

jetSI™ 10 mM *in vivo* siRNA Delivery Protocol into the Brain

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Company Information

Technical Assistance and Scientific Advice

Contact the friendly Polyplus technical support *via*:

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Product Information

jetSI™ 10 mM is a powerful siRNA 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 duplexes to mammalian cells and is particularly well suited for *in vivo* delivery into the brain. jetSI™ 10 mM and siRNA 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 duplexes from degradation and favors rapid endosomal release into the cytoplasm. Publications using jetSI™ 10 mM can be searched on the Product Citation Database, available on the Polyplus website, www.polyplus-transfection.com, under the Products/Technical Resources heading.

Ordering information

Cat #	Size	Comment
403-05	0.5 ml	Sufficient for <i>in vivo</i> delivery of 2.5 mg of siRNA

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).

Additional material required

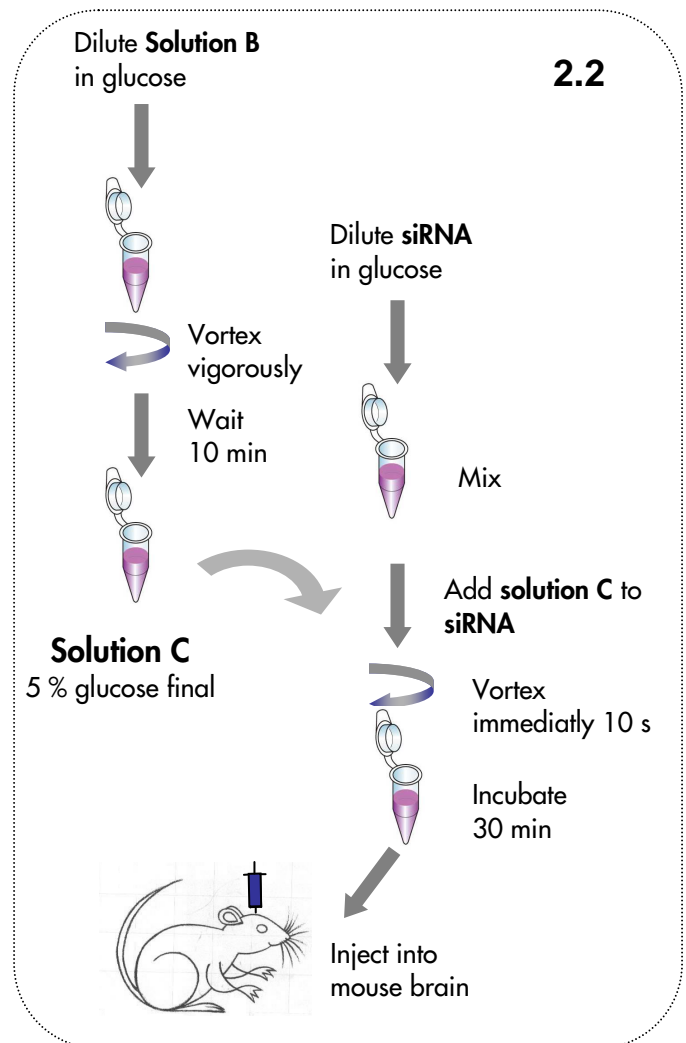
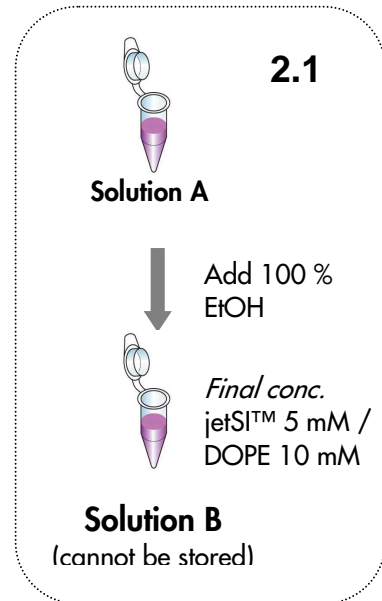
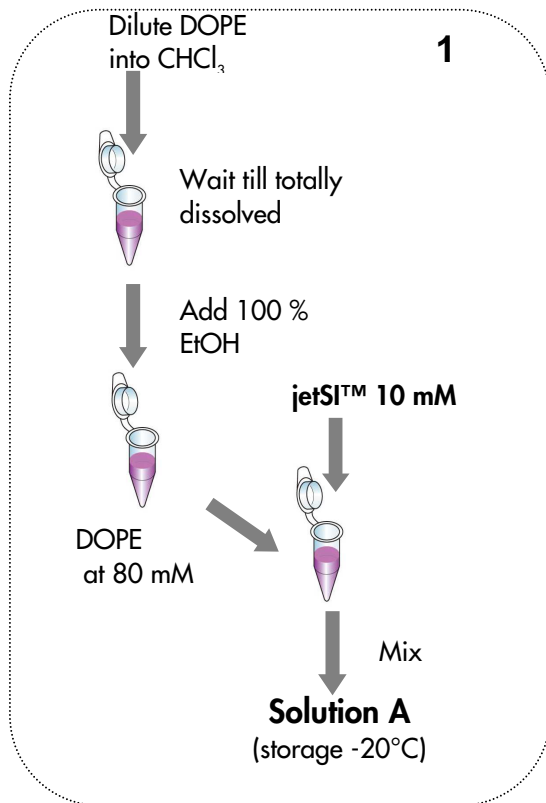
DOPE is required to formulate jetSI™ 10 mM for delivery into the brain. It can be purchased as powder from your regular chemical supplier or from Sigma, Cat P1223.

Reagent use and Limitations

For research use only. Not for use in humans.

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 the tube after use to avoid evaporation.** jetSI™ 10 mM is stable for 6 months at 4°C.



Protocol

siRNA delivery for gene silencing in the mouse brain

1. Preparation of jetSI™/DOPE stock solution (solution A)

Dilute 100 mg of DOPE (to be purchased separately, see p3) 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.

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)*

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 can not be stored.

If using a frozen aliquot of solution A, ensure the aliquot is thawed and equilibrated at room temperature before use.

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:

$$\mu\text{l of solution B} = \frac{\mu\text{g of siRNA} \times 1.8}{[\text{conc. of jetSI}^{\text{TM}} \text{ in solution B}]}$$

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.

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Troubleshooting

Observations	Actions
Low delivery efficiency	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• 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.

Notes