BrainFectIN™ Transfection Reagent

INSTRUCTION MANUAL

In vivo Gene Delivery

Nucleic Acid delivery into central nervous system of small animals

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The art of delivery systems

BrainFectIN™

Instruction Manual

BrainFectIN™ is an original and efficient transfection reagent dedicated to Nucleic Acid delivery (DNA, mRNA, siRNA) into small animal brain by stereotaxy technique.

List of **BrainFectIN™** kits

Catalog Number	Description	Volume	Number of injections*
IV-BF30100	**BrainFectIN™** 100	100 µL	20-30 injections
IV-BF30250	**BrainFectIN™** 250	250 µL	50-80 injections
IV-BF30500	**BrainFectIN™** 500	500 µL	100-160 injections

*represent an average number of injection in mice and rats.

Use the content of the list above to determine the appropriate catalog number for your needs. You can order these products by contacting us (phone, fax, email & website). For all other information, do not hesitate to contact our technical support (tech@ozbiosciences.com or techusa@ozbiosciences.com).

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1. Technology

1.1. Description

BrainFectIN™ transfection reagent is very efficient to deliver nucleic acids 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

1.2. Kits Content, Stability and Storage

Kits content differs according to their volumes:

- 1 tube containing 100 µL of **BrainFectIN™** suitable for 20-30 injections.
- 1 tube containing 250 µL of **BrainFectIN™** suitable for 50-80 injections.
- 1 kit containing 500 µL of **BrainFectIN™** suitable for 100-160 injections - 2 tubes, 250 µL each.

Stability, Storage and Shipping conditions:

Storage: Upon receipt and for long-term use, store at -20°C.

Stability: **BrainFectIN™** reagent is stable for at least 12 months at the recommended storage temperature.

Shipping condition: room temperature.

2. Applications

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.

3. Protocols

3.1. 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.

3.2. 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

3.3. 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.

4. Appendix

Our dedicated and specialized technical support team will be pleased to answer any of your requests and to help you with your experiments at tech@ozbiosciences.com or techusa@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

4.1 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
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 14 days.
Biological Activity	*In vitro* transfection efficiency. Every lot shall have an acceptance specification of > 85% of the activity of the reference lot.
In vivo grade	Endotoxin Quantification. Endotoxins level shall be < 0.1 EU/mL for every lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low efficiency	1- **Nucleic acid amount.** Use different quantity of DNA with the recommended transfection reagent / nucleic acid ratio. 2- **DNA quality.** Nucleic acids should be as pure as possible, free of contaminants (proteins, phenol, ethanol…) and endotoxins free. It must be "transfection grade". 3- **Injection volume.** Optimize the volume of injection to your application. 4- **Speed of injection.** Transfection efficiency could be dependent on the injection speed, adapt your speed of injection to your target tissue. 5- **Transfection reagent temperature.** Reagents must be at room temperature and be vortexed prior to use. 6- **Old transfection reagent / DNA complexes.** The transfection reagent / DNA complexes must be freshly prepared every time. Complexes prepared and stored for longer than 2 hours can be aggregated.

	7- **Incubation time.** Optimal time range between transfection and assay varies with tissue, target gene, etc. Transfection efficiency can be monitored after 48h by analyzing the gene expression.
Toxicity	1- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes injected by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and never inject complexes in which precipitate has formed. 2- **DNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free.
Precipitate formation	1- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes injected by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and never inject complexes in which precipitate has formed. 2- **DNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Prefer water rather than buffer (TE, TRIS) for your DNA preparation.

5. Related Products

MAGNETOFECTION TECHNOLOGY

***In vivo* Transfection/Tranduction reagents**:
In vivo PolyMag & *In vivo* DogtorMag -for all nucleic acids
In vivo ViroMag -for all viral vectors
In vivo SilenceMag - for siRNA application

***In vitro* Transfection/Transduction reagents**:
NeuroMag - *dedicated for neurons*
SilenceMag - *for siRNA application*
PolyMag Neo - *for all nucleic acids*
ViroMag R/L - *specific for Retrovirus and Lentivirus*
AdenoMag - *for Adenoviruses*

PLASMIDS PVECTOZ

pVectOZ-LacZ, Luc, CAT, GFP, SEAP

ASSAY KITS

Bradford – Protein Assay Kit
OZBlue cell viability kit
β-Galactosidase assay kits (CPRG/ONPG)
Luciferase assay kit

BIOCHEMICALS

D-Luciferin, K^+ and Na^+
X-Gal / G-418 Sulfate

Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list.
contact@ozbiosciences.com / www.ozbiosciences.com

Purchaser Notification

Limited License for BrainFectIN™
The purchase of BrainFectIN™ Reagents 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 transfection and transduction of nucleic acids and virus 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.

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BrainFectIN™ Reagents and all of its components are developed, designed, intended, and sold for research use only. They are not meant 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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