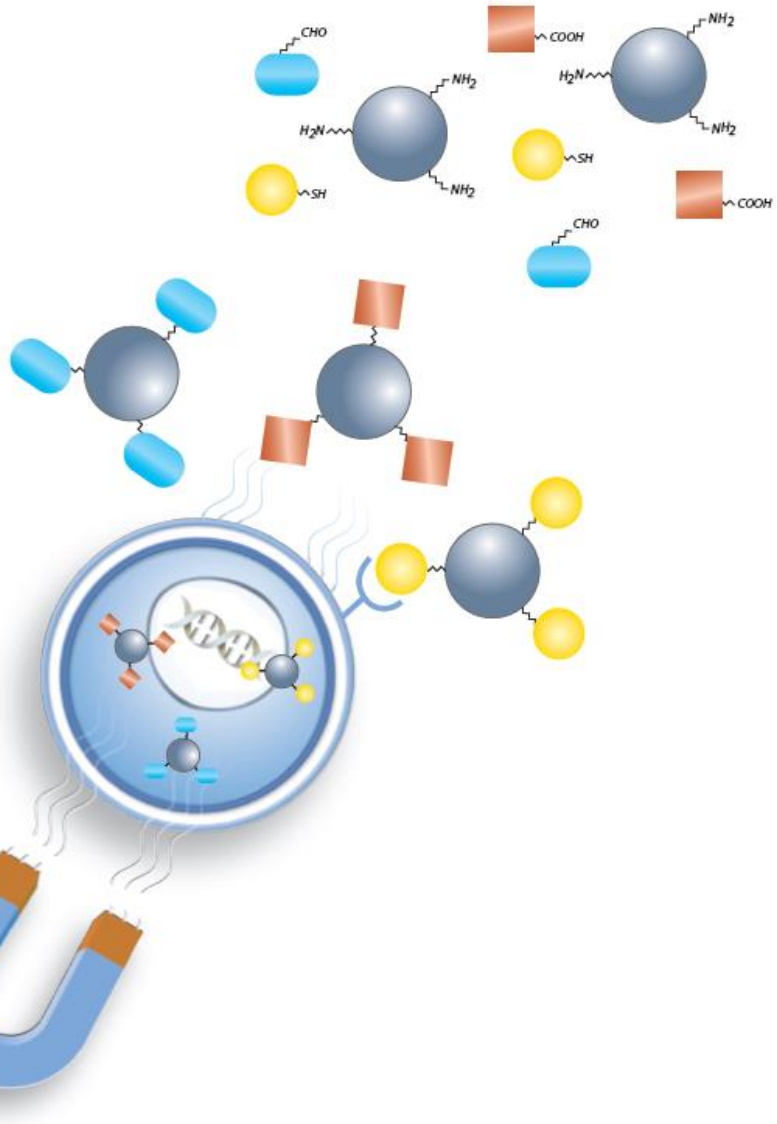
Magnetofection™ SelfMag Amino

INSTRUCTION MANUAL



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Magnetofection™: SelfMag Amino Kit

Instruction Manual

SelfMag has been designed to couple your molecules of interest onto magnetic nanoparticles and deliver them into cells by magnetic targeting.

SelfMag Amino Kit allows you to make your own magnetic delivery system!

List of **SelfMag Amino** Kits

Catalog Number	Description	Content	Number of assays
SA10000	SelfMag Amino Kit[1]	All in one Kit	1 to 50 coupling reactions 1000 delivery assays
SA11000	SelfMag Amino nanoparticles	1 mL	1 to 50 coupling reactions
SA12000	Buffers Kit A[2]	– Buffer A1: 15mL coupling – Buffer A2: 15 mL washing/storage – 100 mg EDC	1 to 50 coupling reactions
SF40000	MagFectin	1 mL	1000 delivery assays
DM30000	MagID (magnetic device)	1 unit	-
MF10000	Super Magnetic Plate	1 unit	-

[1] Contains 1 vial of SelfMag Amino nanoparticles, 1 Buffers Kit (coupling, washing/storage buffers and EDC), 1 MagID device, 1 vial of MagFectin and one Super Magnetic Plate

[2] Contains 1 bottle of coupling buffer A1, 1 bottle of washing/storage buffer A2 and 1 vial of EDC reagent.

1. Technology

1.1. Product Description

Congratulations on your purchase of the **SelfMag Amino Kit**!
SelfMag Amino nanoparticles are colloidal monodispersed superparamagnetic, which are composed of a magnetic core surrounded by a highly cross-linked polymer shell. The SelfMag surface is activated with primary amine functionality (NH_2) making an ideal reagent for coupling a variety of molecules bearing reactive carboxyl, sulfhydryl or aldehyde groups in order to produce your self-made magnetic delivery system. SelfMag Amino nanoparticles are supplied in an aqueous suspension. The hydrophilic surface of SelfMag ensures low non-specific binding, excellent dispersion abilities and easy handling in solution. Optimized coupling and washing/storing buffers, EDC (for carbodiimide activation), a magnetic device MagID for the coupling and washing procedure are also supplied. In addition, a magnetic plate that generates a magnetic field gradient and a specific delivery reagent, MagFectin, allowing the transport into cells of magnetic nanoparticles (Magnetofection) are provided. SelfMag Amino Kit contains all components to make in a simple and easy way your own magnetic delivery system.

1.2. Physical Characteristics of SelfMag Amino Nanoparticles

- **Diameter:** 210 nm (CV max 20%)
- **Relative density:** 1.25 – 1.3
- **Magnetic susceptibility:** 35 – 37 $emu.g^{-1}$
- **Specific surface area per primary amine functionality:** 62.62 $Å^2$
- **Iron oxide content:** approx. 50%
- **NH_2 density:** 70 $\mu mol.g^{-1}$
- **Solid content (stock solution):** 1 mg/mL (0.1%)
- **Beads concentration:** 2×10^{11} beads per mL

1.3. Principle

SelfMag Amino particles are designed as superparamagnetic, nanosized polymer particles bearing primary amino reactive groups on their surface for the coupling and the delivery of your molecules of interest into cells by magnetic targeting. Proteins, peptides, oligonucleotides, small drugs or other various molecules having reactive carboxyl, sulfhydryl or aldehyde groups can be covalently coupled directly onto the surface reactive groups (NH_2) of the nanoparticles and maintain their biological activity. Thereafter, the molecules coupled to the SelfMag Amino nanoparticles can be easily concentrated onto the target cells by exploiting the magnetic field force, and internalized within a few minutes by using a specific MagFectin reagent dedicated to the magnetic nanoparticles delivery into cells (Magnetofection).

1.4. Kit Content

- 1 vial containing 1 mL of SelfMag Amino Nanoparticles (1 mg/mL), good for 1 to 50 coupling reactions
- 2 bottles each containing 15 mL of sterile buffer solutions:
  - Buffer A1: SelfMag Amino Coupling Buffer
  - Buffer A2: SelfMag Amino Washing & Storage Buffer
  - 1 vial containing 100 mg EDC
- 1 vial containing 1 mL of MagFectin delivery reagent good for 1000 delivery assays.
- 1 MagID (Magnetic Isolation Device) for the coupling and washing procedures.
- 1 Super Magnetic Plate for delivery into cells (Magnetofection).

1.5. Magnetic Apparatus

- **MagID (Magnetic Isolation Device) for coupling reactions and washing procedures**

MagID is made from an injection moulded plastic housing incorporating a high-energy neodymium magnet. It is designed to accommodate standard 1.5 mL tubes and is also suitable for some 2 mL tubes. It is ideal for your magnetic nanoparticles coupling reaction and purification. It is adapted to working solutions ranging from 10 µL to 2 mL. This device allows a quick magnetic separation process (< 5 minutes) with a high yield separation. Durable and easy to use, this device, with an open faced design, facilitates aspiration, pipetting etc.

- **Super Magnetic Plate for delivery into cells**

As for all Magnetofection™ reagents, the delivery of coupled SelfMag Amino nanoparticles is supported by an appropriate magnetic field and the **MagFectin** delivery reagent. The special geometry of the **Super Magnetic Plate** produces a strong magnetic field that is suitable for all cell culture dishes (T-75 flasks, 60 & 100 mm dishes, 6-, 12- 24-, 48- and 96-well plates).

Warning and Handling: These products should be handled with care. Avoid direct contact with other magnetic materials and devices. Person with pacemakers/implants should avoid direct contact. Keep loose ferrous material away and do not attempt to disassemble. Keep all magnetic media, watches, and sensitive electronic devices away from these magnetic apparatus. Credit cards, tape and disks can be erased in the presence of a magnetic field. Bodily harm (pinching of hands and skin) can result if magnets are not handled correctly. Maintain distance between two or more magnetic units. Product should be stored in a dry environment and should be cleaned with a damp cloth and mild detergent when exposed to harsh solvents. Do not autoclave.

1.6. Storage / Stability

- **SelfMag Amino nanoparticles**

Storage at +4°C. Upon receipt and for long-term use, store the SelfMag nanoparticles in the fridge. The stock solution of SelfMag Amino nanoparticles is stable for at least one year at the recommended storage temperature. The SelfMag Amino beads must be maintained in liquid during storage and all handling steps. Drying will result in reduced performance. Precaution should be taken to prevent bacterial contamination of the beads.

  - *Do not freeze the magnetic nanoparticles!*
  - *Do not add anything to the stock solution of magnetic nanoparticles!*

- **Buffer Kits and MagFectin reagent**

Upon receipt and for long-term use, store sterile buffer solutions and MagFectin at +4°C and EDC at -20°C. These reagents are stable for at least 1 year at the recommended storage temperature. EDC should be kept dried.

- **Magnetic device and plate**

They can be stored at room temperature (dry environment is preferred) away from any electronic, informatics or magnetic materials.

Shipping condition: Room Temperature.

2. Instruction for Coupling the Molecules

2.1. General Considerations

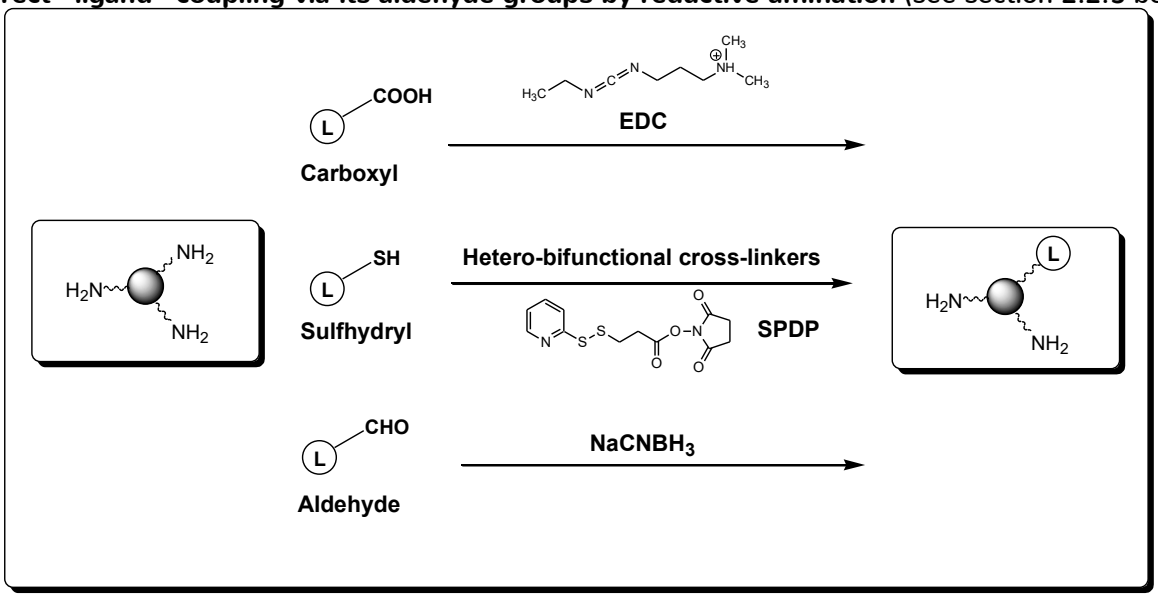
For coupling your molecules of interest on SelfMag Amino nanoparticles, we recommend to use between 0.5 and 5 nmol of "ligand" (molecules) per mg of SelfMag Amino. The nanoparticles should be used at a final concentration comprised between 0.5 and 1 mg per mL during the coupling reaction. However, protocols may be adapted to meet your requirements. Both molecules and nanoparticles concentration can be adjusted depending on the stock concentration, solubility and availability of your "ligand". Higher nanoparticles concentrations increase the coupling efficiency, and higher "ligand" concentrations increase the number of molecules coupled onto the nanoparticles. The suggested protocol described below has been optimized for a specific application: the delivery of molecules into cells. It illustrates an example using 100 µL of SelfMag Amino particles at 1 mg/mL (100 µg of beads), but can be scaled up and down to suit specific needs. The "ligand" concentration was set to 10 µmol/L but can be adapted according to your needs. It is recommended to manipulate SelfMag Amino nanoparticles under sterile conditions for using with cells.

Important note: The nature and the ionic strength of the buffers (for coupling, washing and storage of SelfMag) are critical to obtain a high coupling efficiency rate and to avoid the nanoparticles aggregation. In this context, we highly recommend to only use the buffers provided in the kit (**Buffer A1** for coupling and **Buffer A2** for washing and storage) and NOT other buffers.

2.2. Coupling Procedures

Alternative coupling principles:

- **Activation of the "ligand" with a carbodiimide**. If the molecules to be coupled carried a reactive carboxylic acid groups, they may be activated with a carbodiimide and then reacted with the nanoparticles, resulting in a direct amide bound formation between the particles and the "ligand" (see section 2.2.1 below).
- **Activation of the nanoparticles with a hetero-bifunctional cross-linker**. The SPDP is an amine reactive cross-linker that introduces a linker with a terminal sulfhydryl function (SH) on the surface of the nanoparticles making them suitable for coupling sulfhydryl-containing molecules (see sections 2.2.2 below).
- **Direct "ligand" coupling via its aldehyde groups by reductive amination** (see section 2.2.3 below).



Optionally, isothiocyanate-containing molecules can react directly with SelfMag Amino nanoparticles at alkaline pH (sodium carbonate buffer, pH9). However, this coupling procedure will not be treated in this instruction manual.

2.2.1. Coupling of carboxyl-containing molecules via a carbodiimide (EDC)

Molecules having reactive carboxylic acid groups can be easily conjugated onto the surface of the SelfMag Amino nanoparticles by the formation of an amide bound mediated by carbodiimide activation. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, MW 191.7) is the carbodiimide most commonly used. Its water solubility allows for direct addition to a reaction without prior organic solvent dissolution. The excess of reagent and the by-product (isourea) resulting from the reaction are both water-soluble and can be easily removed. A stock solution of EDC must be prepared to facilitate the addition of a small molar amount to the reaction mixture and used immediately to prevent extensive loss of activity. EDC is not stable in water and thus must be prepared freshly for each reaction. *Optionally*, N-Hydroxysuccinimide (NHS, MW 115.09) may be introduced with EDC to improve the coupling efficiency.

1. Before each use, resuspend the SelfMag Amino nanoparticles by pipetting up and down or by vortexing 1 minute. Avoid foaming.

2. Transfer 100 µL of the SelfMag Amino nanoparticles in a microtube.

3. Place the SelfMag microtube in the MagID device, let stand until the supernatant clears up and remove it by pipetting (aspiration), leaving the nanoparticles pellet undisturbed.

4. Remove the microtube from the MagID device and resuspend the SelfMag nanoparticles in 100 µL of **Buffer A1** by pipetting up and down.

5. Repeat steps 3 and 4 one more time.

6. Prepare 100 µL of the molecule to be coupled ("ligand") at a concentration of 10 µmol/L in the **Buffer A1**. If your molecule is already in a buffered solution, we recommend you to dialyse it against water before use, since some salts containing buffer could potentially cause the aggregation of the nanoparticles.

7. Add between 5 and 50 µL of the "ligand" solution to the 100 µL of SelfMag prepared previously. Then vortex to ensure good mixing.

8. Immediately prior to use, prepare a 10X stock solution of EDC at a concentration of 10 mg/mL in water. Then, dilute 10 µL of the 10X EDC solution to 100 µL with the **Buffer A1** in order to obtai 5 DC solution.
   Option: prepare a solution of EDC with NHS. In this case, prepare a 10X EDC/NHS solution at a concentration of 10 mg/mL of EDC and 15 mg/mL of NHS, then dilute 10 µL of the 10X EDC/NHS solution to 100 µL with the **Buffer A1** in order to obtain a 1X EDC/NHS solution.

9. Add 5 to 10 µL of 1X EDC (or EDC/NHS) solution to the SelfMag / "ligand" mixture. Vortex to ensure a good mixing.

10. Shake (agitator, mixer) the mixture for at least 2 hours at room temperature (or overnight at 4°C depending on the nature of the molecule to be coupled).

11. Then, proceed to the washing protocol as described below (2.3).

The suggested protocol described above is provided for the coupling of 100 µg of nanoparticles. However, this protocol can be scaled up or scaled down depending on the quantity of your molecules or on your specific needs. Refer to the Table 1 below for suggested quantities of SelfMag nanoparticles, molecules to be coupled and EDC.

Table 1: Quantities of SelfMag nanoparticles, molecules and reagents suggested for coupling

SelfMag Amino nanoparticles (1 mg/mL)		Molecule to be coupled (10 µmol/L)		EDC			Washing & Storage volume (µL)
Quantity (µg)	Solution volume (µL)	Quantity (mole)	Solution volume (µL)	Quantity (mole)	Solution concentration (mg/mL)	Solution volume (µL)	
20	20	10 – 100 pmol	1 – 10	5 – 10 nmol	1	1 – 2	20
50	50	25 – 250 pmol	2.5 – 25	12.5 – 25 nmol	1	2.5 – 5	50
100	100	50 – 500 pmol	5 – 50	25 – 50 nmol	1	5 – 10	100
500	500	0.25 – 2.5 nmol	25 – 250	0.13 – 0.25 µmol	10	2.5 – 5	500
1000	1000	0.5 – 5 nmol	50 - 500	0.25 – 0.5 µmol	10	5 - 10	1000

- **<u>Coupling with NHS-Ester activated carboxyl-containing ligand</u>:**

If the carboxylic function(s) of your "ligand" is (are) already activated with N-succimidyl ester, it may be directly linked to SelfMag Amino nanoparticles without using EDC according to the following rapid protocol:

1. Before each use, resuspend the SelfMag Amino nanoparticles by pipetting up and down or by vortexing 1 minute. Avoid foaming.

2. Transfer 100 µL of the SelfMag Amino nanoparticles in a microtube.

3. Place the SelfMag microtube in the MagID device, let stand until the supernatant clears up and remove it by pipetting (aspiration), leaving the nanoparticles pellet undisturbed.

4. Remove the microtube from the MagID device and resuspend the SelfMag nanoparticles in 100 µL of **Buffer A1** by pipetting up and down.

5. Repeat steps 3 and 4 one more time.

6. Prepare 100 µL of the molecule to be coupled ("ligand") at a concentration of 10 µmol/L in the **Buffer A1**. If your molecule is already in a buffered solution, we recommend you to dialyse it against water before use, since some salts containing buffer could potentially cause the aggregation of the nanoparticles.

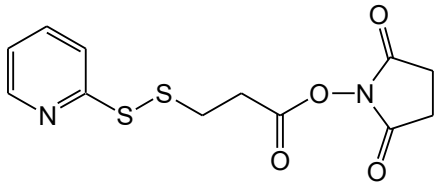
7. Add between 5 and 50 µL of the "ligand" solution to the 100 µL of SelfMag prepared previously. Then, vortex to ensure a good mixing and shake (agitator, mixer) the mixture for at least 2 hours at room temperature (or overnight at 4°C depending on the nature of the molecule to be coupled).

8. Then, proceed to the washing protocol as described below (2.3).

2.2.2. Coupling of sulfhydryl-containing molecules via an hetero-bifunctional cross-linker: SPDP

The most commonly used activating agents are NHS-esters cross-linkers. A variety of different NHS-esters are commercially available for cross-linking. Depending on the nature of the cross-linker, they can react with various chemical groups. Reactive groups include sulfhydryl, carboxyl, hydroxyl, amine as well as non-selective photoreaction. Hetero-bifunctional cross-linkers will be preferred to link the "ligand" onto the nanoparticles, since the use of homo-bifunctionnal cross-linkers can cause the aggregation of the particles.

N-Succinimidyl 3-(2-pyridyldithio)propionate (SPDP) is one of the most popular hetero-bifunctional cross-linkers. The 2-pyridyldithiol group end of SPDP reacts with sulfhydryl residues on the molecules to be coupled to form a disulfide linkage. This conjugation is reversible and the disulfide bond can be cleaved in reducing conditions. SPDP is water-insoluble and must be pre-dissolved in an organic solvent (DMSO or DMF) before use. The final concentration of the organic solvent in the buffered reaction should not exceed 10%. The use of a 10-fold molar excess of SPDP compared to the amount of ligand to be conjugated is recommended. Note that other hetero-bifunctional cross-linkers can be used to couple a sulfhydryl-containing ligand onto nanoparticles such as SMTP, SMCC, MBS and SIAB.



N-Succinimidyl 3-(2-pyridyldithio)propionate (SPDP)

1. Before each use, resuspend the SelfMag Amino nanoparticles by pipetting up and down or by vortexing 1 minute. Avoid foaming.
2. Transfer 100 µL of the SelfMag Amino nanoparticles in a microtube.
3. Place the SelfMag microtube in the MagID device, let stand until the supernatant clears up and remove it by pipetting (aspiration), leaving the nanoparticles pellet undisturbed.
4. Remove the microtube from the MagID device and resuspend the SelfMag nanoparticles in 100 µL of **Buffer A1** by pipetting up and down.
5. Repeat steps 3 and 4 one more time.
6. Immediately prior to use, prepare a SPDP solution in DMSO at a concentration of 0.3 mg/mL (1 mmol/L solution).
7. Add 5 µL of the SPDP solution to the 100 µL of SelfMag prepared previously. Then, vortex to ensure a good mixing and shake (agitator, mixer) the mixture 30 minutes at room temperature.
8. Place the SelfMag microtube in the MagID device, let stand until the supernatant clears up and remove it by pipetting (aspiration), leaving the nanoparticles pellet undisturbed.
9. Remove the microtube from the MagID device and resuspend the SelfMag nanoparticles in 100 µL of **Buffer A1** by pipetting up and down.
10. Prepare 100 µL of the sulfhydryl-containing molecule to be coupled ("ligand") at a concentration of 10 µmol/L in the **Buffer A1**. If your molecule is already in a buffered solution, we recommend you to dialyse it against water before use, since some salts containing buffer could potentially cause the aggregation of the nanoparticles.

11. Add between 5 and 50 µL of the "ligand" solution to the 100 µL of the SelfMag mixture. Then, vortex to ensure a good mixing.
12. Shake (agitator, mixer) the mixture over night at room temperature.
13. Then, proceed to the washing protocol as described below (2.3).

Note: The suggested protocol described above is provided for the coupling of 100 µg of beads. However, this protocol can be scaled up or scaled down depending on the availability of your ligand or to suit specific needs.

2.2.3. Coupling of aldehyde-containing molecules

Coupling of an aldehyde-containing molecule to the SelfMag Amino nanoparticles can be achieved by Schiff base (imine) formation and reductive amination. Aldehyde groups can easily be prepared by sodium periodate oxidation of sugar residues in glycoproteins, or cleavage of carbon-carbon bounds with adjacent hydroxyl groups in polysaccharides. The reductive amination is achieved by using a reducing agent like cyanoborohydrid.

1. Before each use, resuspend the SelfMag Amino nanoparticles by pipetting up and down or by vortexing 1 minute. Avoid foaming.
2. Transfer 100 µL of the SelfMag Amino nanoparticles in a microtube.
3. Place the SelfMag microtube in the MagID device, let stand until the supernatant clears up and remove it by pipetting (aspiration), leaving the nanoparticles pellet undisturbed.
4. Remove the microtube from the MagID device and resuspend the SelfMag nanoparticles in 100 µL of **Buffer A1** by pipetting up and down.
5. Repeat steps 3 and 4 one more time.
6. Prepare 100 µL of the aldehyde-containing molecule to be coupled ("ligand") at a concentration of 10 µmol/L in the **Buffer A1**. If your molecule is already in a buffered solution, we recommend you to dialyse it against water before use, since some salts containing buffer could potentially cause the aggregation of the nanoparticles.
7. Add between 5 and 50 µL of the "ligand" solution to the 100 µL of SelfMag prepared previously. Then, vortex to ensure a good mixing.
8. Prepare a solution of cyanoborohydrid in 0.1 M NaOH at a concentration of 0.5 mol/L (0.5 M). Note that cyanoborohydrid is highly toxic, so use a fume hood to manipulate it.
9. Add 1 µL of the cyanoborohydrid solution to the SelfMag Amino nanoparticles / "ligand" mixture. Vortex to ensure a good mixing.
10. Shake (agitator, mixer) the mixture for 2 hours at room temperature.
11. Place the SelfMag microtube in the MagID device, let stand until the supernatant clears up and remove it by pipetting (aspiration), leaving the nanoparticles pellet undisturbed.
12. Block unreacted aldehyde groups by adding 100 µL of 0.1 M ethanolamine adjusted to pH 7.4. Then, shake (agitator, mixer) the mixture for 15 minutes at room temperature.
13. Then, proceed to the washing protocol as described below (2.3).

Note: The suggested protocol described above is providing for the coupling of 100 µg of beads. However, this protocol can be scaled up or scaled down depending on the availability of your ligand or to suit specific needs.

2.3. Washing and Storage of Coupled Nanoparticles

All coupling procedures require washing of the molecule-conjugated SelfMag Amino nanoparticles to remove the excess of "ligand" and reagents.

1. Place the mixture microtube in the MagID device, let stand until the supernatant clears up and remove it by pipetting (aspiration), leaving the nanoparticles pellet undisturbed.
2. Remove the microtube from the MagID device and resuspend the SelfMag nanoparticles in 100 µL of **Buffer A2** by pipeting up and down.
3. Repeat steps 1 and 2 two or three times.

The use of MagID device avoids significant lost of nanoparticles during this procedure. Consequently**, 100 µg of coupled SelfMag Amino nanoparticles are obtained at a final concentration of 1 mg/mL.** Store the coupled nanoparticles at +4°C. Coupled SelfMag can usually be stored for several weeks at this temperature, depending on the stability of the coupled molecules. However, it is recommended to use them rapidly after coupling. For every delivery experiment, resuspend the nanoparticles by pipeting up and down prior to use.

3. Protocol for Delivering Coupled SelfMag Nanoparticles into Cells

The instructions given below represent a model of protocol that was applied successfully to deliver coupled nanoparticles into several cells. We recommend you to start by following this general protocol as guideline and then optimized the conditions if required (depending on the nature of the molecule coupled and the cell type). Please refer to the table 2 below as a starting point for experimental conditions.

1. The day prior the delivery experiment, plate the cells in your tissue culture dish. The suitable cell density will depend on the growth rate and the conditions of the cells. Cells should not be less than 60 % confluent (percentage of growth surface covered with cells) at the time of delivery (see the suggested cell number in the table 2). The correct choice of optimal plating density also depends on the planned time between delivery and analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.
2. Before each use, vortex the molecule-coupled SelfMag nanoparticles solution. Add the suggested amount of nanoparticles (see Table 2) to a microtube or microwell (U-bottom well is preferred to get a better mixing).
3. In another microtube, dilute the MagFectin delivery reagent in serum and supplement-free culture medium (see Table 2 for volume of MagFectin and dilution volume).
4. Add the MagFectin solution to the coupled nanoparticles and mix immediately by pipeting up and down.

Table 2: Experimental conditions suggested for delivering the coupled nanoparticles into cells

Tissue Culture Dish	Cells to plate		Coupled SelfMag solution volume (µL) (C=1 µg/µL)	MagFectin diluted solution		Total Volume/well
	Adherent Cell Number	Seeding Volume		MagFectin Volume(µL)	Dilution Volume (µL)	
96 well	0.05 – 0.2 x 10^5	150 µL	0.5 - 1	0.2 – 0.5	50	200 µL
24 well	0.5 – 1 x 10^5	400 µL	2 - 4	0.8 - 2	100	500 µL
12 well	1 – 2 x 10^5	800 µL	4 - 8	1.6 - 4	200	1 mL
6 well	2 – 5 x 10^5	1.6 mL	8 - 16	3 - 8	400	2 mL
60 mm dish	5 – 10 x 10^5	3.2 mL	20 - 40	8 – 20	800	4 mL
90 - 100 mm	10 – 30 x 10^5	7 mL	60 - 120	25 – 60	1000	8 mL
T-75 flask	20 – 50 x 10^5	9 mL	70 - 130	35 - 70	1000	10 mL

5. After 20 minutes of incubation, add the complexes (coupled nanoparticles/MagFectin) to the cells. The total volumes per well (culture medium + complexes) are indicated in Table 2.
6. Place the cell culture plate upon the Super Magnetic Plate for 15-20 minutes.
7. Remove the Super Magnetic Plate.
8. Cultivate the cells under standard conditions until assays are performed (from 3 to 48 h).

4. Example of Application

4.1. Protocol for Coupling IgG-FITC

1. Resuspend the SelfMag Amino nanoparticles by pipeting up and down.
2. Transfer 100 µL of the SelfMag Amino nanoparticles in a microtube and washed them with **Buffer A1** as described in section 2.2.1. above.
3. Prepare 100 µL of an IgG-FITC solution at 10 µmol/L (1.5 mg/mL for a 150 kD IgG-FITC) in the **Buffer A1**.
4. Add 30 µL (45 µg, 0.3 nmol) of the IgG-FITC solution to the 100 µL of SelfMag prepared previously. Then, vortex to ensure a good mixing.
5. Prepare a 1X EDC solution (1 mg/mL) in the **Buffer A1** as described in section 2.2.1.
6. Add 10 µL of 1X EDC solution (10 µg) to the SelfMag Amino nanoparticles / IgG-FITC mixture. Vortex to ensure a good mixing.
7. Shake (agitator, mixer) the mixture for 2 to 24 hours at room temperature.
8. Wash the coupled nanoparticles with **Buffer A2** as described in section 2.3 above.
9. Finally, store the IgG-FITC coupled SelfMag in 100 µL of **Buffer A2** at a concentration of 1 mg/mL.

4.2. Intracellular Delivery of IgG-FITC Coupled Nanoparticles

The instructions given below represent an example of protocol to deliver the IgG-FITC coupled SelfMag into cells. The following procedure can be adapted to deliver other proteins or molecules.

1. The day prior the intracellular delivery experiment, seed 100,000 HeLa cells / well in a 24 well-plate in 400 µL of DMEM complete culture medium (with serum).
2. Mix the IgG-FITC coupled SelfMag nanoparticles. Add 2 to 4 µL (2 to 4 µg) of IgG-FITC coupled SelfMag to a microtube.
3. Dilute 1 to 2 µL of MagFectin delivery reagent to 100 µL with serum and supplement-free DMEM culture medium.
4. Add the 100 µL of the MagFectin solution to the IgG-FITC coupled SelfMag nanoparticles and mix immediately by pipeting up and down.
5. After 20 minutes of incubation, transfer the 100 µL of complexes onto cells. The total volume per well (DMEM medium + complexes) is 500 µL.
6. Place the cell culture plate upon the Super Magnetic Plate for 20 minutes.
7. Remove the Super Magnetic Plate.
8. Cultivate the cells under standard conditions during 3 to 24 h.
9. The intracellular localization of IgG-FITC into cells was observed by fluorescence microscopy. Moreover, the quantity of fluorescein delivered into cells was estimated using a spectrofluometer (λ exc = 495 nm; λ em = 530 nm).

5. Appendix

5.1. Quality Controls

To guarantee the performance of **SelfMag Amino Kit** produced, we qualify each lot using rigorous standards.

Components	Standard Quality Controls
SelfMag Amino Nanoparticles	1. Quality and size homogeneity of the magnetic nanoparticles. 2. Stability of the magnetic nanoparticles formulation. 3. Sterility. Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
MagFectin	1. Sterility. Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days. 2. Intracellular delivery assay. MagFectin efficacy to deliver IgG-FITC coupled SelfMag nanoparticles on Hela cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.
Buffer solutions and EDC	1. Sterility. Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days. 2. Coupling assay. All buffers and EDC are tested for their efficiency to couple IgG-FITC onto SelfMag. Every lot shall have an acceptance specification of > 90% of the activity of the reference lot.
MagID and Super Magnetic Plate	1. Tests of solidity. 2. Test of the magnetic field force.

5.2. Troubleshooting

6. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)* / 3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g X-Gal powder 1g / G-418, Sulfate 1g

7. Purchaser Notification

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