jetSI 10 mM

in vivo siRNA/miRNA delivery to the brain

PROTOCOL

DESCRIPTION

jetSI 10 mM is a powerful siRNA/miRNA delivery reagent that ensures good gene silencing in the brain. jetSI 10 mM is synthesized and purified at Polyplus-transfection. jetSI 10mM efficiently delivers siRNA/miRNA duplexes to mammalian cells and is particularly well suited for *in vivo* delivery into the brain

jetSI 10 mM and siRNA/miRNA duplexes form positively charged particles capable of interacting with anionic proteoglycans at the cell surface and entering cells by endocytosis. Due to its properties, it protects siRNA/miRNA duplexes from degradation and favors rapid endosomal release into the cytoplasm.

Publications using jetSI 10 mM can be found on the Polyplus-transfection Database, available on the Polyplus website, www.polyplus-transfection.com.

1 GENE SILENCING IN THE MOUSE BRAIN PROTOCOL

1.1 PREPARATION OF jetSI/DOPE STOCK SOLUTION (SOLUTION A)

Dilute 100 mg of DOPE (to be purchased separately, see p7) into 300 µl of chloroform in a capped glass tube and wait until it is totally dissolved. Add 1.36 ml of 100% ethanol to obtain a solution at 80 mM. Mix 20 µl of jetSI 10 mM and 5 µl of DOPE 80 mM to obtain 25 µl of solution A. Solution A can be stored at -20°C for one month.

1.2 PREPARATION OF COMPLEXES AND TRANSFECTION PROCEDURE

Note:

- *a- Check the concentration of the siRNA duplexes (even if provided by the manufacturer)*
- *b- Use desalted siRNA as the presence of salt favours aggregates formation, inhibiting in vivo siRNA delivery*
- *c- Use RNase and pyrogen free materials (Tips, tubes, low binding microtubes, solutions)*

1.2.1. Preparation of solution B

Just before use, add 15 µl of 100% ethanol to the 25 µl of solution A in order to obtain a final solution of 40 µl of jetSI 5 mM/DOPE 10 mM called solution B. Solution B cannot be stored. If using a frozen aliquot of solution A, ensure the aliquot is thawed and equilibrated at room temperature before use.

1.2.2. Preparation of the complexes

The following protocol is sufficient to prepare material for 50 brain injections in mice using siRNA duplexes at 0.1 µg/µl for a total injection volume of 1 µl per mouse. Refer to Table 1 for other siRNA delivery conditions. Preparation of the siRNA/reagent mix is usually performed in a 50 µl total volume as larger volumes tend to form aggregates.

• Dilute 2 µl of solution B and 5 µl of 25% glucose into 18 µl of pure RNAse free water in order to obtain a total volume of 25 µl with a final concentration of 5% glucose called solution C. **Vortex vigorously** (important: do not pipet to mix) and wait for 10 min (important: do not exceed 30 minutes).

• Dilute 5.6 µg siRNA duplexes into a final volume of 25 µl with a final glucose concentration of 5%. Vortex gently.

• Add the 25 µl solution C into the 25 µl siRNA duplexes solution all at once (important: do not mix the solutions in the reverse order).

• **Immediately** vortex-mix the solution for 10 seconds.

• Incubate for 30 minutes at room temperature to allow complexes to form (do not exceed 1 hour).

• Inject 1 µl of the solution C/siRNA mixture per mouse using stereotaxic injection procedure (Note that the procedure was optimized on newborn mouse brains using the intracerebroventricular route).

• Gene silencing is measured at suitable time points (usually between 24 h – 1 week)

Detailed transfection conditions

The volume of solution B needed according to the quantity of siRNA delivered can easily be calculated following to the formula:

$$\text{µl of solution B} = \frac{\text{µg of siRNA} \times 1.8}{[\text{conc. of jetSI} \quad \text{in solution B}]}$$

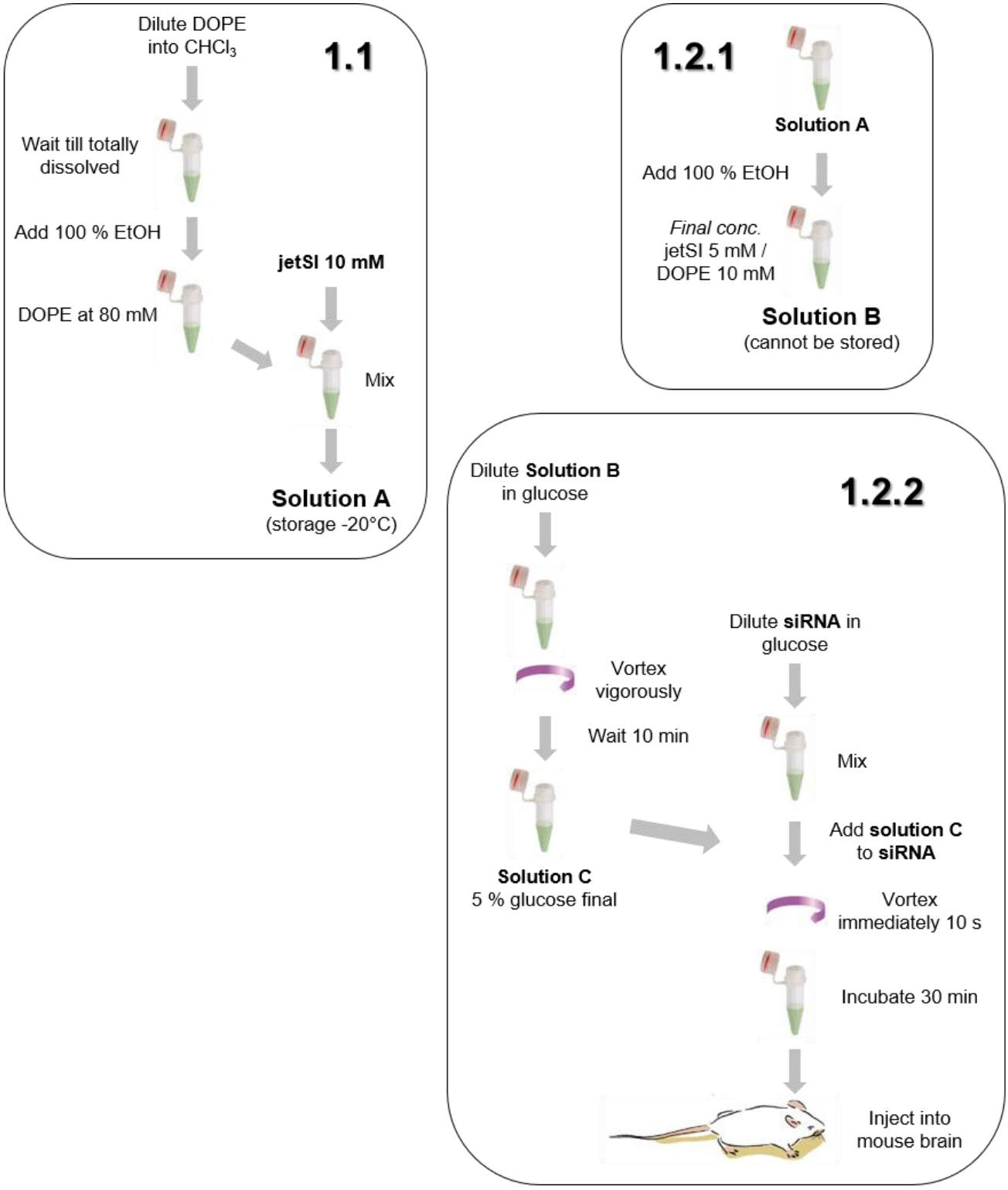
Dilute DOPE into $CHCl_3$
1.1
Wait till totally dissolved
Add 100 % EtOH
DOPE at 80 mM
jetSI 10 mM
Mix
Solution A
(storage -20°C)
1.2.1
Solution A
Add 100 % EtOH
Final conc.
jetSI 5 mM /
DOPE 10 mM
Solution B
(cannot be stored)
Dilute Solution B in glucose
1.2.2
Vortex vigorously
Wait 10 min
Solution C
5 % glucose final
Dilute siRNA in glucose
Mix
Add solution C to siRNA
Vortex immediately 10 s
Incubate 30 min
Inject into mouse brain

Table 1: Volumes of solution B required according to various siRNA amounts.

	400 pmoles (5.6 µg) siRNA	200 pmoles (2.8 µg) siRNA	100 pmoles (1.4 µg) siRNA	80 pmoles (1.2 µg) siRNA	60 pmoles (0.84 µg) siRNA	40 pmoles (0.56 µg) siRNA	20 pmoles (0.28 µg) siRNA
Volume of solution B	2 µl	1 µl	0.5 µl	4 µl*	3 µl*	2 µl*	1 µl*
Volume of RNAse free water	18 µl	19 µl	19.5 µl	16 µl	17 µl	18 µl	19 µl
Volume of 25 % glucose	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl
Total volume of solution C	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl
Volume of 20 µM siRNA	20 µl	10 µl	5 µl	4 µl	3 µl	2 µl	1 µl
Volume of RNAse free water	0 µl	10 µl	15 µl	16 µl	17 µl	18 µl	19 µl
Volume of 25 % glucose	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl
Total volume of diluted siRNA	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl

* use a 1/10 dilution of solution B into 100% ethanol.

IMPORTANT: 0.1 µg/µl is the maximum recommended siRNA concentration to be injected.

2 TROUBLESHOOTING

Observations	Actions
Low delivery efficiency	Optimize the amount of siRNA used.
	Use high-quality siRNA (PAGE purified and desalted) in order to prevent aggregation.
	Optimize the solution B / siRNA duplexes ratio.
Cellular toxicity	Decrease the amount of siRNA used, always keeping the solution B / siRNA duplexes ratio constant.
	Check that silencing of the target gene does not affect animal survival.

3 PRODUCT INFORMATION

3.1 ORDERING INFORMATION

Ref #	Size	Comment
403-05	0.5 ml	Sufficient for *in vivo* delivery of 2.5 mg of siRNA

3.2 CONTENT

0.5 ml of jetSI 10 mM is sufficient to deliver up to 2.5 mg of siRNA in the mouse brain (c.a. 100 ng of siRNA per injection).

3.3 ADDITIONAL MATERIAL REQUIRED

DOPE is required to formulate jetSI 10 mM for delivery into the brain. It can be purchased as powder from your regular chemical supplier or from Sigma, Ref P1223.

A 25% glucose solution is required and should be prepared by dissolving ≥99.5% of D-glucose in RNAse and DNAse free Ultrapure water, and further sterilized using a 0.22 µm filter.

3.4 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

3.5 FORMULATION AND STORAGE

jetSI 10 mM transfection reagent is provided at a concentration of 10 mM. jetSI 10 mM should be stored at **4°C** upon arrival (tightly capped). **Do not freeze. Always ensure the tube is tightly closed after use to avoid evaporation.** jetSI 10 mM is stable for 6 months at 4°C.

3.6 TRADEMARKS

Polyplus-transfection is a registered trademark of Polyplus-transfection.

How to cite us: “jetSI (Polyplus-transfection S.A, Illkirch, France)”

3.7 TECHNICAL ASSISTANCE AND SCIENTIFIC ADVICE

Contact the friendly Polyplus technical support *via*:

- The Polyplus website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87