siRfficient™ siRNA Transfection Reagent

REF WU2001, WU2002, WU2003, WU2004, WU2005

Product Description

siRfficient™ is a proprietary nanotechnology formulation developed and optimized for gene silencing experiments. It provides highest efficiencies for delivery of siRNA, microRNA or antisense RNA into the cytoplasm of a wide variety of cell types including Neuro 2a, Jurkat, K562 and LLC-1 cells. siRfficient™ is **easy to use, robust, and exhibits very low toxicity**. One mL is sufficient for approximately 125 transfections on wells of 6-well cell culture plates.

Package Contents

WU2001	WU2002	WU2003	WU2004	WU2005
size	size	size	size	size
0.1 mL	0.3 mL	1.0 mL	6 X 1.0 mL	12 X 1.0 mL

Storage Conditions / Store at 4°C (do not freeze)

Required Materials

- siRNA, microRNA or antisenseRNA (> 0.5 µg/µL or > 38 µM)
- 5% dextrose
- Microcentrifuge tubes

Table 1

Suggested amounts of siRNA and siRfficient for transfection of siRNA into adherent and suspension cells.

Tissue Culture Plates	Surface Area Per Well	Plating Medium Per Well	siRNA Per Well	siRfficient Per Well	RNA-siRfficient Complex Per Well
6-well	9 cm^2	2000 µL	1 µg (in 50 µL 5% dextrose)	8 µg (in 50 µL 5% dextrose)	50-100 µL
12-well	4 cm^2	2000 µL	0.5 µg (in 25 µL 5% dextrose)	4 µg (in 25 µL 5% dextrose)	25-50 µL
24-well	2 cm^2	500 µL	0.25 µg (in 12.5 µL 5% dextrose)	2 µg (in 12.5 µL 5% dextrose)	12-25 µL
96-well	0.3 cm^2	200 µL	0.05 µg (in 2.5 µL 5% dextrose)	0.4 µg (in 2.5 µL 5% dextrose)	3-5 µL

Optimizing Your Transfection

- It is important to optimize transfection conditions to obtain the highest transfection efficiency with lowest toxicity for various cell types.
- We recommend starting with the volumes and concentrations outlined in Table 1 for different plate formats.
- You can optimize your transfection efficiency by increasing or decreasing the volume of RNA-siRfficient complex that is added to each plate.
- When varying the siRNA concentration, keep siRNA mass to siRfficient volume proportional (e.g. 1 µg siRNA: 8 µL siRfficient).

Troubleshooting

1. Higher transfection efficiencies are normally achieved if the transfection medium is not removed. However, if toxicity is a problem, aspirate the transfection medium after 6 hrs of transfection and replace with fresh growth medium.
2. If varying the volume of RNA-siRfficient complex or concentration of siRNA does not achieve the desired results, then the transfection protocol can be performed in the absence of serum. To do this, make the following adjustments to the protocol.
   a. Prior to adding the RNA-siRfficient complex to the wells (STEP 3), remove the growth medium in each well and replace with 225 µl medium **without serum**.
   b. Add 25 µL of RNA-siRfficient complex to each well.
   c. Incubate the cells for 5 hrs.
   d. Replace the medium in each well with 500 µl growth medium containing the normal concentration of serum.
   e. Assay gene activity after 24 to 48 hrs.
3. To reduce cytotoxicity, transfect the cells at a higher confluence or reduce RNA-siRfficient complex volume that is added to each well.

For Research Use Only

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Protocol

Use the following procedure to transfect siRNA, microRNA or antisense RNA into mammalian cells in a 24-well format. For other plate formats, scale up or down the amounts of RNA and siRfficient proportionally to the total transfection volume (Table 1).

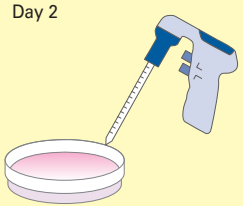
Day 1 — Step 1

Adherent cells

Plate the cells (roughly 80,000 cells/well) in 500 µL of growth medium **without antibiotics** so that they are approximately 70-95% confluent next day.

Suspension cells

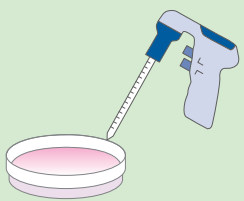
Check suspension cells for Day 2



Day 2 — Step 2

Adherent cells

Use the cells plated on Day 1



Suspension cells

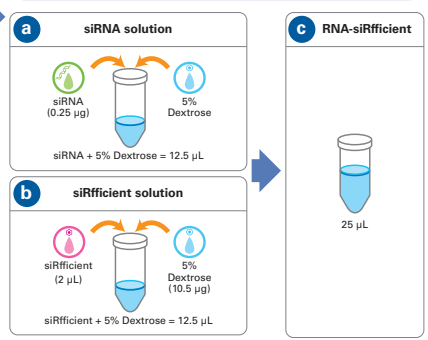
Just prior to preparing RNA-siRfficient complex, plate 160,000 cells/well in 500 µL of growth medium **without antibiotics**.

Prepare RNA-siRfficient complexe as follow

a. Dilute 0.25 µg (≈19 pmol) of siRNA in a microfuge tube containing 5% dextrose up to a total volume of 12.5 µL.

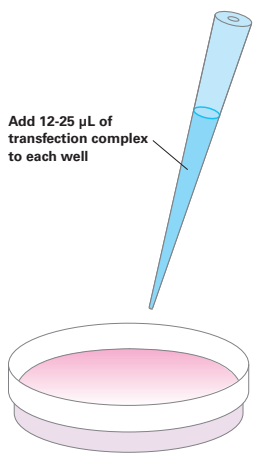
b. In a separate microfuge tube, add 2 µL of siRfficient into 10.5 µL 5% dextrose for a total volume of 12.5 µL.

c. Transfer the diluted siRNA solution to the diluted siRfficient solution (total volume = 25 µL). Mix gently and incubate at room temperature for 10 min.



Step 3

Add RNA-siRfficient complex to the adherent or suspension cells, incubate at 37°C in a CO_2 incubator for 1-3 days.



Day 3-5 — Step 4

Assay cells for gene activity.