Accelerate the transduction process
4. Infect non permissive cells
5. Significantly improve virus infectivity with extremely low vector doses
6. Synchronize cell adsorption / infection
7. Target / confine transduction to specific area (magnetic targeting)

Based upon a validated and recognized magnetic drug targeting technology this innovative method is:

- Highly Efficient
- Suitable for all Adenoviruses and AAV Serotypes
- Economical, Simple & Rapid
- Universal (primary cells, hard-to-transfect cells, cell lines and non-permissive cells)
- Serum compatible & Non toxic
- Amenable to high throughput automation

1.2. Kit Contents

Kit contents vary according to their size:

- 1 tube containing 0.1 mL of **AdenoMag** suitable for 50 - 100 assays in a 24-well plate (for a MOI of 1).
- 1 tube containing 0.2 mL of **AdenoMag** suitable for 100 to 200 assays in a 24-well plate (for a MOI of 1).
- 1 tube containing 1 mL of **AdenoMag** suitable for 500 to 1000 assays in a 24-well plate (for a MOI of 1).

Stability and Storage

Storage +4°C. Upon receipt and for long-term use, store all tubes in the fridge. Magnetofection kits are stable for at least one year at the recommended storage temperature.

- **DO NOT FREEZE THE MAGNETIC NANOPARTICLES!**
- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF MAGNETIC NANOPARTICLES!**

Shipping condition: Room Temperature

2. Applications

2.1. Cell Types

AdenoMag is applicable on numerous cell types. This technology has been tested successfully on a variety of cells.

| <i>Cell Line</i> | <i>Cell Type</i> | <i>Source</i> |
|----------------------------|-------------------------------|---------------|
| 181RDB | Pancreatic | Human |
| 293, HEK-293, 293-T, -EBNA | Transformed embryonic kidney | Human |
| C6 | Glioma cells | Rat |
| CHO-K1 | Epithelial-like (ovary) | Hamster |
| COS-7 | Fibroblast (kidney) | Green Monkey |
| HeLa | Cervical epithelial carcinoma | Human |
| HMEC-1 | Endothelial cells | Human |
| NIH3T3 | Fibroblasts | Mouse |
| RAW | Macrophages | Mouse |

2.2. Virus Types

AdenoMag reagent can usually be combined with any Adenoviruses or AAV.

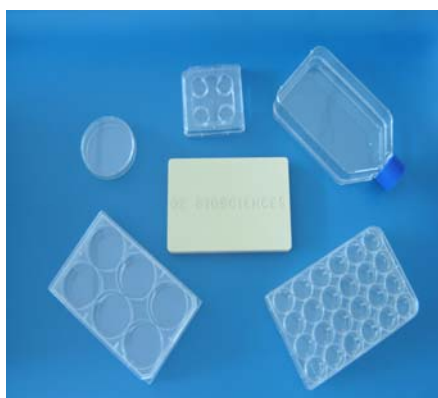
| <i>Virus Type</i> | <i>Virus name</i> | <i>Application</i> |
|-----------------------------|-------------------|--|
| Adenovirus | Ad5-LacZ, Ad5-GFP | Increase transduction, infect non permissive cells |
| Oncolytic adenovirus | Ad520 | Concentrate and boost oncolytic activity |
| AAV serotype 6 | AAV6-PGK1-GFP | Increase transduction, infect non permissive cells |

3. Magnetofection™ Apparatus

As for all Magnetofection™ reagents, **AdenoMag** requires appropriate magnetic fields. Magnetic plates especially designed for Magnetofection are provided to exert these specific magnetic fields. Their special geometry produces strong magnetic fields that are suitable for all cell culture dishes (T-75 flasks, 60 & 100 mm dishes, 6-, 12- 24-, 48- and 96-well plates). Two standard magnetic plates: 96-Magnet plate and Super Magnetic plate and one Mega Magnetic plate are available. Super Magnetic plate suits for all cell culture supports and Mega Magnetic plate is designed to hold up to 4 culture dishes. 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.



96-Magnet Plate



Super Magnetic Plate



Mega Magnetic Plate

4. Protocol

4.1. General Considerations

The instructions given below represent example protocols that were applied successfully with a variety of cells and adenoviruses. Our R&D team has tested and optimized the **AdenoMag** reagent in order to provide you with the most straightforward and efficient procedure. Therefore, we recommend you to start by following our general protocol as guidelines to obtain good data rapidly and if necessary, we advise you to optimize the experimental condition parameters in order to achieve the best effects. Optimal conditions do vary from cell to cell and are highly dependent upon the type of adenovirus used, its titer, the composition of the viral solution, and cell culture conditions. Consequently, the amount, concentration and ratio of the individual components (adenovirus and AdenoMag), the time course and the number of cells may have to be adjusted to get the best results. Several optimization protocols are available in the Appendix.

4.2. Cell Culture

It is recommended to plate the cells the day prior transduction, however cells can also be prepared few hours before infection. 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 (see the suggested cell number in the table 1).

Table 1: Recommended cell number.

| Culture vessel | Number of adherent cells (day of infection) | Number of suspension cells (day of infection) | Final Transduction Volume* |
|------------------|--|--|-------------------------------|
| 96-well | 0.05 – 0.15 x 10 ⁵ | 0.5 – 1 x 10 ⁵ | 150 µL |
| 24-well | 0.5 – 1 x 10 ⁵ | 2 – 5 x 10 ⁵ | 500 µL |
| 12-well | 1 – 2 x 10 ⁵ | 2.5 – 10 x 10 ⁵ | 1 mL |
| 6-well | 2 – 5 x 10 ⁵ | 1 – 2 x 10 ⁶ | 2 mL |
| 60 mm dish | 5 – 10 x 10 ⁵ | 2.5 – 5 x 10 ⁶ | 4 mL |
| 90 – 100 mm dish | 15 – 30 x 10 ⁵ | 5 – 10 x 10 ⁶ | 8 mL |
| T-25 flask | 5 – 10 x 10 ⁵ | 2.5 – 5 x 10 ⁶ | 5 mL |
| T-75 flask | 20 – 50 x 10 ⁵ | 5 – 15 x 10 ⁶ | 10 mL |

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

As described in the following procedure, it is preferable to prepare the adenovirus / **AdenoMag** mixture in medium without serum and supplement or in physiological saline (formation of complexes in serum containing medium is also feasible). These mixtures, prepared in serum-free conditions or saline buffer, are then added to the cells that are cultured with complete medium. Therefore, the addition of this cocktail will result in the dilution of supplements such as serum, antibiotics or other additives of your standard culture medium. Although a medium changed after Magnetofection is not required for most cell types, it may be necessary for cells that are sensitive to serum/supplement concentration.

4.3. AdenoMag Procedure

Viral Magnetofection is carried out in the same manner as standard transductions with the following exceptions:

- Adenovirus preparations are mixed with **AdenoMag** prior to transduction
- Cell culture plate is positioned upon the magnetic plate for 20-30 minutes before incubation
- Polybrene or other additives must NOT be used in combination with AdenoMag.

The protocol is straightforward. For instance, 10 to 20 µL of **AdenoMag** magnetic particles have been found sufficient to bind 1x10⁶ infectious adenoviral particles. Thus, the particle amounts listed in **Table 2** will be mostly sufficient to bind virus doses which are usually applied in transduction experiments. Depending on the viral vector type, the quantity of virus and the cell type used, this protocol would have to be adjusted (see appendix for optimization protocol).

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.

- 1) Plate the cells the day before infection or just before infection in your appropriate culture dish as suggested in Table 1.
- 2) Add a suitable amount of **AdenoMag** (see table 2) in a tube large enough to contain the volume of virus preparation added in step 3. For small volume (< 1µL) **AdenoMag** can only be diluted with deionized water. The amount of **AdenoMag** depends on the type and dose of virus used. As a starting point, the “suggested AdenoMag quantity” indicated in the table 2 can be used. In order to get the best efficiency AdenoMag should be optimized (section 5.2 – optimization protocol).
- 3) Add your virus preparation to the tube(s) containing **AdenoMag** and mix immediately by pipetting up and down. Adenovirus solution made in serum free medium or salt-containing buffers are preferable.

Note 1: If required, dilute the aliquot of your adenovirus preparation to be used for transduction with serum-free cell culture medium or other salt-containing buffer (HBS, PBS). Alternatively, you can directly use an aliquot of culture supernatant from a producer cell line.

Note 2: The ratios adenovirus / **AdenoMag** should be adjusted according to the viral titers and cell types used.

Table 2 Recommended volume of **AdenoMag** depending on the infectious adenoviral particles number

| Infectious Adenoviral Particles | AdenoMag Quantity (µL) “Starting Point” | AdenoMag Quantity (µL) Suggested range of testing |
|---------------------------------|---|---|
| 10 ³ | 0.02 µL | 0.01 µL – 0.04 µL |
| 10 ⁴ | 0.2 µL | 0.1 µL – 0.4 µL |
| 10 ⁵ | 2 µL | 1 µL – 4 µL |
| 10 ⁶ | 20 µL | 10 µL – 30 µL |
| 10 ⁷ | 200 µL | 100 µL – 300 µL |

- 4) Incubate 15 to 25 minutes at room temperature.
- 5) Add the **AdenoMag** / adenovirus mixture dropwise to the cells to be transduced.
- 6) Place the cell culture plate upon the magnetic plate for 20-30 minutes. Longer incubation time (30 to 60 minutes) or shorter (5 to 15 minutes for synchronization) can also be used. Optionally, after this incubation, a medium change can be performed while maintaining the magnetic plate under the cell culture.
- 7) Remove the magnetic plate and cultivate the cells under standard conditions until evaluation of the transduction experiment. Optionally a medium change can be performed after 24 hours.

4.4. Suspension Cells Protocol

- 1) The composition and preparation of **AdenoMag** / adenovirus mixtures are performed exactly as described above from steps 1 to 4 (section 4.3 above).
- 2) While the **AdenoMag** / adenovirus mixtures incubate (step 4 above), prepare the cells to be transduced (as suggested in Table 1). For example, dilute the cells to 5 x 10⁵ - 1 x 10⁶ / 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.
 - a. Seed the cells on polylysine-coated plates and use the protocol for adherent cells, **OR**
 - b. Briefly, centrifuge the cells (2 minutes) to pellet them and use the protocol for adherent cells, **OR**
 - c. 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.
- 3) 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.

- 4) Remove culture plate from magnetic plate.
- 5) Then, cultivate the cells as desired until evaluation of the transduction experiment.

4.5. Example of straightforward protocol

The following protocol is suitable for various cell lines (NIH-3T3, C6, COS7, HeLa etc.) in a 24 well-plate infected with an Ad-GFP at a MOI of 1.

- 1) Plate 4×10^4 cells the day before infection in a 400 μ L volume. This should lead to about 8×10^4 cells the day of infection.
- 2) Add 1.6 μ L of AdenoMag in 50 μ L of DMEM without serum.
- 3) Dilute 8×10^4 infectious adenoviral particles in 50 μ L of serum free DMEM.
- 4) Add the adenovirus solution to the AdenoMag solution and incubate at room temperature for 25 min.
- 5) Add these 100 μ L drop wise to the cell culture (total transduction volume: 400 μ L of culture medium plus 100 μ L of complexes).
- 6) Place the cell culture upon the magnetic plate and incubate for 25 min.
- 7) Remove the magnetic plate, cultivate under standard conditions and analyze GFP expression.

Table 3: Successful examples of **AdenoMag** experimental procedure with Ad5-GFP and Ad5-LacZ

| Cell types (1×10^5 cells) | Titer | AdenoMag Quantity (μ L) | Culture Vessel |
|--|-------|---------------------------------|-------------------|
| C6 | MOI 1 | 2 μ L | 24 well |
| NIH-3T3 | MOI 1 | 1 μ L | 24 well |
| COS 7 | MOI 1 | 2 μ L | 24 well |
| CHO | MOI 1 | 1 μ L | 24 well |
| HMEC-1 | MOI 1 | 1.5 μ L | 24 well |
| RAW | MOI 1 | 1 μ L | 24 well |

5. Appendix

5.1. Critical Parameters for best performance

- 1) Cell culture conditions: Best results are achieved when cells are 60–80 % confluent at the time of the transduction. If necessary, you can wash the culture medium containing the transduction mixture after 8-24 hours and replace it by fresh medium.
- 2) AdenoMag quantity. We often observed good effects at very low doses of AdenoMag (0.8 – 2 μ L / well for a 24-well plate). However the efficiency may depend on the cell line and the virus type used. Consequently, we suggest you to start by testing a range of AdenoMag volumes in order to obtain the best experimental conditions.
- 3) Adenovirus purity and quality. AdenoMag has been designed to work with culture supernatant-produced adenovirus. However to gain a better efficiency, Adenoviruses can be purified and/or concentrated by CsCl density gradient or bed chromatography before mixing with AdenoMag. To be noted: plasmid expression or gene silencing efficiencies will highly depend on adenovirus construction and on the type of promoter.
- 4) Time course. The infection time course depends on the amount/concentration of virus used. Indeed, longer incubation under the magnetic field is required with very low viral titers whereas with high viral dose short incubation times are sufficient. We recommend monitoring infection 24 to 96 hours post-infection.

5.2. Protocol Optimization

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

- AdenoMag dose & ratio of AdenoMag to Virus
- Cell density and incubation time

OZ Biosciences team has investigated numerous factors. Based on our experience, we recommend that you optimize one parameter at a time and start from the experimental procedures described above (section 4).

- 1) Start by optimizing the **AdenoMag** dose with a **fixed amount of adenovirus**. 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 0.4 μ L of AdenoMag in a 96-well plate.
- 2) Next, you can inverse the procedure by optimizing the dose of virus with a **fixed amount of reagent**.
- 3) After having identified the correct quantity of **AdenoMag** and virus, you could pursue the process by optimizing the **cell number** (density) and **time course of incubation**, between AdenoMag and viruses (section 4.3.4) and under the magnetic field (section 4.3.6).

Table 4: Recommended optimization conditions for a MOI of 1:

| Culture Vessel | Adenoviral Infectious Particles | Suggested AdenoMag Quantity (μ L) | Volume of AdenoMag for optimization | Final Transduction Volume* |
|----------------|---------------------------------|--|--------------------------------------|----------------------------|
| 96 well | 0.05 – 0.15 x 10 ⁵ | 0.1 – 0.3 μ L | 0.05 / 0.1 / 0.2 / 0.3 / 0.4 μ L | 150 μ L |
| 24 well | 0.5 – 1 x 10 ⁵ | 1 – 2 μ L | 0.5 / 1 / 1.5 / 2 / 3 μ L | 500 μ L |
| 12 well | 1 – 2 x 10 ⁵ | 2 – 4 μ L | 1 / 2 / 3 / 4 / 6 μ L | 1 mL |
| 6 well | 2 – 5 x 10 ⁵ | 4 – 10 μ L | 2 / 4 / 6 / 10 / 12 μ L | 2 mL |
| 60 mm dish | 5 – 10 x 10 ⁵ | 10 – 20 μ L | 5 / 10 / 15 / 20 / 30 μ L | 4 mL |
| 90-100 mm dish | 15 – 30 x 10 ⁵ | 30 – 60 μ L | 15 / 30 / 45 / 60 / 90 μ L | 8 mL |
| T-25 flask | 5 – 10 x 10 ⁵ | 10 – 20 μ L | 5 / 10 / 15 / 20 / 30 μ L | 5 mL |

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

5.3. "Troubleshooting"

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

| Problems | Comments and Suggestions |
|-----------------------------|--|
| Low transduction efficiency | <ol style="list-style-type: none"> 1. Adenovirus titer. Use higher titers of adenovirus with the recommended or optimized AdenoMag/adenovirus ratio (see table 4). 2. AdenoMag / Adenovirus ratio. Optimize the reagent/adenovirus ratio by using a fixed MOI of Adenovirus and vary the amount of AdenoMag as suggested in table 4. 3. Cell density. A non-optimal cell density at the time of transfection can lead to poor efficiency. The optimal confluency should range from 50 to 70% (true confluency, corresponding to 90% visual confluency) but most favorable cell density may vary according to the cell type; preferably mid-log growth phase. 4. Adenovirus quality. Even though AdenoMag has been designed to work with cell supernatants containing viral particles, for the best efficiency, Adenoviruses should be as pure as possible. |

| | |
|--|---|
| | <ol style="list-style-type: none"> 5. Type of promoter. Ensure that the promoter can be expressed by the cells to be infected. Another viral-driven promoter can be used as a control. 6. Cell condition. Use freshly thawed cells that have been passaged at least once. Cells should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) can alter the transduction efficiency. 7. Medium used for preparing AdenoMag/Adenovirus complexes. It is better to use serum-free medium or buffer (HBS, PBS) during preparation of the complexes. 8. Cell culture medium composition. For some cells, the infection efficiency can be increased without serum or under reduced serum condition. Thus, infect these cells in serum-free medium during the first 4-5h of incubation. 9. Incubation time and transduction volume. 1) The optimal time range between transduction and assay varies with cells, promoter, expression product, etc... The transduction efficiency can be monitored after 24 – 96h by analyzing the gene product. Several reporter genes can be used to quantitatively monitor gene expression kinetics. 2) To increase transduction efficiency, transfection volume suggested can be reduced for the first 24 hours. 10. Old AdenoMag / adenovirus complexes. The AdenoMag / adenovirus complexes must be freshly prepared every time. Complexes prepared and stored for longer than 1 hour can be aggregated. 11. Transgene detection assay. Ensure that your post-transduction assay is properly set up and includes a positive control. 12. Reagents temperature. Reagents should have an ambient temperature and be vortexed prior to use. 13. Reagents storage. AdenoMag should be stored at +4°C. |
| Low infection Level | <ol style="list-style-type: none"> 1. Adenoviral storage. Adenoviral stocks should be properly conserved at -80°C. Freeze/thaw cycles reduce viral titers and thus, must be avoided. Use the adenovirus solution within hours after thawing. Diluted adenoviral preparation shouldn't be conserved for further assay. 2. Multiplicity of Infection (MOI). Be sure that the MOI is calculated for infectious particles and not for physical particles. There could be a 1/10 to 1/1000 ratio between physical and infectious particles. |
| Cellular toxicity | <ol style="list-style-type: none"> 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. Infection is toxic. Most of the adenoviruses used are not replicative. Be sure that the cell line doesn't express the missing region for replication. It should be noted that even if adenovirus can't replicate into the cells, it can still express viral proteins that can be toxic and cause cytopathic effect. Oncolytic viruses kill cells. 3. Adenovirus quality – Presence of contaminants. Ensure that adenovirus is pure and contaminant-free. Contaminants can lead to cell death. Adenovirus from cellular supernatants can contain cellular contaminants toxic for the targeted cells. 4. Concentration of AdenoMag / adenovirus too high. Decrease the amount of adenovirus / AdenoMag complexes added to the cells by lowering the adenovirus or the reagent concentration. Complexes aggregation can cause some toxicity; prepare freshly and adjust the ratio as outlined previously. 5. Incubation time. Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4 h to 24 h. 6. Key gene silencing. If the targeted gene is essential for cell survival or if a key gene is non-specifically silenced by the shRNA, this can lead to cell death. |
| No or weak gene silencing effect (adenovirus encoding shRNA) | <ol style="list-style-type: none"> 1. shRNA design. The design of an efficient shRNA is a crucial step. Ensure to use a validated shRNA sequence encoded in the expression vector. If a validated shRNA cannot be used, assay your sequence in an easy to transfect cell line (if possible) in order to validate. 2. Incubation time. Perform a time-course experiment to set up the optimal incubation time since gene silencing is dependent on the gene expression and the protein turnover rate. 3. Medium used for preparing AdenoMag/Adenovirus complexes. It is preferable to use serum-free medium of buffer (HBS, PBS) during the complexes preparation. |

5.4. Quality Controls

To assure the performance of each batch of **AdenoMag** produced, we qualify each lot using rigorous standards. *In vitro* assays are conducted to qualify the quality and activity of each kit component.

| Components | Standard Quality Controls |
|-----------------------|--|
| AdenoMag | <ol style="list-style-type: none"> 1. Quality and size homogeneity of the magnetic nanoparticles. 2. Stability of the magnetic nanoparticles formulations. 3. AdenoMag transduction efficacies with a recombinant adenovirus on NIH-3T3 or HeLa cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot. 4. Sterility. Thioglycolate assay: absence of fungal and bacterial contamination shall be obtained for 14 days. |
| Magnetic Plate | <ol style="list-style-type: none"> 5. Tests of solidity and Test of the magnetic field force. |

6. Related Products

| Description | Reference |
|--|-----------|
| Magnetofection Technology | |
| Mega Magnetic Plate | MF14000 |
| Super Magnetic Plate | MF10000 |
| Magnetic Plate 96-magnets | MF10096 |
| PolyMag 1mL (<i>for all nucleic acids</i>) | PN31000 |
| PolyMag Neo 1mL (<i>for all nucleic acids</i>) | PG61000 |
| CombiMag 1mL (<i>to boost transfection reagent</i>) | CM21000 |
| SilenceMag 1mL (<i>for siRNA application</i>) | SM11000 |
| NeuroMag 1mL (<i>for transfection of neurons</i>) | NM51000 |
| ViroMag 1mL (<i>for all viral applications</i>) | VM41000 |
| ViroMag R/L 1mL (<i>for retrovirus and Lentivirus</i>) | RL41000 |
| SelfMag Amino Kit | SA10000 |
| SelfMag Carboxy Kit | SC20000 |
| FluoMag-P 100µL | FP10100 |
| FluoMag-C 100µL | FC10100 |
| FluoMag-S 100µL | FS10100 |
| FluoMag-V 100µL | FV10100 |
| Protein Delivery Systems | |
| Ab-DeliverIN 1 mL | AI21000 |
| Pro-DeliverIN 1 mL | PI11000 |
| Tee-Technology (lipid-based reagents) | |
| Lullaby siRNA transfection reagent | LL71000 |
| DreamFect Gold Transfection reagent 1mL | DG81000 |
| DreamFect Transfection reagent 1mL | DF41000 |
| VeroFect Transfection Reagent 1mL | VF61000 |
| FlyFectin Transfection Reagent 1mL | FF51000 |
| Gene & Protein Tools | |
| Bradford – Protein Assay Kit | BA00100 |
| GeneBlaster selection kit | GB20010 |
| β-Galactosidase (ONPG) assay kits | GO10001 |
| β-Galactosidase (CPRG) assay kits | GC10002 |
| X-Gal Staining Kit | GX10003 |

Limited License

The purchase of the AdenoMag and other Magnetofection™ Reagents 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 and transduction of nucleic acids and virus 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 AdenoMag and other Magnetofection™ Reagents. 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 AdenoMag and other Magnetofection™ Reagents material and documentation to OZ Biosciences, or by destroying all AdenoMag and other Magnetofection™ Reagents components. Purchasers are advised to contact OZ Biosciences with the notification that a AdenoMag and other Magnetofection™ Reagents 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 AdenoMag and other Magnetofection™ Reagents 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.

Product Use Limitations

The AdenoMag and other Magnetofection™ Reagents 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.

For more information, or for any comments on the terms and conditions of this License, please contact:



Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it