

EXOBX® siRNA Transfection Reagent

Cat. no. R2102

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EXOBX® siRNA Transfection Reagent

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Cat. No.	Reagent In Kit	Size	Storage
R2102	EXOBX® siRNA Transfection Reagent	1 mL	4°C

1. Transportation and Storage

- This product is transported at room temperature and should be stored at 4°C. Do not freeze.

2. Important Information (Please read before using the kit)

- 1) Use logarithmic phase cells for siRNA transfection. Typically, transfection should be initiated 24-36 hours post-passaging to ensure high transfection efficiency.
- 2) Cell density significantly impacts transfection efficiency. Logarithmic phase cells with 30-70% confluence are recommended for siRNA transfection to achieve optimal results. Overly high or low cell densities reduce transfection efficiency.
- 3) Dissolve siRNA powder and aliquot before storing at -80°C to prevent degradation due to repeated freeze-thaw cycles.
- 4) The incubation time after mixing siRNA with R2102 should not be too short. A 15-30 minute incubation (preferably 30 minutes) ensures adequate formation of transfection complexes, enhancing efficiency.
- 5) The recommended dosage of R2102 in Table 1 is sufficient for effective siRNA transfection. Do not exceed the maximum recommended dosage to avoid cytotoxicity.**
- 6) For suspension cells, siRNA uptake is slower. Avoid media replacement within the first 6 hours post-transfection. Change the medium after 24-36 hours to allow full uptake of the transfection complexes.
- 7) Fluorescently labeled siRNA (e.g., siRNA-FAM) becomes detectable under a fluorescence microscope at 6 hours post-transfection, peaks at 12 hours, and diminishes by 24-48 hours. To assess gene knockdown efficiency, collect cell samples at 24-36 hours post-transfection.
- 8) Compared to green fluorescent protein (GFP), fluorescently labeled siRNA (e.g., siRNA-FAM) appears as smaller and dimmer fluorescence spots under a microscope. Increasing excitation intensity can enhance fluorescence intensity and detection efficiency.
- 9) R2102 should be stored at 4°C. Freezing reduces transfection efficiency or may render the reagent ineffective.
- 10) This product is for research use only!

3. Product Introduction

The EXOBX® siRNA Transfection Reagent is a transfection reagent specifically designed for siRNA delivery. It exhibits high transfection efficiency and low cytotoxicity across various cell lines and primary cells.

4. Features and Advantages

- 1) High transfection efficiency: Comparable to Lipofectamine 2000 and RNAi Max.
- 2) Low cytotoxicity: No significant toxicity observed across multiple cell lines and primary cells.
- 3) Ease of use: The reagent is not affected by serum or antibiotics, allowing transfection without medium replacement.

5. Experimental Procedure

For the transfection experiment, adherent cells cultured in a 6-well plate are used as the experimental system. The procedure is as follows:

- 1) Cell Culture: Collect cells using trypsinization, count, and seed into a 6-well plate. Incubate for 24 hours until cell density reaches 30-70% (High cell density will reduce the transfection efficiency).
- 2) siRNA Preparation: Dissolve siRNA powder in RNase-free ddH₂O to a final concentration of 20 μ M.
- 3) Transfection Complex Preparation: Mix siRNA with R2102 in OPTI-MEM medium and incubate for 30 minutes to form transfection complexes.
- 4) Cell Transfection: Add the transfection complex dropwise to the cell culture medium, gently mix, and continue incubating in a CO₂ incubator.
- 5) Media Replacement: At 6-12 hours post-transfection, replace with fresh culture medium. If no cytotoxicity is observed, media replacement is optional.
- 6) Gene Expression Analysis: At 24-36 hours post-transfection, analyze mRNA expression levels via qPCR or protein expression via Western blot.

Table 1. Recommended R2102 and siRNA Dosage

Culture Plate	20 μ M siRNA (μ L)	R2102 (μ L)	OPTI-MEM (μ L)
24-well plate	1-3	0.5-1	50-100
6-well plate	4-10	2-4	100-200
Ø 35 mm dish	4-10	2-4	100-200
Ø 60 mm dish	8-20	4-8	200-400

6. Transfection Efficiency Evaluation

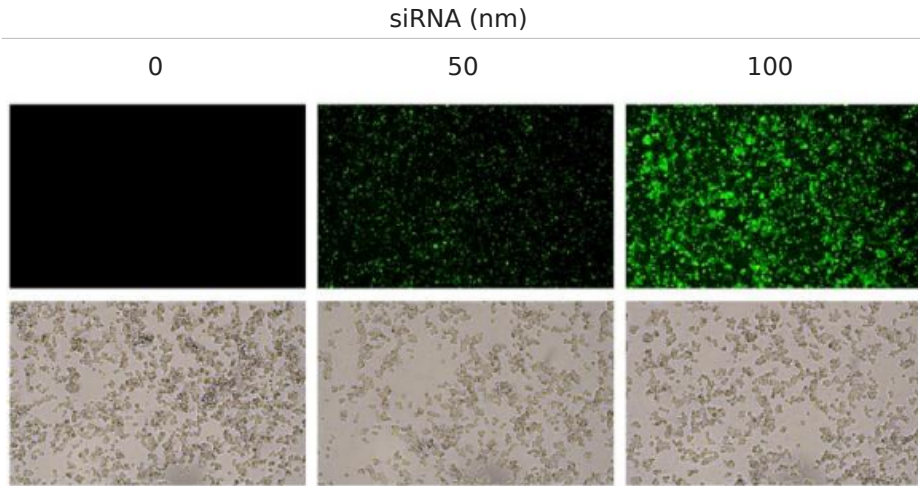


Fig. 1. Evaluation of siRNA Transfection Efficiency in NALM-6 Cells

NALM-6 cells were passaged and seeded into a 6-well plate. After 24 hours, the cell density reached 30-50%. The cells were transfected with FAM (green fluorescent probe)-labeled control siRNA using R2102. Three groups were established: a control group (0 nM siRNA), a group with 4 μ L R2102 + 50 nM siRNA-FAM, and a group with 4 μ L R2102 + 100 nM siRNA-FAM.

After 24 hours of transfection, fluorescence intensity was observed under a fluorescence microscope and recorded via imaging. The results demonstrated that R2102 effectively delivered siRNA into the cells.

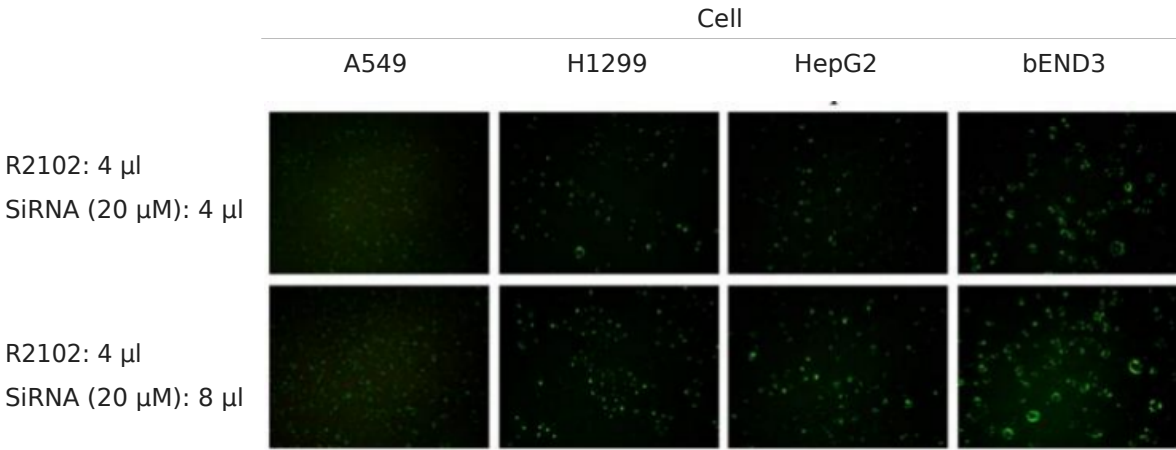


Fig. 2. Evaluation of siRNA Transfection Efficiency Using R2102

Adherent cell lines, including A549, H1299, HepG2, and bEND3, were seeded into 6-well plates and cultured until they reached 30-50% confluence after 24 hours. Cells were transfected with FAM-labeled control siRNA using R2102. Two experimental groups were established: one with 4 μ L R2102 + 40 nM siRNA-FAM and the other with 4 μ L R2102 + 80 nM siRNA-FAM.

Fluorescence microscopy performed at 24 hours post-transfection revealed efficient intracellular delivery of siRNA by R2102. Additionally, the results indicated that, when the R2102 concentration was fixed (4 μ L), transfection efficiency increased in a siRNA concentration-dependent manner.

7. Troubleshooting

Issue	Possible Cause	Solution
Low gene knockdown efficiency	Inefficient siRNA design or selection.	Redesign and synthesize a new siRNA or use a validated, high-efficiency siRNA.
	Insufficient siRNA concentration.	Increase siRNA concentration. Concentrations up to 100 nM are effective without causing significant off-target effects.
	Inadequate R2102 dosage.	Increase the amount of R2102.
	Incorrect siRNA-to-R2102 ratio.	Mix siRNA and R2102 at a 1:1 volume ratio (20 μ M siRNA:R2102), or increase the siRNA amount while maintaining this ratio.
	Suboptimal cell density.	Adjust cell density to 30-70% confluence at the time of transfection. Very high or low densities reduce transfection efficiency.
	Improper timing for gene expression analysis.	Collect cells at 24-36 hours post-transfection for mRNA analysis and at 36-48 hours post-transfection for protein analysis.
	Insufficient transfection complex incubation time.	Incubate siRNA and R2102 at room temperature for 30 minutes to allow adequate complex formation. Do not mix again before adding to cells, as this may disrupt the complexes.
	siRNA degradation.	Use RNase-free tubes and pipette tips to prevent RNase contamination. After dissolving, aliquot siRNA and store at -80°C to avoid degradation from repeated freeze-thaw cycles.
High Cytotoxicity	Certain cell lines are sensitive to R2102.	Reduce the amount of R2102 and change the culture medium 6 hours after transfection.
	Low cell density at transfection.	Ensure cell density is at least 50% at the time of transfection.
	Low siRNA purity.	Use high-purity siRNA, such as PAGE-purified siRNA.
	Poor cell condition.	Improve cell culture conditions or use a healthier cell batch.
	Excessive R2102 concentration.	Follow the recommended R2102 dosage in Table 1 and do not exceed the maximum suggested amount.