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RNAifectin™

Store at 4°C

Cat. No.	Description	Quantity
G073	RNAifectin	1.0ml

Description

RNAifectin™ is a transfection reagent specially formulated with multiple cationic polymers. It is suitable for the transfection of RNAi oligos into cultured eukaryotic cells.

Transfection protocol

Use the following conditions as guidelines to transfect mammalian cells in a 6-well or 35mm dish format. For other culture vessels, please refer to Table 1.

1. Eighteen to twenty-four hours prior to transfection, seed cells at a density of 1-3 x 10^5 cells per well in 2.0ml of appropriate growth medium (with serum and antibiotics if normally required). Incubate the cells at 37˚C in a CO_2 incubator until cells are 70% to 90% confluent at the time of transfection.

   Suspension Cells: Just prior to preparing complexes, plate 3-5 x10^5 cells in 0.8ml of serum-free medium without antibiotics.
   Since transfection efficiency is sensitive to culture confluence, it is important to maintain a standard seeding protocol throughout all of your experiments.

2. For each transfection sample, prepare the complexes in sterile micro centrifuge tubes as follows:

   Solution A: Dilute 1-3µg of RNAi oligos into 125µl of serum-free, antibiotic-free medium.

   Solution B: Mix RNAifectin™ reagent thoroughly prior to use, then dilute 4-10µl of reagent in 125µl serum-free, antibiotic-free medium.

   Incubate solution A and B separately at room temperature for 5 minutes.

3. Combine solutions A and B, mix thoroughly to ensure uniform distribution and incubate for 20 minutes at room temperature to allow RNA-liposome complexes to form.

4. ***Adherent cells ONLY*** *(For suspension cells, go directly to step 5b):*
   Add 0.8ml of serum-free, antibiotic-free medium to the RNAifectin™ -RNA complex. Mix solution gently.

5a. ***Adherent cells:*** Remove growth medium from the cells and add 1.0ml of RNAifectin™ -RNA solution to the each well containing cells. Incubate at 37˚C.

5b. ***Suspension cells:*** Add 0.2ml of the RNAifectin™ -RNA solution into each well containing suspension cells in 0.8ml serum-free, antibiotic-free medium. Incubate at 37˚C.

6. After 5-8 hours, remove transfection solution and add 2.0ml of the appropriate growth medium (with serum and antibiotics) or add 0.1ml of FBS directly into each vessel. Incubate the cells at 37˚C for a total of 18-24 hours.

7. Assay cell extracts for marker gene activity 24-72 hours after the start of transfection depending on cell type and promoter activity.

8. A similar procedure can be used to transfect an RNAi vector for stable expression. Seventy-two hours after transfection passage the cells 1:10 or higher into an appropriate selective medium.

Optimizing transfection for specific cell lines

To achieve the maximum transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density along with RNA oligo and RNAifectin™ concentrations. Optimal results have been observed when cells are 70-90% confluent and RNA(µg): RNAifectin™ (µl) ratios are 1:1 to 1:5.

Table 1: Reagent quantities for different culture vessels

Culture Vessel	Surface area per well (cm^2)	Volume of plating medium	RNAi oligos (µg) in medium volume (µl)	DNAfectin™ in medium volume	Transfection medium vol.
24-well	2	500 µl	0.2-0.4 µg in 25 µl	2-4 µl in 25 µl	0.4 ml
12-well	4	1 ml	0.5-0.8 µg in 100 µl	5-8 µl in 100 µl	0.6 ml
6-well	10	2 ml	1.0-3.0 µg in 125 µl	4-10 µl in 125 ul	0.8 ml
35mm	10	2 ml	1.0-3.0 µg in 125 µl	4-10 µl in 125 µl	0.8 ml
60mm	20	5 ml	3.0-6.0 µg in 500 µl	12-30 µl in 500 µl	2.4 ml
100mm	60	10 ml	8.0-16.0 µg in 1.5 ml	32-80 µl in 1.5 ml	6.4 ml

Notes

- Do not add antibacterial agents to the media during transfection

- Cells that will not tolerate the absence of serum for 2-24 hours can be transfected in the presence of serum. This is done by preparing the oligo-liposome complexes for 45 minutes in serum-free medium, followed by diluting the complexes with serum-containing medium before adding to the cells. It is extremely important that the amount of RNAifectin™ be re-optimized as the optimal amount of lipid under these conditions may be different from that observed for serum-free transfection.