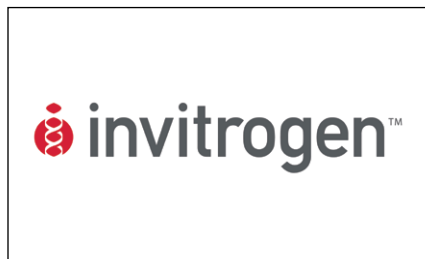


Stealth RNAi™ siRNA brain infusion



Invitrogen

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www.biotechniques.com/protocols/113289

Stealth RNAi™ siRNAs are highly potent, specific, and stable siRNA duplexes that are particularly suited for in vivo applications. This protocol describes the in vivo delivery of Stealth RNAi™ siRNA to mouse brain using an osmotic minipump.

Materials

Mouse (six months old)

Reagents: **BLOCK-iT™ Alexa Fluor® 555 Red Fluorescent Control**. In vitro–validated **Stealth RNAi™ siRNA** against gene of interest and **Stealth RNAi™ siRNA Negative Control**. **Osmotic minipumps** (Alzet model 1007D and 1002, Cupertino, CA, USA)

Methods

1. Design Stealth RNAi™ siRNAs against the target of interest. See www.invitrogen.com/rnai for design and ordering. Select the most potent Stealth RNAi™ siRNA in vitro by transfecting it into a cell line expressing the gene of interest. If no cell line is available, the most potent Stealth RNAi™ siRNA can be selected by doing a co-transfection with Stealth RNAi™ siRNA and the plasmid DNA containing the ORF of your gene. In addition, the gene of interest can be cloned behind the *lacZ* reporter gene to facilitate the screening (www.invitrogen.com).

It is important to titrate the siRNA to a concentration (pmol range) to be able to select the most potent Stealth RNAi™ duplex. For siRNA transfection, we recommend using Lipofectamine® RNAiMAX Transfection Reagent, and for a co-transfection study, we recommend using Lipofectamine® 2000 Transfection Reagent.

2. For any in vivo experiment, we recommend using in vivo–purity Stealth RNAi™ siRNAs. For this protocol, you will need ~600 µg Stealth RNAi™ siRNA per mouse. The doses used were based on preliminary studies published by Thakker et al. (1). Dilute Stealth RNAi™ siRNAs in saline solution to a final concentration of 250 µM (4.25 mg/mL).

3. Preload 150 µL of a 250 µM (4.25 mg/mL) solution of Stealth RNAi™ siRNA diluted in saline, in the Alzet minipump. Use an Alzet minipump 1007D for infusion of the BLOCK-iT™ Alexa Fluor® 555 Red Fluorescent Control (1 week at 6 µL per

day) and the Alzet minipump 1002 for infusion of the Stealth RNAi™ siRNA for 2 weeks at 6 µL per day. Place mice under anesthesia on a Kopf stereotaxic apparatus, and determine coordinates by means of the Franklin and Paxinos atlas. Stereotaxically place the Alzet minipump with a brain infusion cannula in the lateral ventricle following manufacturer's instructions. To allow diffusion of the solution into the brain tissue, leave the cannula for 2 weeks at a flow rate of 6 µL/day.

4. Characterize levels of target protein by Western blot and ELISA analysis after intracerebral delivery of Stealth RNAi™ siRNA.

5. For immunohistochemistry analysis, remove brains and post-fix in phosphate-buffered 4% PFA (pH 7.4) at 4°C for 48 hr for neuropathological analysis as previously described (2).

References

1. Thakker, D.R., Natt, F., Hüskén, D., et al. 2004. Neurochemical and behavioral consequences of widespread gene knockdown in the adult mouse brain by using nonviral RNA interference. *Proc Natl Acad Sci USA* 101:17270–17275.

2. Singer, O., Marr, R.A., Rockenstein, E., et al. 2005. Targeting BACE1 with siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model. *Nat. Neurosci.* Oct. 8:1343–1349.

Contact

Invitrogen, part of Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008, USA. 800 955 6288, www.invitrogen.com

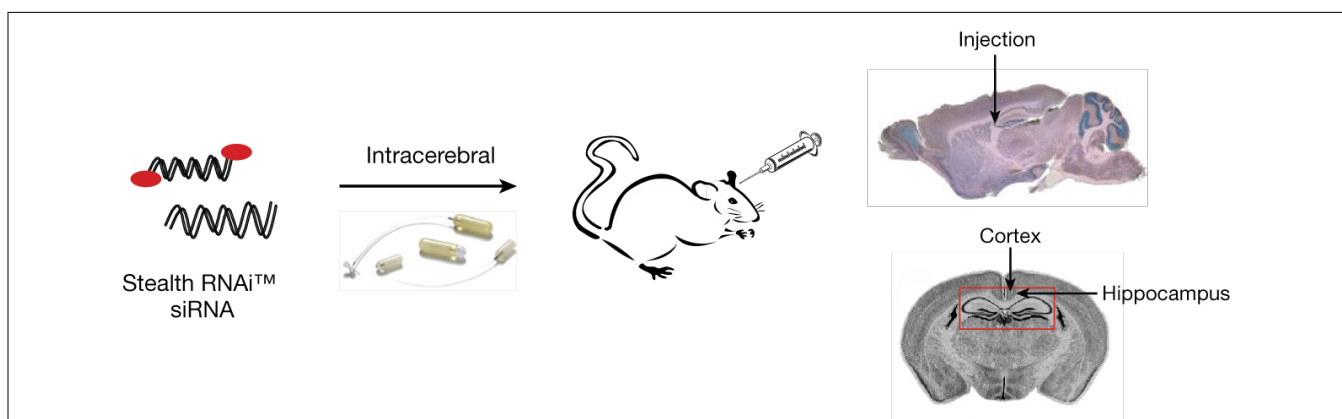


Figure 1. Stealth RNAi™ in vivo delivery to mouse brain. A cannula was inserted into the lateral ventricle of the brain. Stealth RNAi™ siRNA solution (4.75 mg/mL in PBS) was infused via cannula at 6 µL/day for 2 weeks in the lateral ventricle using an osmotic mini pump. For data and further information, contact rnairesearcher@invitrogen.com.