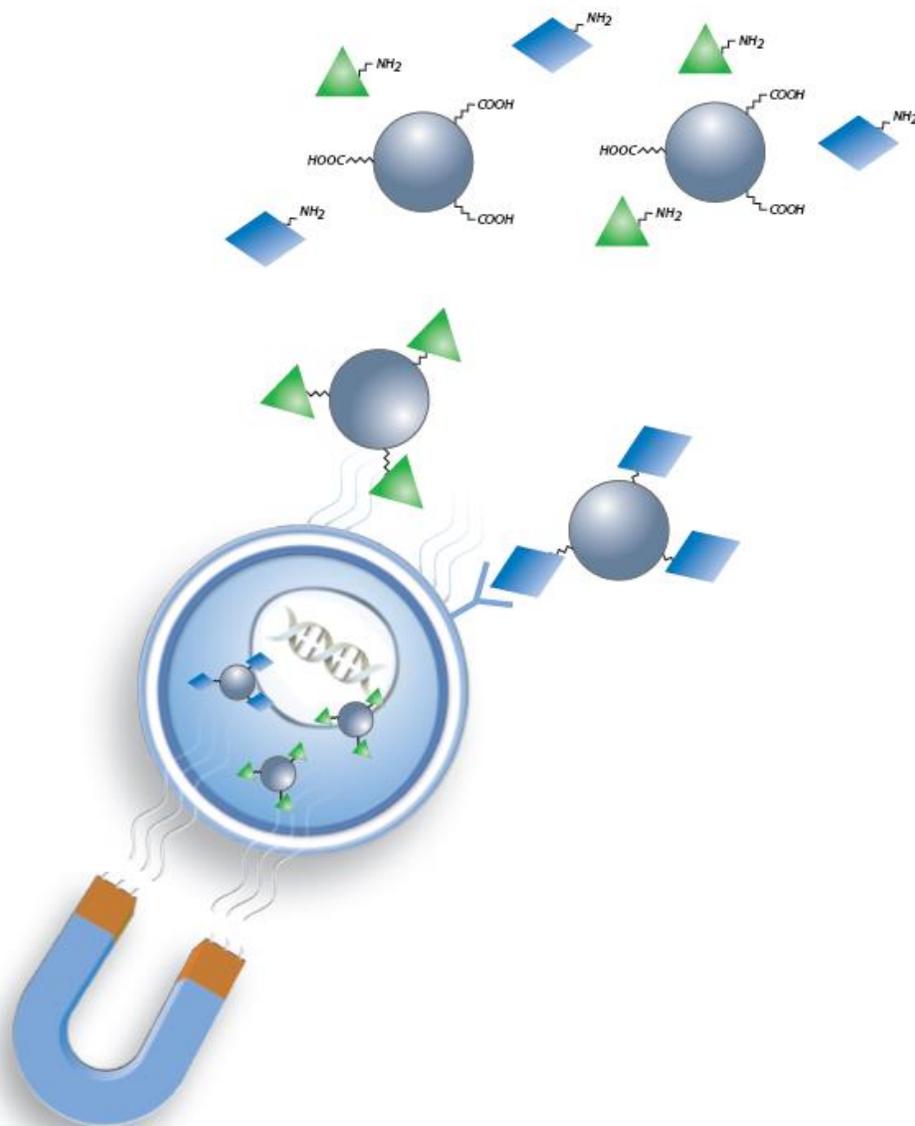


Magnetofection™ SelfMag Carboxy

INSTRUCTION MANUAL



Magnetofection™: SelfMag Carboxy 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 Carboxy Kit allows you to make your own magnetic delivery system!

List of SelfMag Carboxy Kits

Catalog Number	Description	Content	Number of assays
SC20000	SelfMag Carboxy Kit ¹	All in one Kit	1 to 50 coupling reactions 1000 delivery assays
SC21000	SelfMag Carboxy nanoparticles	1 mL	1 to 50 coupling reactions
SC22000	Buffers Kit C ²	– Buffer C1: 15mL coupling – Buffer C2: 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 Carboxy 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 C1, 1 bottle of washing/storage buffer C2 and 1 vial of EDC reagent.

1. Technology

1.1. Product Description

Congratulations on your purchase of the **SelfMag Carboxy Kit!**

SelfMag Carboxy 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 carboxylic acid functionality (COOH) making an ideal reagent for coupling any molecules bearing an amino reactive group in order to produce your self-made magnetic delivery system. SelfMag Carboxy 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 Carboxy Kit contains all components to make in a simple and easy way your own magnetic delivery system.

1.2. Physical Characteristics of SelfMag Carboxy Nanoparticles

- **Diameter:** 160 nm (CV max 20%)
- **Relative density:** 1.25 – 1.3
- **Magnetic susceptibility:** 35 – 37 emu.g⁻¹
- **Specific surface area per carboxylic acid functionality:** 34.38 Å²
- **Iron oxide content:** approx. 50%
- **COOH density:** 150 µmol.g⁻¹
- **Solid content (stock solution):** 1 mg/mL (0.1%)
- **Beads concentration:** 2.5 × 10¹¹ beads per mL

1.3. Principle

SelfMag Carboxy particles are designed as superparamagnetic, nanosized polymer particles bearing carboxy 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 amino groups can be covalently coupled directly onto the surface reactive groups (COOH) of the nanoparticles and maintain their biological activity. Thereafter, the molecules coupled to the SelfMag Carboxy 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 Carboxy Nanoparticles (1 mg/mL), good for 1 to 50 coupling reactions
- 2 bottles each containing 15 mL of sterile buffer solutions:
 - Buffer C1: SelfMag Carboxy Coupling Buffer
 - Buffer C2: SelfMag Carboxy 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 μ L 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 Carboxy 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 Carboxy nanoparticles**

Storage at +4°C. Upon receipt and for long-term use, store the SelfMag nanoparticles in the fridge. The stock solution of SelfMag Carboxy nanoparticles is stable for at least one year at the recommended storage temperature. The SelfMag Carboxy 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

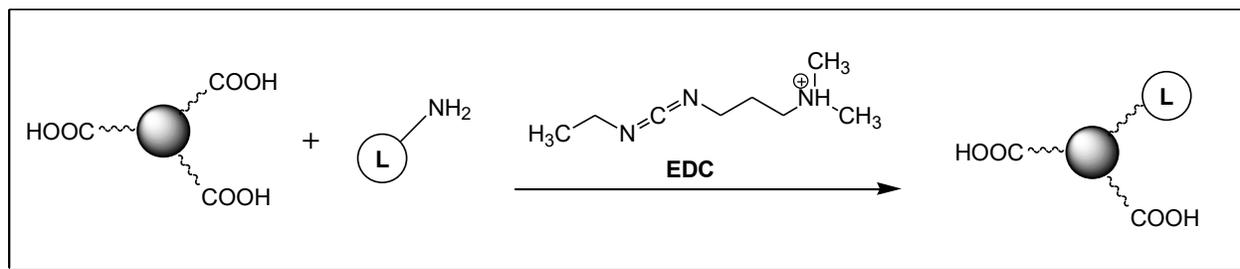
2.1. General Considerations

For coupling your molecules of interest on SelfMag Carboxy nanoparticles, we recommend to use between 0.5 and 5 nmol of "ligand" (molecules) per mg of SelfMag Carboxy. 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 μ L of SelfMag Carboxy particles at 1 mg/mL (100 μ g of beads), but can be scaled up and down to suit specific needs. The "ligand" concentration was set to 10 μ mol/L, but can be adapted according to your needs. It is recommended to manipulate SelfMag Carboxy 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 C1** for coupling and **Buffer C2** for washing and storage) and NOT other buffers.

2.2. Activation and Coupling of the Molecules

Formation of an amide bond between a primary amino group of the molecule of interest and a carboxylic acid group on the surface of the SelfMag Carboxy nanoparticles is 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.



1. Before each use, resuspend the SelfMag Carboxy nanoparticles by pipetting up and down or by vortexing 1 minute. Avoid foaming.
2. Transfer 100 μ L of the SelfMag Carboxy 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 μ L of **Buffer C1** 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 $\mu\text{mol/L}$ in the **Buffer C1**. 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 15-30 minutes at room temperature.
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 C1** in order to obtain a 1X EDC solution.
9. Add 5 to 10 μL of 1X EDC solution (5 to 10 μg) to the SelfMag Carboxy nanoparticles / "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).

2.3. Washing and Storage of Coupled Nanoparticles

All coupling procedures require washing of the molecule-conjugated SelfMag Carboxy nanoparticles to remove the excess of "ligand", EDC and the resulting by-product (isourea).

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 C2** by pipeting up and down. *Optionally*, in order to quench the non reacted activated carboxylic acid groups, resuspend the coupled nanoparticles with 100 μL of 1 mM Tris pH 7.4 instead of **Buffer C2**, then shake the mixture for 15 minutes at room temperature.
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 C2** by pipeting up and down.
5. Repeat steps 3 and 4 one or two times.

The use of MagID device avoids significant lost of nanoparticles during this procedure. Consequently, **100 μg of coupled SelfMag Carboxy 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.

2.4. Scale up/down of the Coupling Reaction

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 Carboxy 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 µmol	1 – 10	5 – 10 nmol	1	1 – 2	20
50	50	25 – 250 µmol	2.5 – 25	12.5 – 25 nmol	1	2.5 – 5	50
100	100	50 – 500 µmol	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

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. Please refer to the table 2 below as a starting point for experimental conditions. However, some optimization may be needed depending on the nature of the molecule coupled and the cell type.

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.

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

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.
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 β -Galactosidase

1. Resuspend the SelfMag Carboxy nanoparticles by pipeting up and down.
2. Transfer 100 μ L of the SelfMag Carboxy nanoparticles in a microtube and washed them with **Buffer C1** as described in section 2.2 above.
3. Prepare 100 μ L of a β -galactosidase solution at 5 μ mol/L in the **Buffer C1**.
4. Add 50 μ L (25 μ g, 0.2 nmol) of the β -galactosidase solution to the 100 μ L of SelfMag prepared previously. Then, vortex to ensure a good mixing and shake (agitator, mixer) the mixture 30 minutes at room temperature.
5. Prepare a 1X EDC solution (1 mg/mL) in the **Buffer C1** as described in section 2.2.
6. Add 5 μ L of 1X EDC solution (5 μ g) to the SelfMag Carboxy nanoparticles / β -galactosidase mixture. Vortex to ensure a good mixing.
7. Shake (agitator, mixer) the mixture for 2 hours at room temperature.
8. Wash the coupled nanoparticles with **Buffer C2** as described in section 2.3 above.
9. Finally, store the β -galactosidase coupled SelfMag in 100 μ L of **Buffer C2** at a concentration of 1 mg/mL.

The amount of β -Galactosidase coupled on the SelfMag was monitored by using the ONPG β -Galactosidase assay kit (OZ Biosciences, cat# GO10001). Results showed 0.45 pmol (54 ng) of linked β -Galactosidase per μ g of nanoparticles which correspond to a coupling efficiency of 23%. Efficiency can be optimized by varying various parameters such as incubation time, β -Galactosidase concentration, nanoparticles quantity and incubation temperature.

4.2. Intracellular Delivery of β -Galactosidase Coupled Nanoparticles

The instructions given below represent an example of protocol to deliver the β -Galactosidase 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 NIH-3T3 cells / well in a 24 well-plate in 400 μ L of DMEM complete culture medium (with serum).
2. Mix the β -Galactosidase-coupled SelfMag nanoparticles. Add 2 to 4 μ L (2 to 4 μ g) of β -Galactosidase 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 β -Galactosidase-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 delivery of β -galactosidase into cells was revealed using OZ Biosciences' X-Gal staining kit (cat # GX-10003) or OZ Biosciences' ONPG β -galactosidase assay kit (cat # G010001).

5. Appendix

5.1. Quality Controls

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

Components	Standard Quality Controls
SelfMag Carboxy Nanoparticles	<ol style="list-style-type: none"> 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	<ol style="list-style-type: none"> 1. Sterility. Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days. 2. Intracellular delivery assay. MagFectin efficacy to deliver β-Galactosidase-coupled SelfMag nanoparticles on NIH-3T3 cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.
Buffer solutions and EDC	<ol style="list-style-type: none"> 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 β-Galactosidase onto SelfMag. Every lot shall have an acceptance specification of > 90% of the activity of the reference lot.
MagID and Super Magnetic Plate	<ol style="list-style-type: none"> 1. Tests of solidity. 2. Test of the magnetic field force.

5.2. Troubleshooting

6. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate <i>(standard size for all cell culture support)</i> Mega Magnetic plate <i>(mega size to hold 4 culture dishes at one time)</i>
Transfection reagents:
PolyMag Neo <i>(for all nucleic acids)</i>
Magnetofectamine™ <i>(for all nucleic acids)</i>
NeuroMag <i>(dedicated for neurons)</i>
SilenceMag <i>(for siRNA application)</i>
Transfection enhancer:
CombiMag <i>(to improve any transfection reagent efficiency)</i>
Viral Transduction enhancers:
ViroMag <i>(to optimize viral transduction)</i>
ViroMag R/L <i>(specific for Retrovirus and Lentivirus)</i>
AdenoMag <i>(for Adenoviruses)</i>
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby <i>(siRNA transfection reagent)</i>
DreamFect Gold <i>(Transfection reagent for all types of nucleic acids)</i>
VeroFect <i>(for Vero cells)</i>
FlyFectin <i>(for Insect cells)</i>
i-MICST TECHNOLOGY
Viro-MICST <i>(to transduce directly on magnetic cell purification columns)</i>
3D TRANSFECTION TECHNOLOGY
3Dfect <i>(for scaffolds culture)</i> / 3DfectIN <i>(for hydrogels culture)</i>
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit <i>(for High Yield Protein Expression)</i>
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN <i>(delivery reagent for antibodies)</i> Pro-DeliverIN <i>(delivery reagent for protein in vivo and in vitro)</i>
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

Limited License

The purchase of this product 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 purposes 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 this product. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, please contact us.

Buyers may end this License at any time by returning the product and documentation to OZ Biosciences, or by destroying all components. Purchasers are advised to contact us with the notification that the product 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 the 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

This product 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.



www.bocascientific.com

(781) 686-1631

info@bocascientific.com