

FREQUENTLY ASKED QUESTIONS (FAQ)

Q: HOW DOES THE MAGNET-ASSISTED TRANSFECTION TECHNOLOGY FROM nanotherics WORK?

A: The nanotherics technology is easy-to-use, fast, simple and highly efficient and uses magnetic forces acting on magnetic nanoparticles to promote uptake of plasmid DNA, siRNA and other biomolecules into the cell. An overview of the process is as follows:

- Biomolecules, such as nucleic acids, are mixed with magnetic nanoparticles made of biodegradable iron oxides coated with cationic molecules in a simple one-step process to form a biomolecule/nanoparticle complex.
- The biomolecule/nanoparticle complex is added to the cells to be transfected in a multi-well plate.
- A magnetic force is applied beneath the cells to be transfected using a magnet array position beneath the multi-well plate. This draws the biomolecule/nanoparticle complex onto cells on the bottom of the multi-well plate. The complex is drawn towards the cells by magnetic forces and the oscillation of the magnet array promotes efficient endocytosis. This leads to rapid, efficient transfection without disturbing membrane architecture, causing chromosomal damage or leaving holes in cell membranes, therefore maintaining higher levels of cell viability. This is in stark contrast to other physical transfection methods, including electroporation or biolistic methods, that damage, create holes or electroshock the cell membranes causing cell death.
- The magnetic nanoparticles are biodegradable and non-toxic at the recommended doses.
- The complete biomolecule dose is concentrated on the cells within minutes and 100% of the cells are generally in contact with significant biomolecule dose.
- After delivery into the cells, the biomolecules are released into the cytoplasm by different mechanisms depending on the formulation used.

Q: HOW IS THE nanotherics MAGNET-ASSISTED TRANSFECTION DIFFERENT?

A: The nanotherics technology is totally unique in that the magnetic field created is designed to oscillate which promotes more efficient uptake of the biomolecule into the cell, resulting in improvement of transfection efficiencies and viability compared to other transfection techniques.

Q: WHAT IS THE BENEFIT TO THE OSCILLATING MAGNETIC FIELD?

A: The magnefect-LT operates at 2 Hz frequency and 0.2 mm displacement. The mechanical stimulation caused by the oscillation promotes uptake of the biomolecule/nanoparticle complex into cells to enable improved transfection. The duration of oscillation can also be controlled up to a maximum of 6 hours 59 minutes to optimise experiments. The magnefect-nano II is fully programmable and can operate between 0.1 and 5 Hz and between 0.1 and 4 mm. This enables a high level of optimisation in terms of frequency, displacement and duration selection to enable full flexibility to optimise protocols with even the most difficult to transfect cells to get best possible results. The duration of oscillation can also be controlled up to a maximum of 9 hours 59 minutes to optimise experiments.

Q: WHAT IS THE EFFICIENCY OF THE nanotherics MAGNET-ASSISTED TRANSFECTION TECHNOLOGY?

A: The nanotherics magnet-assisted transfection technology is a highly efficient transfection method suitable for use with the broadest range of cell types (Cell Lines and Primary Cells), including difficult to transfect cells (e.g. Stem Cells, Neuronal Cells). As with all transfection methods, transfection efficiency varies between cell types. Transfection efficiencies using this technique depend on a range of parameters including cell type, cell density, nature of the biomolecule to be transfected, nature of the magnetic nanoparticle used, cell culture conditions, media used etc. The frequency, displacement and duration of oscillation can also be used to improve transfection efficiencies. The system is therefore highly flexible and easy to optimise to enable the highest levels of transfection efficiency to be achieved.

Q: WHAT EQUIPMENT IS REQUIRED?

A: To perform transfection with the nanotherics magnet-assisted transfection technology, you only need a nanotherics magnefect device (either the magnefect-LT, the magnefect-nano II or the DUO device) and the magnetic nanoparticles (nTMag, nTMag PLUS, NeuroMag or SilenceMag), depending on your needs. There is no further equipment, nor additional reagents or special media required, so the technique is very inexpensive to run (<0.1\$ per well for a 96-well plate).

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Q: HOW LONG DOES IT TAKE?

A: Transfection takes place in less than 30 minutes and the total protocol time (inclusive of reagent preparation) is less than 60 minutes.

Q: WHAT HAPPENS TO THE MAGNETIC NANOPARTICLES AFTER TRANSFECTION AND ARE THEY TOXIC?

A: The magnetic nanoparticles are coated with cationic polymers and associate with biomolecules by salt-induced aggregation and electrostatic interaction. The magnetic nanoparticle / biomolecule complex is then concentrated on the target cells by the magnetic field generated by the magnets on the nanoTherics magnefect transfection device. This magnet-assisted transfection method therefore enables concentration of the biomolecule onto the cell surface to promote uptake into the cell. The magnetic nanoparticle / biomolecule complex enters the cell by endocytosis, which is a natural biological process. This leads to rapid, efficient transfection without disturbing the cell membrane or causing chromosome damage, therefore maintaining higher levels of cell viability. This is in stark contrast to physical transfection methods, including electroporation or biolistic methods, that damage, create holes or electroshock the cell membranes causing cell death.

The biomolecules are released into the cytoplasm by different mechanisms depending upon the formulation used: **1)** via a proton sponge effect caused by the cationic polymer coating on the nanoparticles promoting endosome osmosis, endosome swelling, disruption of the endosome membrane and the intracellular release; **2)** via destabilisation of the endosome by the cationic lipid coating on the magnetic nanoparticles that release the biomolecules into cells. The magnetic nanoparticles, which are made of biodegradable iron oxide with a cationic polymer coating, are not toxic to the cells at the recommended concentrations nor are they thought to influence cell function. Any observed toxic effects that relate to particle dose are likely effects of the plasmid being used.

Q: WHAT SIZE ARE THE MAGNETIC NANOPARTICLES?

A: The magnetic particles used are 100 to 200 nm in diameter.

Q: WHAT IS THE DIFFERENCE BETWEEN THE nTMAG, nTMAG PLUS, NeuroMag AND SilenceMag MAGNETIC NANOPARTICLES?

A: nTMag, nTMag PLUS, NeuroMag and SilenceMag magnetic nanoparticles are comprised of biodegradable iron oxide magnetic nanoparticles associated with proprietary cationic polymers. They each have different properties and have been designed for specific applications. nTMag is a universal, general reagent suitable for use with all types of nucleic acids (plasmid DNA, RNA, siRNA).

nTMag Plus is a magnetic nanoparticle preparation designed for use in combination with any commercially available transfection reagent such as polycations and lipids and can be used with plasmid DNA, antisense oligonucleotides, siRNAs or viruses. NeuroMag is a neuron specific transfection reagent. SilenceMag is a powerful and very efficient siRNA delivery reagent.

Q: CAN nTMAG AND nTMAG PLUS BE USED FOR CO-TRANSFECTION?

A: Yes, it is possible to perform co-transfection experiments using these reagents.

Q: CAN I TRANSFECT AT ANY STAGE AND RE-TRANSFECT?

A: Yes, we have found that with some cell types transfection efficiency can be improved by conducting re-transfection at various stages. This addresses some of the challenges faced with cell types such as transfection of Mature Neuronal Cells which we have shown we can transfect after up to 21 days. We have also shown that it is possible to conduct re-transfection to boost transfection levels. With Neuronal Cells we have also illustrated advantages of transfecting before and/or after differentiation. This method therefore provides excellent opportunities for flexibility to optimise experiments for specific cell types to maximise efficiencies.

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Q: IS THE nanotherics MAGNET-ASSISTED TRANSFECTION TECHNOLOGY SUITABLE FOR STABLE AND TRANSIENT TRANSFECTIONS?

A: The nanotherics magnet-assisted transfection technology is suitable for both stable and transient transfection.

Q: CAN THE nanotherics MAGNET-ASSISTED TRANSFECTION TECHNOLOGY BE USED WITH SERUM-CONTAINING AND SERUM-FREE MEDIA?

A: The nanotherics magnet-assisted transfection technology is compatible with serum-containing and serum-free media so it is possible to use your normal cell culture conditions.

A benefit of the method is that serum-containing media does not interfere with magnet-assisted transfection techniques. Only the incubation of the biomolecule with the magnetic nanoparticles should be conducted in serum/supplement-free media to avoid interference with vector assembly. The technique is highly flexible so optimisation steps can be performed quickly and easily. For example, a serum-free incubation time during transfection can be limited to a few minutes without significant loss of efficiency. After this short incubation time cells can be supplemented with complete medium and it is possible to change medium just before transfection with fresh medium with or without serum. It is also possible to change medium after transfection. With some cell types serum starvation can result in improved transfection efficiency.

Q: CAN YOU USE THE nanotherics MAGNET-ASSISTED TRANSFECTION TECHNOLOGY FOR SUSPENSION AND ADHERENT CELLS?

A: The nanotherics technology is particularly well suited for transfection of adherent cells. We also have a range of optimised protocols for use with semi-adherent and suspension cells. Depending on the cell type used, different types of coated plates may be required to ensure best transfection efficiency.

Q: CAN THE nanotherics MAGNET-ASSISTED TRANSFECTION TECHNOLOGY BE USED WITH DIFFERENT CELL CULTURE MULTIWELL PLATES (96-WELLS, 24-WELLS, 6-WELLS)?

A: The magnefect devices come in 96-well, 24-well and 6-well formats. The magnet holder plates with magnets for each of these formats are designed in the appropriate well plate format and contain the appropriate number of small magnets which can be aligned under each well of the cell culture plate used. The magnefect-nano II is designed so that the system is fully interchangeable so you can switch between magnet array configurations (6-, 24- and 96-well plate). The magnefect-LT is available as a choice of one magnet array configuration (6-, 24- or 96-well plate).

We provide optimised starting protocols for each of these well configurations including appropriate recommendations for DNA concentrations and DNA/particle ratios.

Q: ARE THERE ANY SPECIAL CONDITIONS REQUIRED FOR NUCLEIC ACID USE?

A: The nanotherics magnet-assisted transfection technology is suitable for transfection of any kind of nucleic acid. Purity of the nucleic acid can, however, affect transfection efficiency so nucleic acids should be used at the highest purity possible and free from impurities such as toxins, nucleases, etc. Depending on the plasmid and cell type some optimisation of the plasmid concentration may also be required but identification of the ideal concentration to use can easily be conducted using the 96-well plate format.

Q: DOES THE nanotherics TECHNOLOGY WORK WITH PRIMARY CELLS?

A: The nanotherics technology has been successfully used for transfection of a range of primary cells, including primary neuronal cells such as Primary Hippocampal Neuronal Cells, with high efficiency and high levels of cell viability.

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Q: CAN THE TECHNOLOGY BE USED FOR TRANSFECTION OF NEURONAL CELLS? WHAT ABOUT MATURE NEURONAL CELLS?

A: The technology has shown to be successful with a range of Neuronal Cells including Cortical, Hippocampal and Retinal Neuronal Cells. Higher levels of transfection together with higher levels of cell viability have been observed when compared with traditional transfection techniques. In addition, we have also shown that we can successfully transfect Mature Neuronal Cells (>DIV 21).

Q: DOES IT WORK FOR STEM CELL TRANSFECTION?

A: Yes, the nanoTherics technology has been proven to show successful transfection with ESC, MSC and Neural Stem Cells.

Q: CAN THE nanoTherics TECHNOLOGY BE USED IN siRNA APPLICATIONS?

A: The technology can be used with all types of nucleic acids including DNA, plasmid DNA siRNA, and synthetic miRNA.

Q: DOES IT WORK WITH LARGE DNA CONSTRUCTS?

A: The nanoTherics technology has been successfully tested for transfection of constructs of up to 18Kb in size.

Q: FOR A CELL TYPE WHERE YOU DO NOT HAVE A CELL PROTOCOL LISTED HOW DO I SELECT THE BEST CONDITIONS FOR OPTIMAL RESULTS?

A: When testing a cell type that is not listed in our current list of cells protocols we provide a tried and tested optimisation protocol which provides step by step recommendations for optimisation steps that can be performed to enable improvements of transfection efficiencies so you can get the best possible results for your particular cell type. These recommendations include varying DNA concentration, amount and nature of magnetic nanoparticles used, assessing serum and serum-free conditions as well as testing a range of oscillating frequencies, displacements and durations all of which can affect efficiencies.

Q: CAN YOU USE THIS TECHNOLOGY WITH NON-MAMMALIAN CELLS?

A: We have routinely used the technique with mammalian cells. There is no reason why other cells may not be transfected with this method.

Q: CAN I PERFORM OTHER EXPERIMENTS AFTER THE TRANSFECTION?

A: Use of magnet-assisted transfection should not affect any experimental tests which follow on from the transfection such as flow cytometry, luminescence, colourimetric assays, and qPCR/qRT-PCR.

Q: HOW MANY TRANSFECTIONS CAN BE MADE WITH 100 µl OF REAGENT?

A: It depends on the quantity of DNA used per transfection. If you use 1 µg of DNA per transfection, you get 100 assays with 100 µL of reagent.