Applied Biological Materials Inc.

1-866-571-7226
info@abmGood.com
www.abmGood.com

RNAifectin™

Store at 4°C

Cat. No.	Description	Quantity
G073	Cationic Polymers	1.0ml

Description

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

Protocol

These conditions are recommended as guidelines only. A 6-well or 35mm dish is adequate for most applications, but larger vessels are sometimes required. In that case, consult table 1.

1. In a 6-well plate, seed cells at a density of $1\text{-}3x10^5$ per well in 2.0ml of the appropriate growth medium (with serum if cells are cultured in presence of serum). Incubate the cells at 37°C until cells are 60-90% confluent. This will usually take 18-24 hours, depending on cell types. Since transfection efficiency is sensitive to culture confluence, it is important to maintain a standard seeding protocol from experiment to experiment.

2. Prepare the following solutions in sterile 12x75 mm or microcentrifuge tubes:

   Solution A: For each transfection, dilute 1-3µg of RNAi oligoes into 125µl serum-free medium.

   Solution B: For each transfection, dilute 4-10µl of RNAifectin™ reagent into 125µl of serum-free medium.

   (Vortex RNAifectin™ reagent before use)

3. Combine the solutions, mix thoroughly and incubate at room temperature for 20 mins to allow DNA-lipsome complexes to form.

4. Remove growth medium from the cells and add 800µl of serum-free medium to the cells.

5. Add the RNAifectin™-oligo complexes to the cells, mix gently to ensure uniform distribution and incubate at 37°C for 2-24 hours. We recommend starting with 5 hours.

6. Remove the transfection solution and add 2.0ml of the appropriate complete growth medium (with serum) to each well. Incubate cells at 37°C for a total of 24-72 hours.

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

8. A similar procedure can be used to transfect an RNAi vector for stable expression. At 72 hours after transfection, split the cells at a ratio of 1:10 into the selective medium for the marker gene transfected.

Table 1. Reagent Quantities for different sized culture vessels

Dishes (mm)	Dilution volume (µl)	RNAifectin™ volume (µl)	Oligo amount (µg)	Medium volume (ml)
35	125	4-10	1-3	0.8
60	375	12-30	3-9	2.4
100	1,000	32-80	8-24	6.4

Transfection Optimization

Optimization of transfection conditions is essential for high efficiency transfections and low toxicity. Lipid and oligo concentrations, cell number and time of exposure of cells to oligo-liposome complexes are conditions that should be optimized.

Notes

- Lower cell yield or toxicity is often associated with transfection activity regardless of the transfection method. Cell yield can be increased by transfecting cultures at a higher confluence by using less RNAifectin™ reagent or oligo in the transfection or by including serum during exposure of cells to oligo-liposome complexes.

- 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 oligo-liposome complexes for 45 mins 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 lipid be re-optimized as the optimal amount of lipid under these circumstances may be different from that observed for serum-free transfection.

This product is distributed for laboratory research only.
CAUTION: Not for clinical use.
For technical questions about this product, phone the ABM helpline at 1-866-571-7226 or visit our website at www.abmGood.com.

CERTIFICATE OF ANALYSIS