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RNAiCarrier™ – *siRNA transfection reagent*

(Cat. No.: 31-00130)

I. Description

RNAiCarrier™ -siRNA transfection reagent is a formulation of a polycationic lipid and a neutral, non-transfecting lipid compound in sterile water. This transfection reagent is effective in both serum-containing medium and serum-free medium, offering high efficiencies with minimal cytotoxicity.

RNAiCarrier™ has been used successfully to transfect siRNA into HEK293, HuH-7 (human hepatoma), B16 melanoma, CHO-K1, COS-1, COS-7, NIH3T3, SF-9, S2, primary human keratinocytes, primary rat aortic smooth muscle (WKY3M), and other cell lines.

II. Working Concentration

Enough RNAiCarrier™ is provided for approximately 250 transfections in 35mm plates. RNAiCarrier™ is provided as a sterile suspension in water at a concentration of 1 mg/ml.
Store at 4°C or above, DO NOT FREEZE.

III. Determining optimal conditions

The optimal conditions for achieving the highest transfection efficiency involves several parameters including cell confluence, the composition of the siRNA/liposomal complex and the duration of transfection.

A. Cell Confluence

Cell growth should cover 50-70% of the total plating area before performing a transfection. In general, it is easier to control the level of growth by using larger well plates.

B. siRNA/Liposomal Complex

The optimal siRNA to liposome ratio used can be determined by beginning with a 1µg amount of siRNA duplex per 35 mm well. Vary the amount of lipid over a range of 2-8 µl, in 0.5 or 1.0 µl increments, per 35 mm well. Once the optimal siRNA to liposome ratio for a given cell type is identified, the appropriate amount of complex used per well should be determined. For example, if the optimal siRNA/liposome ratio in the initial experiment was 1 µg/2µl, then amounts ranging from 0.5 µg/1.0 µl, 1.5 µg/3.0 µl and 2.0 µg/4.0 µl should be tested to determine which amount provides the highest level of transfection efficiency.

C. Duration of Transfection

An additional factor to consider when optimizing transfection is the duration that cells are exposed to the siRNA/liposome complex. As a general rule, the longer the exposure time, the higher the efficiency rate. However, since transfection is usually performed with low serum-containing or serum-free medium, prolonged exposure to these reagents can result in cell detachment or death. Therefore, it is recommended that an initial transfection time of 5 to 18 hours be used. Longer exposure times may be possible depending on the cell line.

D. Controls for siRNA experiments

Controls should always be included in siRNA experiments. An siRNA with a scrambled sequence can be used as a negative control. 3'-Fluorescently labeled sense strand siRNA allows for visualization of oligos during transfection.

E. Assessing the gene-silencing effect

Do not correlate observed biological effect with the gene-silencing effect. Rather demonstrate down regulation of the targeted mRNA (ex. northern blot, Real-time Q-PCR, or cDNA/oligo array) or protein level (e.g. western blot or antibody). The same endpoints can be made for *in vivo* experiments, while also assessing behavioral endpoints.

IV. Transfection Protocol

1. In a six well or 35mm tissue culture plate, seed ~2*10_5 cells per well in 2ml of DMEM with 10%FBS.
Note: Adjust volumes proportionately for wells or dishes of different sizes

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2. Incubate the cells at 37°C in a CO2 incubator until the cells are 60-80% confluent. This will usually take 18-24 h.
Note: Since transfection efficiency is sensitive to cell confluence, it is important to maintain a standard seeding protocol from experiment to experiment.

3. Prepare the following solutions:
Solution A: For each transfection, dilute 1µg siRNA duplex into 100µl serum-free or low protein medium without antibiotics.
Solution B: For each transfection, dilute 2-8µl of lipid reagent into 100µl serum-free or low protein medium without antibiotics. Peak activity should be at about 6µl.
Note: Some chemically defined serum-free medium formulations may inhibit transfection efficiency

4. Add the siRNA duplex solution directly to the dilute lipid using a pipet. Mix gently by pipeting the solution and incubate the mixture 15-45 minutes at room temperature.

5. Wash the cells once with 2ml of low protein or serum-free and antibiotics free medium.
Note: Do not use PBS since the residual phosphate may compete with siRNA and bind lipid thereby reducing transfection efficiency.

6. For each transfection, add 0.8ml of low protein or serum-free and antibiotics free medium to each tube containing the siRNA:lipid complex. Mix gently and overlay the diluted complex solution onto the washed cells.

7. Incubate the cells 5-7 h at 37°C in a CO2 incubator.

8. Add 1ml of medium containing 2 times the normal serum and antibiotics concentration (2x medium) without removing the transfection mixture. If toxicity is a problem, remove the transfection mixture and replace with normal growth medium.
Note: addition of the 2x medium results in a higher level of transfection. Furthermore the 2x medium restores cells to their normal growth conditions.

9. Incubate the cells an additional 18-24 h.

10. Aspirate the medium and replace with fresh 1x medium containing the additives normally used to culture the cells.

11. Assay the cells using the appropriate protocol 24-72 h after the addition of fresh medium in step 10.
Note: This time frame is dependent upon the cell type and promoter activity.

Stability and Storage:
RNAiCarrier™ Is a Research Use Only product and is not intended for *in vitro* Diagnostic Use. The product should be stored 4°C or above and protected from light. Normal precautions should be taken when working with this or any other research product. A Material Safety Data Sheet is available upon request.

Storage Temperature: Store at 4°C or above.
DO NOT FREEZE

Related Products:
GenCarrier-1™: Cat. No. 31-00110, 1ml
GenCarrier-2™: Cat. No. 31-00120, 1ml