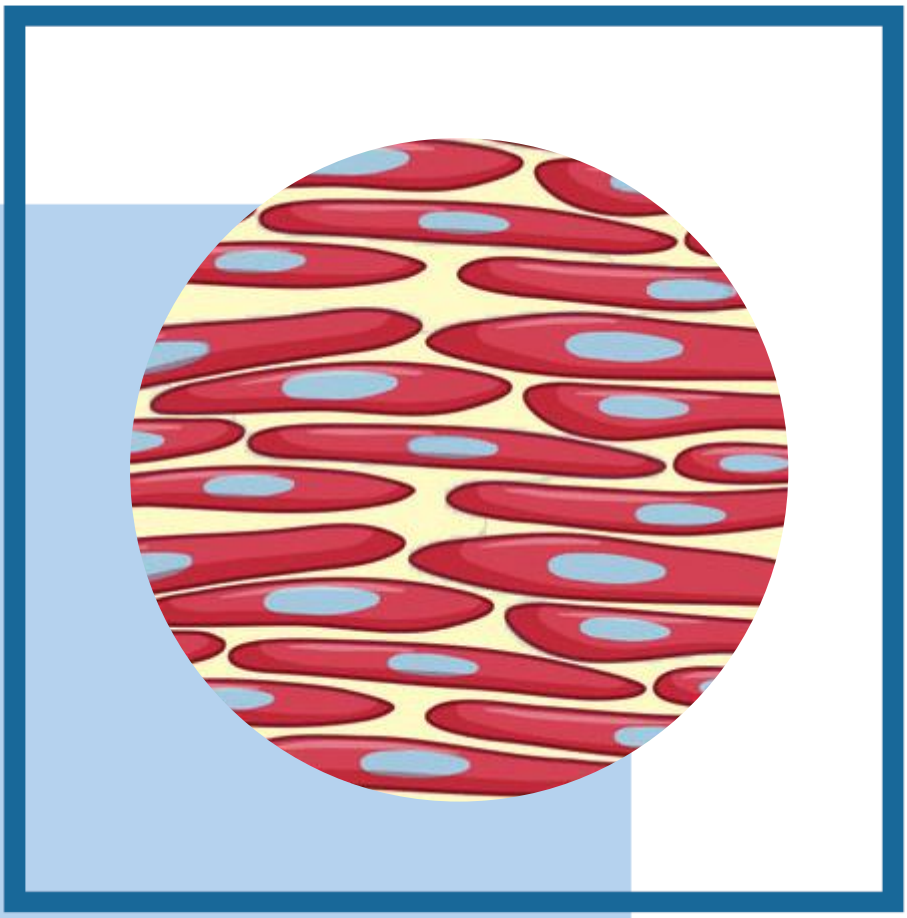
XPMag - Transfection Reagent

INSTRUCTION MANUAL



Reverse Magnetofection

This reagent needs to be used with magnetic plate

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IMPORTANT NOTES – Before you begin

- ✓ XPMag, is a novel magnetic nanoparticles formulation dedicated to gene transfection in organotypic cultures of explant by "Reverse Magnetofection".
- ✓ "Reverse Magnetofection" provides a novel and non-toxic strategy for nucleic acids (DNA & siRNA) based gene therapy in the retina that can be translated to a wide variety of organ explants.
- ✓ XPMag, associated with Reverse Magnetofection allows the delivery of nucleic acids (NA) up to the deepest explant layers.
- ✓ The magnetic force, delivered by a specific magnetic plate, guides and concentrates complexes of NA and XPMag magnetic nanoparticles from the culture media to the tissue.
- ✓ This novel method of transfection associating XPMag and Reverse Magnetofection is fully biocompatible and does induce neither apoptosis nor inflammatory reactions.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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XPMag Reagent | Specifications

Package content	**XP00250**: 250 µL of XPMag reagent **XP00500**: 500 µL of XPMag reagent **KXP0250**: XPMag Starting Kit - 250 µL of XPMAg reagent + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the XPMag transfection reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	XPMag is a novel magnetic nanoparticles formulation dedicated to gene transfection in organotypic cultures of explant by "Reverse Magnetofection".
Important notice	For research use only. Not for use in diagnostic procedures

Applications and Protocols

1. General Considerations

- Allow reagents to reach room temperature before starting
- This reagent needs to be used with a dedicated magnetic plate delivering a specific magnetic field
- It is mandatory to use medium without any supplement for the preparation of complexes
- Replace culture medium 24h after transfection; perform assay 48 to 96h after Reverse Magnetofection (depending on the layer to reach and on the gene to express or silence).

2. Organotypic culture of Explants

Use your already-settled protocol for preparing *ex vivo* cultures of explants; we recommend placing organotypic culture of explants on 0.4 µm polycarbonate membrane culture insert into 1 mL of culture medium. Considering retinal explant, place RPE layer on the membrane side down facing the medium and ganglion layer cells up.

Tissue culture dish format	Insert diameter per well[1]	Thickness	Recommended volume for culture	Recommended volumes of XPMag
0.4 µm polycarbonate membrane	24 mm	10 µm	1 mL	2.5-3.5 µL

[1] diameter may vary depending on the manufacturer.

Table 1: Recommended volume of XPMag for complexes preparation

3. Standard Protocol

The following protocol sets the basis to deliver nucleic acids (DNA and siRNA) in explant using XPMag. We recommend starting with a 200 µL suspension of 50 nM siRNA or 2 µg DNA with respectively 2.5 and 3.5 µL XPMag according to the table below. Refer to paragraph 2.4 to optimize transfections.

	XPMag magnetic nanoparticles volumes:
50 nM siRNA in 200 µL	2.5 µL
2 µg DNA in 200 µL	3.5 µL

Table 2: Recommended volumes of XPMag depending on the nucleic acid to use

1) Dilute nucleic acid in **200 µL** medium without any supplement according to table 2
2) Prepare a tube containing **2.5** or **3.5 µL** of XPMag
3) Add nucleic acid suspension to XPMag
4) Incubate **30 min** at RT to ensure a correct formation of complexes
5) Add complexes to the **800 µL** of culture medium
6) Place the culture membrane support carrying explant onto the medium containing the complexes
7) Apply **Reverse Magnetofection** by placing the magnetic plate **above the lid** of the culture plate
8) Incubate **30 min** at **37°C**
9) Remove the Magnetic plate and incubate under standard culture conditions
10) Replace medium with fresh medium after 24 hours.
11) Incubate 24 h to 96 h.

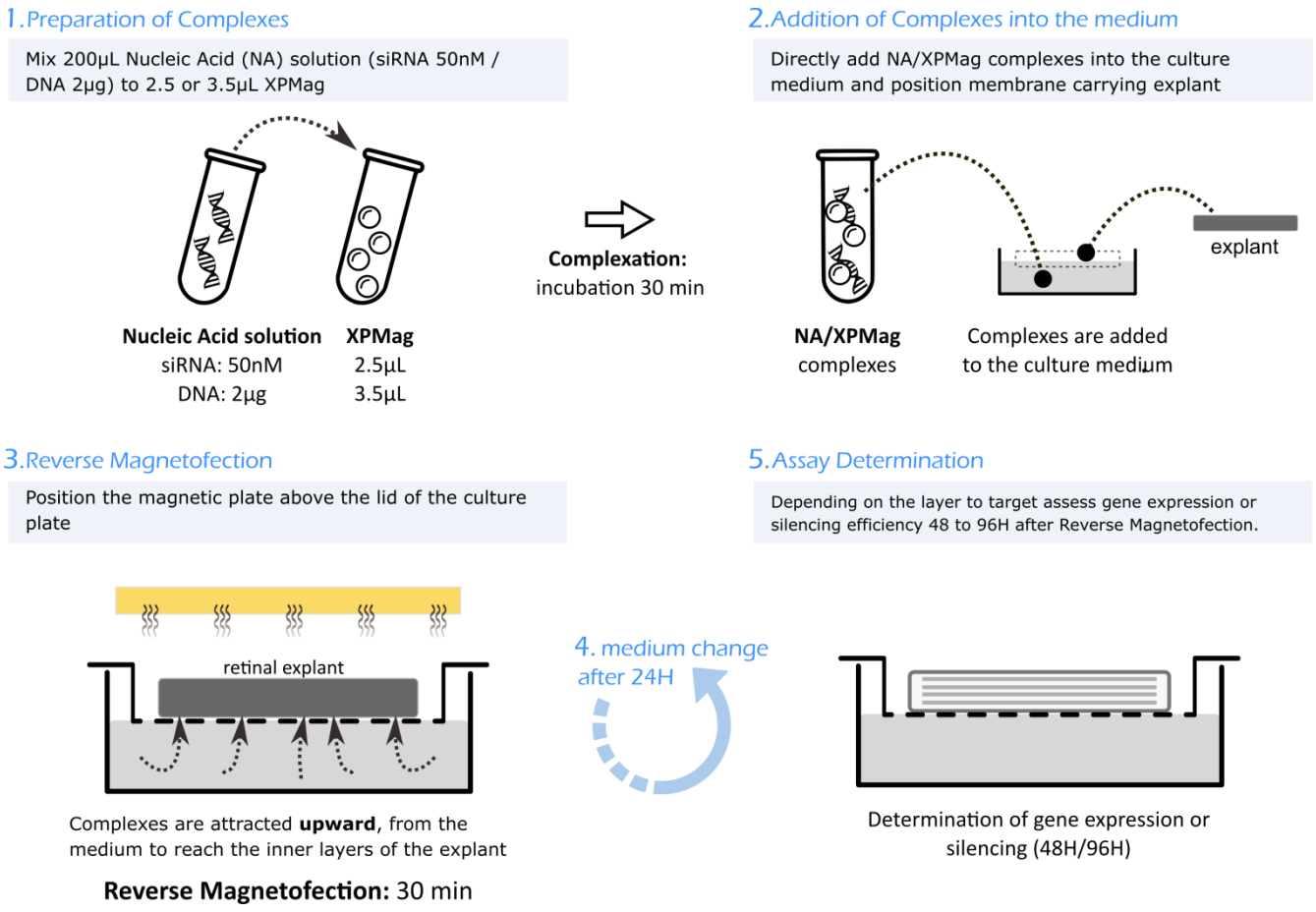

Figure 1: XPMag standard operative procedure for transfection of explant by Reverse Magnetofection. (1) Complexes of nucleic acids (recommended: siRNA 50 nM / DNA 2 µg) and XPMag (recommended: siRNA 2.5 µL / DNA 3.5µL per 1mL culture medium for cultivating explants on 24 mm transwell membrane inserts) are formed and incubated 30 minutes at RT. (2) After complexation, NA/XPMag solution is added directly into the medium underneath the explant and a magnetic plate is place above the lide of the culture plate for 30 min RT (3). After 24H, the medium is replaced with fresh medium (4) and depending on the layer and/or on the gene to express or target, gene expression or silencing can be monitored from 48 h to 96 h after reverse Magnetofection (5).

Optimization Protocol

Transfection conditions for Reverse Magnetofection may vary depending on the nucleic acid (siRNA/DNA…), the gene to express or silence, and inner layer to reach, (…). It is thus highly recommended to optimize the conditions in order to find the best nucleic acid concentrations with the ideal volume of XPMag magnetic nanoparticles; Table 3 below gives an example of volumes to try.

	XPMag magnetic nanoparticles volumes:		
siRNA concentration in 200µL: 25nM / 50nM /100nM	2 µL	2.5 µL	3 µL
DNA amount in 200µL: 1µg / 2µg / 3µg	3 µL	3.5 µL	4 µL

Table 3: Recommended volumes of XPMag depending on the nucleic acid to use

Additional products

- **NeuroMag** dedicated to neurons transfection
- **Glial-Mag** dedicated to microglial cell lines and primary cells.
- **BrainFectIN** for *in vivo* transfection in central nervous system

Purchaser Notification

Limited License
The purchase of the XPMag kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the XPMag kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all XPMag kit reagents and documentation to OZ Biosciences, or by destroying all XPMag components. Purchasers are advised to contact OZ Biosciences with the notification that a XPMag kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the XP Mag kit 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
XPMag kit 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.

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