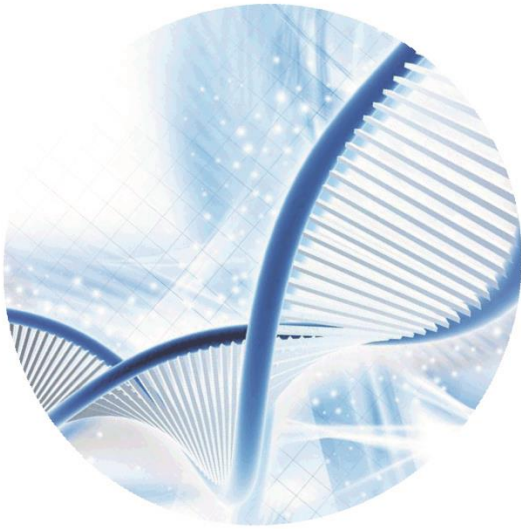




Transfection reagent

BrainFectIN™

Protocol

IMPORTANT NOTES – Before you begin

- ✓ **BrainFectIN™** transfection reagent is very efficient to deliver nucleic acids (DNA, mRNA, siRNA) into central nervous system of small animals.
- ✓ This non-viral formulation - rapid, easy and safe - allows high transfection efficiency of target area following stereotaxic injection.
- ✓ **BrainFectIN™** has been designed by our R&D team to meet *in vivo* grade quality (reagents performed under high manufacturing and quality standards and tested by strict quality controls).

BrainFectIN™ main advantages:

1. Good transfection efficiency
2. Targeting of specific regions (stereotaxic injection)
3. Reduction of the injected volume
4. Reduced DNA doses
5. Minimized toxicity
6. Low immunogenicity
7. Rapid and long-term transgene expression

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



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Any questions?



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Package content	IV-BF30100: 1 tube containing 100 µL of BrainFectIN™ suitable for 20-30 injections. IV-BF30250: 1 tube containing 250 µL of BrainFectIN™ suitable for 50-80 injections. IV-BF30500: 1 kit containing 500 µL of BrainFectIN™ suitable for 100-160 injections
Shipping conditions	Room temperature.
Storage conditions	Upon receipt and for long-term use, store at -20°C .
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

BrainFectIN™ has been developed for *in vivo* delivery of DNA, mRNA or siRNA into the central nervous system. The instructions given hereunder represent a protocol that was successfully applied in several studies. Nevertheless, optimal conditions may vary depending on the nucleic acid and the animal model.

1. General protocol for DNA transfection

The nucleic acid solution and the BrainFectIN™ reagent should be at room temperature. We recommend injecting 0.25µg to 1µg of DNA into Rat with **1.5 µL of BrainFectIN™ per µg of DNA**.

1. Reagents preparation.

- Due to dead volume, we suggest to always prepare extra-volume of **BrainFectIN/DNA** mix per animal (recommendation: 5 to 10 µL of mixture par animal)
- DNA preparation should be in water at the concentration of 2 to 3 µg/µL to reduce the injected volume.
- Before each use, vortex **BrainFectIN™** vial and add the required volume (1.5 µL of BrainFectIN™ per µg of DNA) into a sterile microtube.

2. Complexes formation.

- Add DNA solution directly to the **BrainFectIN™** and mix immediately by pipetting up and down.
- Incubate the complexes for 15 to 20 min at room temperature.

3. Animal preparation

- Determine the coordinates used for the injection.
- Inject anesthesia and analgesic solutions to your animal.
- Stabilize the animal on stereotaxic frame and proceed to surgical procedure.

4. Injection procedure:

- Slowly inject 1µL of mix **BrainFectIN/DNA** complexes over 4 min (rate 250 nL/min). A range of 1 to 3 µL of mix can be injected into a rat, depending on the location, the age of the animal, the number of transfected cells expected etc. Generally, 1, 1.5, or 2 µL is well tolerated by the rat.
- Wait 4 more minutes to ensure a correct diffusion of the mix after injection.
- Slowly pull the needle up.

5. Monitor gene expression at least 48h after injection.

Important notes:

- Prepare DNA as pure as possible, free of endotoxins and prepared in water.
- Do not inject complexes if precipitates have formed.
- Use high concentrated DNA solution (2-3µg/µL) to reduce the mix volume.
- Due to dead volume during injection, always prepare an extra-volume of **BrainFectIN/DNA** mix.
- We recommend to prepare 5 to 10 µL of complexes par animal. Volume of injection can be between 1 to 3 µL for a rat.

The amount of DNA and maximum injection volume depend on the nucleic acid and the animal model.

2. Example of protocol for DNA transfection in young rat hippocampus

- Protocol for one animal:
- Prepare a DNA solution at 2µg/µL in H₂O
- Prepare a microtube containing 6µL of BrainFectIN™
- Admix 2µL of DNA into BrainFectIN solution
- Mix well and incubate 20 min at room temperature
- Prepare your animal for stereotaxic injection
- Slowly inject 1µL of BrainFectIN/DNA mixture (injection rate 250nL/min) – 0.5 µg of DNA injected.
- Wait 4 min before to slowly pull the needle up
- Proceed to analyses at least 48h after injection

3. Optimization protocol for DNA transfection

Some optimization may be needed in order to obtain the maximum efficiency. Several parameters can be optimized.

1. Amount of nucleic acid used

To achieve the optimal transfection efficiency, the DNA amount can be increased or decreased. We recommend injecting between 0.25 to 1 µg of DNA per injection in a rat.

2. Ratio of BrainFectIN™ to nucleic acid

BrainFectIN™ amounts can vary from 1µL to 3µL per µg of DNA.

3. Volume injected

Depending on the targeted brain area, the volume of mix injected can be increased or decreased. Volume of injection can be between 1 to 3 µL into a rat. Larger or smaller volume can be considered depending on the animal.

4. Read-out time point

Depending on your DNA sequence, promoter activity, transgene expression product, the optimal time point for read-out may vary. Usually, the transfection efficiency can be monitored 48h up to 1 month after injection.

Appendix

1. Quality Controls

To guarantee the performance of each lot of **BrainFectIN™** produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
<i>Sterility</i>	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 14 days.
<i>Biological Activity</i>	<i>In vitro</i> transfection efficiency. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.
<i>In vivo grade</i>	Endotoxin Quantification. Endotoxins level shall be < 0.1 EU/mL for every lot.

Additional products for *In vivo* delivery

- ***In vivo ViroMag***, is an optimized nanoparticles formulation dedicated to viral vectors that allows reduction of titer virus. It is particularly suitable for Lentiviral/Retroviral, Adenoviral and Adeno-Associated Viral (AAV) vectors.
- ***In vivo PolyMag***, a cationic polymer-based magnetic nanoparticles formulation, designed for *in vivo* targeted transfection of nucleic acids.
- ***In vivo DogtorMag***, a cationic lipid-based magnetic nanoparticles formulation, designed for *in vivo* targeted transfection of nucleic acids.
- ***In vivo SilenceMag***, a cationic lipid-based magnetic nanoparticles formulation, designed to transfect small RNA, into target cell/ tissue *in vivo*.

Purchaser Notification

Limited License

The purchase of the BrainFectIN™ 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 BrainFectIN™. 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 BrainFectIN™ reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a BrainFectIN™ 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 BrainFectIN™ 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

BrainFectIN™ 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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