Product Number(s): NI35150, NI35750

NEUROMICS

i-Fect™ siRNA Transfection Kit

SUPPLEMENT: Intrathecal Delivery of siRNA Instruction Manual

METHODS AND PROCEDURES

i-Fect Protocol for Intrathecal Administration of siRNA

Protocol developed in collaboration with Miaw-Chyi Luo, Ph.D. and Josephine Lai, PhD, University of Arizona.

Reference:

Luo MC, Zhang DQ, Ma SW, Huang YY, Shuster SJ, Porreca F, Lai J. **An efficient intrathecal delivery of small interfering RNA to the spinal cord and peripheral neurons.** *Mol Pain. 2005 Sep 28; 1:29.*

siRNA Preparation

To date, we have tested exclusively siRNA and control RNA that are synthesized initially as 2 separate complementary strands by solid phase synthesis, de-protected and RNase-free HPLC purified. The RNA preparations are reconstituted separately to a concentration of 200 µM in annealing buffer as described (Elbashir et al, 2001). RNA duplexes are formed by mixing equal volume of the RNA solutions for 3 min at 90°C followed by 60 min at 37°C to yield a final concentration of 100 µM double stranded RNA. The RNA duplexes are aliquoted (typically in 20µL) and stored in –80°C. Always avoid repeated freezing and thawing of the samples.

Reference:

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001) **Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells.** Nature 411:494-8.

Catheter implantation for the intrathecal delivery of siRNA into rats

The surgical procedure for intrathecal catheter implantation that we have exclusively employed is that described by Yaksh and Rudy (1976).

The catheter is directed to the lumbar region of the spinal cord and the external opening should be heat-sealed until use. It is highly recommended that the experimental animals should be allowed to recover for 5 to 7 days post-surgery before any further experimental manipulations. It is also critical that the patency of the catheters is verified prior to experiment. Patency of the catheters declines over time, thus optimally siRNA administration should begin soon after the allotted recovery time stated above.

Reference:

Yaksh TL, Rudy TA (1976) **Chronic catheterization of the spinal subarachnoid space.** Physiol. Behav. 17:1031-6.

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Preparation of siRNA for intrathecal administration

On the day of the injection, thaw an aliquot(s) of the siRNA and control RNA ON ICE. At the same time, warm the desired quantity of i-Fect to room temperature.

IMPORTANT: The i-Fect is used undiluted without the addition of the siRNA diluent.

To inject a 2 µg (for a 21 mer, this is equivalent to 0.14 nmol) dose of siRNA or control RNA per rat for a group size of 6 rats, mix 12 µL of the 100 µM RNA stock with 60 µL of i-Fect (1:6 v/v). This is equivalent to 15 µg of RNA in 60 µL of i-Fect (1:5 w/v) to yield a total volume of 72 µL of RNA/i-Fect solution.

Mix the RNA and i-Fect by GENTLY swirling the solution with a sterile pipet tip and allow the mixture to stand for 5 min at room temperature.

IMPORTANT: Add the siRNA to the i-Fect reagent to avoid siRNA precipitate. If i-Fect is added to siRNA solution, precipitation has been observed.

At this 1:5 w/v ratio, the RNA should remain soluble in the transfection reagent; the mixture should be clear to slightly cloudy. More RNA or less volume of i-Fect will cause the RNA to precipitate out of solution and is not recommended.

Intrathecal administration of siRNA

It is highly recommended that the RNA/i-Fect solution be injected WITHIN 30 MINUTES after it is prepared. For intrathecal injection, draw 9 µL of sterile saline solution into a Hamilton syringe, followed by 1 µL of air, and 10 µL of the RNA/i-Fect solution. Inject the content slowly into the catheter, noting the air bubble's movement and that it does not show any compression. We routinely include a vehicle control group, for which each rat receives 10 µL of i-Fect. It should be noted that the volume of intrathecal delivery is typically between 5 µL and 10 µL, and so the 2 µg RNA used in this protocol represents the maximum bolus dose of RNA in i-Fect that can be delivered intrathecally. Treatment of up to 6 doses of either i-Fect or this dose of RNA/i-Fect solution over a period of 72 hours did not precipitate any overt sign of behavioral toxicity.

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