Magnet Assisted Transfection (MATra)

A comprehensive manual

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1 Introduction

1.1 General considerations

Magnet assisted Transfection (MATra) is a new, easy-to-handle and highly efficient method to transfect cells in culture. Using this new technique nucleic acids, such as plasmid DNA, oligonucleotides or siRNA, are in a first step associated with magnetic particles. Exploiting magnetic force the full nucleic acid dose is then rapidly drawn towards and delivered into the target cells leading to efficient transfection. For MATra, cells must adhere to the bottom of the culture vessels which makes special precautions necessary for suspension cells.

Three different procedures according to different initial situations can be followed to apply MATra:

1. MATra for adherent cells (MATra-A)

In this case, the nucleic acid has to be combined to the MATra-A Reagent only and MATra can be performed (s. 3.2).

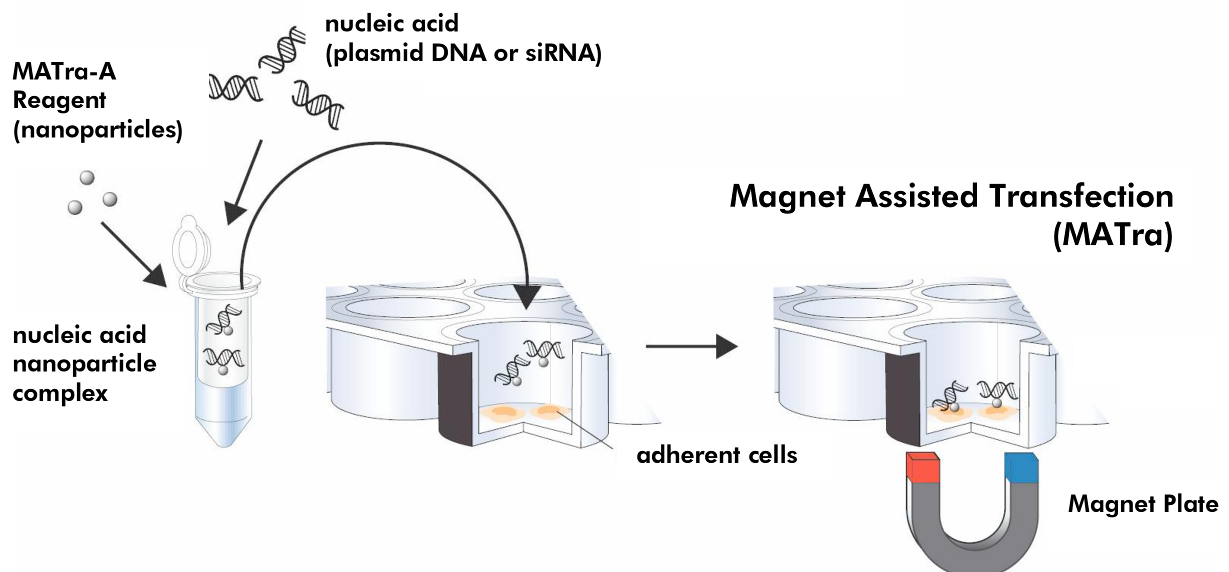
2. MATra for suspension cells (MATra-S)

In this case, cells have to be made adherent first by incubating them with the magnetic reagent MATra-S Immobilizer or by using polylysine plates. Then MATra-A Reagent loaded with the nucleic acid can be applied and MATra can be performed (s. 3.2).

3. MATra in combination with standard transfection reagents (MA-Lipofection)

Transfection with common lipidic (IBAfect, Lipofectamine, Fugene, Geneporter) or polycationic (e.g ExGen500 or Superfect) reagents can be enhanced by magnetic assistance. In this case, the nucleic acid to be transfected has to be combined with MA Lipofection Enhancer in the presence of the common transfection reagent (s. 3.2). The formulation of MA Lipofection Enhancer has been optimized for use with IBAfect.

1.2 Principle of MATra



Exploiting magnetic force the full nucleic acid dose is rapidly drawn towards and delivered into the target cells leading to efficient transfection

2 Necessary materials and reagents for MATra

For adherent cells
Nucleic acid in serum free and supplement free medium (e.g. DMEM)
MATra-A Reagent
Magnet Plate: optionally

- Universal Magnet Plate, 8x13 cm (for 1 plate or flask up to 75 cm^2) or
- Universal Magnet Plate, 26x26 cm (for 500 cm^2 plates, larger flasks or up to 6 plates at a time); Universal Magnet Plates are recommended for all but 96 well plates or
- 96 Magnet Plate (for 96 well plates) or
- 24 Magnet Plate (for 24 well plates)

For suspension cells
Nucleic acid in serum free and supplement free medium (e.g. DMEM)
MATra-A Reagent
MATra-S Immobilizer (can be optionally replaced by the use of polylysine plates)
Magnet Plate (see above)

For enhancing standard transfection
Nucleic acid and standard transfection reagent like IBAfect or others (e.g. Lipofectamine, Fugene, GenePorter, ExGen500, Superfect)
MA Lipofection Enhancer
Magnet Plate (see above)

3 Starting point methods

3.1 General considerations

The instructions given below represent typical protocols that were applied successfully with a large variety of cells. Optimal conditions may vary slightly from cell type to cell type. Therefore, the amount of nucleic acid and the ratios of the individual components may have to be adjusted to achieve optimal results. Thus, an optimum may be found between toxicity and transfection efficiency. If toxicity is high, reduce the amount of nucleic acid and possibly also the duration of MATra.

Cell preparation
Adherent cells are seeded such that they are at 30-60% maximum density at the time of MATra. For a cell type that continues to grow after having reached an apparently confluent monolayer (e.g. HEK293) it is recommended to determine the maximal density it finally reaches. For all other cell types (e.g. COS-7), confluency reflects maximum density. Cells need to undergo another round of cell division after transfection in order to get plasmid DNA in contact with the transcription machinery in the nucleus but excessive rounds of division lead to unnecessary dilution of the transfected DNA. Recommended seeding densities depend on a) the cell line and b) on the time available before transfection. I.e. 2×10^5 HEK293 cells/cm^2 or 4×10^4 CHO-K1 cells/cm^2 respectively work well when transfections are to be carried out the following day.
Suspension cell lines are suitably seeded by using MATra-S Immobilizer immediately before MATra (1.5-6x10^5 cells/cm^2) or by culturing them on polylysine-coated plates prior to MATra (at densities as for adherent cells). Immediately prior to transfection, the medium is replaced with 150 µl of fresh medium (optionally without serum).

Nucleic acid (plasmid, siRNA or oligonucleotides) amount to be applied in different setups
The starting ratio between MATra-A Reagent (µl) and nucleic acid (µg) should be 1:1 in each setting. Usually, a nucleic acid amount of 300 ng per cm^2 culture dish yields good results but optimization is worthwhile if the given setting (cell type, culturing conditions, etc.) is intended to be used as a basis for further investigations.

Assay format*	Surface*	Surface Factor*	Nucleic acid (plasmid or siRNA) amount dissolved in serum free and supplement free medium (e.g. DMEM).	Nucleic acid solution is added to x µl MATra-A Reagent for complex formation	The complex is added to y ml medium supernatant of the cells (may contain serum)
96 well plate	0.32 cm^2	1	0.1 µg in 15 µl medium	0.1 µl	0.15 ml
48 well plate	0.95 cm^2	3	0.3 µg in 25 µl medium	0.3 µl	0.25 ml
24 well plate	1.9 cm^2	6	0.6 µg in 50 µl medium	0.6 µl	0.5 ml
12 well plate	3.8 cm^2	12	1.2 µg in 100 µl medium	1.2 µl	1 ml
6 well plate	9.5 cm^2	30	3 µg in 200 µl medium	3 µl	2 ml
60 mm dish	21 cm^2	66	6.6 µg in 400 µl medium	6.6 µl	4 ml
100 mm dish	55 cm^2	172	17.2 µg in 1000 µl medium	17.2 µl	10 ml
T-75 flask	75 cm^2	235	23.5 µg in 1500 µl medium	23.5 µl	15 ml

* The basis for the above mentioned suggestions were cell culture materials from Corning/Costar. Surfaces may differ for materials from other manufacturers. If other cell culture materials are used, nucleic acid and MATra-A Reagent amount should be adapted according to the difference in surface.

The values given in the table above have to be considered as starting point parameters. In each individual case, optimization may be achieved by performing a titration experiment in the 96 well format (see 3.2). The surface factor may be used for the determination of the appropriate amount of nucleic acid in the desired format. Besides determining the optimal nucleic acid amount and ratio between nucleic acid and MATra-A Reagent (usually not necessary for plasmid DNA transfections with MATra-A Reagent) for MATra, cell density and incubation time are further parameters which may be optimized.

3.2 Standard protocols for MATra-A and MATra-S and titration protocol

Materials and important notes

- Please consider the general remarks under 3.1 prior to starting with the standard protocol, particularly if transfection is intended to be performed in another than the 96 well format.
- Vortex MATra-A Reagent and MATra-S Immobilizer before use!
- If required, MATra-A Reagent can be pre-diluted with water.
- Incubations on the Magnet Plate may be carried out at 37 °C in the cell incubator.
- Incubation times should be followed exactly.

Standard protocol for MATra-S

1. Dilute the cells to be transfected to 5×10^5 - 1×10^6 per ml in medium.
   The medium may contain serum or supplement or may be serum-free; depending on cell type and sensitivity of cells towards serum-free conditions.

2. Mix cell suspension with 30 µl of MATra-S Immobilizer per 1ml of cell suspension.

3. Incubate for 10 - 15 min.

4. Distribute 100 µl of the cell suspension per well of a flat-bottom 96-well plate placed on the 96 Magnet Plate.
   Use the surface factor of the Table under section 3.1 for other formats. Universal Magnet Plates are recommended for all but 96 well plates.

5. Incubate for 15 min.

6. During the incubation continue with the standard protocol for MATra-A.
   Do not perform the medium change of step 3 of the MATra-A protocol. Keep cells on the Magnet Plate until the 15 minute incubation of step 6 of the MATra-A protocol has been terminated.

Standard protocol for MATra-A

1. Dilute the nucleic acid amount to be transfected with serum-free and supplement-free medium (e.g. DMEM) as proposed in Table under 3.1.
   For the transfection of cells grown in one well of a 6 well plate, for example, 3 µg nucleic acid are diluted with DMEM to an end volume of 200 µl.

2. Add the diluted nucleic acid to the respective amount of MATra-A Reagent (e.g. 3 µl for 3 µg nucleic acid of the 6 well plate example given above), mix thoroughly and incubate at ambient temperature for 20 minutes.
   Exceedingly long incubation times lead to inhomogeneous DNA:bead precipitation and thus reduced transfection efficiency.

3. During the incubation, perform a medium change (optional, not for MATra-S protocol).
   The medium supernatant (which may contain supplements and/or serum) should equal to the volume suggested in the Table under 3.1 for the different cell culturing formats. E.g. add 2 ml medium to the cells in each cavity of the 6 well plate.

4. Add the DNA:bead-mixture from step 2 to the cells and mix immediately.
   For the 6 well plate example, add 200 µl of the DNA:bead mixture to the 2 ml supernatant in each well of the 6 well plate and mix. Please notice that the supplements and serum are diluted by this step. If the cells used are sensitive to such alterations, a medium change should be performed as proposed under step 7.

5. After mixing, place immediately the plate or flask on the suitable Magnet Plate.
   Universal Magnet Plates are recommended for all but 96 well plates.

6. Incubate for 15 minutes and then remove Magnet Plate.

7. (optional) Perform a medium change, particularly if transfection has been carried out in serum-free medium.

8. Continue to culture cells as desired until evaluation of transfection efficiency.
   It is important to wait at least 48 hours before exposing the transduced cells to selection media.

3.2.1 Titration protocol

We recommend optimizing transfection conditions in order to get the best results. Several parameters can be optimized:

- Amount of nucleic acid
- Cell density
- Incubation time
- Ratio of MATra-A reagent/MA Lipofection Enhancer to nucleic acid or virus (usually not necessary for plasmid DNA transfections with MATra-A)

The following titration protocol is designed to find the optimal ratio of MATra-A Reagent to nucleic acid and the optimal amount of nucleic acid.

For adherent cells, seed the cells at the desired density in a 96 well plate the day prior or at least several hours prior transfection in a total of 150 µl medium per well. For Plasmid DNA it is recommended to titrate cell density instead of ratio between MATra-A Reagent and nucleic acid amount as in all plasmid DNA transfections tested so far an 1:1 (v/w) ratio emerged optimal.

1. Dilute 21.6 µg nucleic acid to a final volume of 280.8 µl with serum-free and supplement-free medium (e.g. DMEM).

2. Pipet 3.6 µl, 7.2 µl and 14.4 µl MATra-A Reagent in well A1, A5 and A9 of a 96-well plate and bring the volume in wells A1 and A5 to 14.4 µl by adding 10.8 and 7.2 µl sterile water respectively.

3. Add 93.6 µl nucleic acid solution from step 1 to each well A1, A5, A9 containing MATra-A Reagent and mix well by pipetting up and down.
   Incubate for 20 min at ambient temperature. The actual duration should be noted for reproduction of results.

   3a. (optional for adherent cells). Perform a medium change for the cells to be transfected. Remove medium supernatant from the seeded cells and replace with 150 µl fresh medium (with or without serum or supplements).

   3b. In case of suspension cells (for adherent cells proceed immediately to step 4), dilute the cells to be transfected to 5 x 10^5 - 1 x 10^6 per ml in medium (serum- or supplement-containing or serum-free; depending on cell type and sensitivity of cells towards serum-free conditions).

   3c. Mix cell suspension with 30 µl of MATra-S Immobilizer per 1ml of cell suspension. Incubate for 10 - 15 min.

   3d. Distribute 100 µl of the cell suspension per well of a flat-bottom 96-well plate placed on the 96 Magnet Plate. Incubate for 15 min. Use another plate than for performing dilution series of MATra-A Reagent/nucleic acid.

4. In the meantime add 54 µl serum-free and supplement-free medium (e.g. DMEM) to the residual wells of column 1, 5, 9 of the 96-well plate (B1-H1, B5-H5, B9-H9).

5. After the incubation from step 3 transfer 54 µl from well A1, A5, A9 to B1, B5, B9 mix by pipetting, transfer 54 µl from B1, B5, B9 to C1,C5, C9 mix by pipetting, transfer 54 µl from C1, C5, C9 to D1, D5, D9 and so on down to H1, H5, H9.

6. Transfer 15 µl each in triplicates from column 1, 5, 9 to the columns of the cell culture plate (column 2/3/4, 6/7/8, 10/11/12) where the cells to be transfected have been seeded (corresponding to nucleic acid amounts) and mix immediately:
   1000 ng for row A, 500 ng for row B, 250 ng for row C, 125 ng for row D, 62,5 ng for row E, 31 ng for row F, 15 ng for row G and 7.5 ng for row H).

   6a. For suspension cells, the plate remains on the 96 Magnet Plate during this step.

6b. For adherent cells, transfer the culture plate on the 96 Magnet Plate after transfer of the MATra-A Reagent/nucleic acid mixture on the cells.

7. Incubate for 15 min on the 96 Magnet Plate.
   This parameter may be varied between 5 and 20 min for optimization.

8. Remove 96 Magnet Plate.

9. (optional) Perform a medium change, particularly if the transfection has been carried out in serum-free medium.

10. Continue to culture cells as desired until evaluation of transfection efficiency.

The above mentioned titration protocol can also be used for determination of optimal use of MA Lipofection Enhancer when the DNA solution from step 1 has been prepared with IBAfect or other lipofection reagent and if MATra-A Reagent in step 2 is replaced by the MA Lipofection Enhancer.

Following characterization of the transfected cells, the optimal nucleic acid amount and nucleic acid:MATra-A Reagent ratio for efficient MATra can be determined and extrapolated to other formats by using the surface factor mentioned in the Table under section 3.1.

If required, MATra-A Reagent can be pre-diluted with water.

MATra-A Reagent

3.6 µl in A1 + 10.8 µl H_2O | **7.2 µl in A5 + 7.2 µl H_2O** | **14.4 µl in A9 + 0 µl H_2O**

1 2 3 4 5 6 7 8 9 10 11 12

A B C D E F G H

Nucleic acid added to each A1, A5, A9: 7.2 µg in 93.6 µl DMEM

Nucleic acid amount after dilution in columns 1,5,9 (54 µl)	Nucleic acid amount in 15 µl transferred to the cells
3.6 µg	1000 ng
1.8 µg	500 ng
0.9 µg	250 ng
0.45 µg	125 ng
0.23 µg	62.5 ng
0.11 µg	31 ng
0.06 µg	15 ng
0.03 µg	7.5 ng

150 µl cell supernatant + 15 µl of the left-hand dilution assay

Columns 1, 5, and 9: Dilution series with different quantities of MATra-A Reagent. Amount of MATra-A Reagent added in row A is indicated at the top. Final DNA concentrations are given to the right. Final volumes: 54 µl.
Columns 2, 3, 4, 6, 7, 8, 10, 11, and 12: 150 µl cell supernatant + 15 µl of the left-hand dilution assay.

3.2.2 Specific considerations for transfections using large plate formats

The 26 x 26 cm Universal Magnet Plate can be used for transfections of cells grown on larger plates (up to 500 cm^2). While the same general principle applies, a few points should be considered.

- Optimizing conditions can be easily carried out in a small scale calculating cell, number, DNA and MATra amounts for the respective growth surface area
- For larger plates an even liquid level over the cells is even more important. Make sure plates are not inclined during the transfection.
- Even distribution of the DNA:bead complex on large plates can be challenging. An alternative is adding the entire medium to the DNA:bead complex, aspirate the supernatant off the cells and add the premixed solutions to the plate.

3.3 Standard protocol for MA Lipofection

MA Lipofection Enhancer in combination with common transfection reagents, e.g. IBAfect

IBA sells an efficient polycationic transfection reagent based on liposome technology, IBAfect, as well. The efficiency of IBAfect and also that of similar reagents of other suppliers can be enhanced through magnetic assistance simply by mixing them with MA Lipofection Enhancer, usually leading to improved transfection efficiencies. There are two strategies of using MA Lipofection Enhancer: One is to prepare a standard nucleic acid complex with IBAfect, followed by mixing with MA Lipofection Enhancer. The second strategy is to first mix nucleic acid with MA Lipofection Enhancer followed by mixing this complex with IBAfect. Also in this case, the instructions for IBAfect are used with the only exception that instead of nucleic acid alone, a mixture of nucleic acid and MA Lipofection Enhancer is added to the transfection reagent. Depending on the transfection reagent, the mixing order of components may influence the final transfection efficiency. It is recommended to use 1 µl of MA Lipofection Enhancer per microgram of nucleic acid in initial experiments. However, depending on the cell to be transfected and the commercial transfection reagent used, the optimal composition may be found above or below this ratio. A similar titration experiment as described under 3.2 may be performed for determining optimal conditions.

If required, MA Lipofection Enhancer can be pre-diluted with water.

Vortex MA Lipofection Enhancer before use!

Example protocol for IBAfect for a titration experiment in the 96 well format:

1. Add 2.4 µl IBAfect to 97.6 µl serum-free and supplement-free medium (such as DMEM) and mix by pipetting up and down.
2. Dilute 0.8 µg of nucleic acid to 100 µl with serum-free and supplement-free medium (such as DMEM).
3. Mix the nucleic acid solution with the IBAfect dilution by pipetting up and down (do not vortex).
4. Incubate for 15-20 minutes.
5. Add the resulting 200 µl of nucleic acid complex to 0.8 µl of MA Lipofection Enhancer and mix immediately by vigorous pipetting.
6. After further 15 minutes of incubation add 12.5 µl or 25 µl or 50 µl of the resulting mixture, each in triplicates, to the cells (corresponding to 0.05 µg or 0.1 µg or 0.2 µg of nucleic acid per well, respectively) and mix immediately and thoroughly with the medium.
7. Immediately after mixing, place the cell culture plate for 15 min on the 96 Magnet Plate. This parameter may be varied between 5 and 20 min for optimization.
8. Remove the 96 Magnet Plate and cultivate cells under standard conditions until evaluation of transfection efficiency.
9. Optionally perform a medium change.

For other transfection reagents like Fugene, Lipofectamine, GenePorter, ExGen500 or Superfect the same steps as described above are carried out. The primary nucleic acid complex is prepared according to the instructions of the manufacturer. For example, in step 1, 2.4 µl Fugene + 97.6 µl medium, 3.2 µl of Lipofectamine + 96.8 µl medium, or 4.0 µl GenePorter + 96 µl medium are used. The residual protocol remains the same. However, it should be reminded that the alternative mixing order (first mixing nucleic acid and MA Lipofection Enhancer followed by mixing with the common reagent) may be advantageous.

MA Lipofection Enhancer in combination with viruses

Viral infection depends highly on the presence of the respective cell surface receptors (CAR for adenovirus; CD4 for HIV etc.). Unfortunately, many important and interesting target cells for basic research and gene therapy are non-permissive to viral gene delivery as the respective receptors are down regulated or even missing (e.g. tumor tissues and apical surface of lung epithelium may express variable, little or none of the required receptors).

A solution to this problem is the association of viral vectors with MA Lipofection Enhancer to permit efficient infection of non-permissive cells as could already be shown for adenovirus or retroviruses.

Protocol:

1. Cells should be plated in the same manner as required for standard viral gene delivery. For example, the confluency can be high for adenoviral vectors but must be low for retroviral vectors because retroviruses require cell division for infection. Cells must be plated the day prior transfection.

2. Pipet the required amount (see examples in the table below) of MA Lipofection Enhancer in a reaction vessel appropriate to harbor the volume of virus preparation aimed to be added in step 3.

3. Add virus preparation (e.g. retroviral supernatant or purified adenovirus diluted in HBS, PBS or cell culture medium) to the reaction vessel containing MA Lipofection Enhancer and mix immediately by pipetting or gentle vortexing. Incubate 20 minutes at room temperature.

4. The ratio virus:MA Lipofection Enhancer should be adjusted according to the virus titer and cell types used. For optimization, as a starting point the use 1.5 μL, 3 μL, 6 μL and 12 μL of MA Lipofection Enhancer with a fixed quantity of virus preparation:supernatant is recommended.

5. Add the mixture prepared in step 3 in duplicate or triplicate to the cells.

6. Place the cell culture plate on the Magnet Plate for 30 minutes.

7. Remove the Magnet Plate. Optionally perform a medium change.

8. Cultivate cells under standard conditions until evaluation of transgene expression.

9. This protocol would have to be adjusted depending on the viral vector, the quantity of virus and the cell types used.

Cell type	Virus	MA Lipofection Enhancer
K562	adenovirus (200 MOI)	6 μl
human PBL	adenovirus (500 MOI)	3-6 μl
NIH-3T3	adenovirus (200 MOI)	3-6 μl
NIH-3T3	Retrovirus (1-5x10^3 X gal CFU/ml)	3-6 μl

4 Trouble shooting guide

Problem	Suggestion
Cell culturing plate does not fit perfectly onto magnet plate.	- A small spacing between the bottom of the culturing plate and the magnets is not critical. The culturing plate should, however, not be placed inclined on the magnet plate which would lead to inhomogeneous transfection
Transfection is not homogeneous over the whole culturing area	- Reduce time of complex formation between the magnetic beads and nucleic acids - Mix the nucleic acid:MATra bead complex and the cell culturing medium supernatant over the cells thoroughly prior to placing the culturing plate onto the magnet plate. Do not mix by circular motion. It may be recommendable, particularly in case of large surfaces, to add the culture supernatant to the formed bead:nucleic acid complex in a suitable vessel, to mix thoroughly and then to apply the homogenous mixture back to the cells.
Low efficiency	- Avoid serum or other charged macromolecules in incubation step of magnetic particles and nucleic acids - Optimize cell density - Optimize/titrate amount of nucleic acid:bead complex to be applied to the cells - Extend readout time (e.g. 24 h longer than usual) - Incubate cells for prolonged time in the magnetic field - Use other cell line if possible
Toxic effects	- Change medium after performing MATra - Reduce amount of nucleic acid:bead complex to be applied to the cells - Use other cell line if possible

5 Related products

Cat. no.	Description	Amount
7-2001-020	MATra-A Reagent	for 200 μg nucleic acid
7-2001-100	MATra-A Reagent	for 1000 μg nucleic acid
7-2002-020	MATra-S Immobilizer	up to 7 Mio. cells
7-2002-100	MATra-S Immobilizer	up to 35 Mio. cells
7-2003-020	MA Lipofection Enhancer	for 200 μg nucleic acid
7-2003-100	MA Lipofection Enhancer	for 1000 μg nucleic acid
7-2004-000	96 Magnet Plate	1 plate
7-2005-020	IBAfect	for 30-100 μg nucleic acid
7-2005-050	IBAfect	for 75-250 μg nucleic acid
7-2005-100	IBAfect	for 150-500 μg nucleic acid
7-2005-500	IBAfect	for 750-2500 μg nucleic acid
7-2006-000	24 Magnet Plate	1 plate
7-2011-000	Universal Magnet Plate, 8 x 13 cm	1 plate
7-2012-000	Universal Magnet Plate, 26 x 26 cm	1 plate

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