Chemi - Trans™ RNAiBor siRNA Transfection Reagent

（Cat #: T012, T013）

Introduction:

The Chemi - Trans™ RNAiBor siRNA Transfection Reagent is a novel, high biological compacity and biodegradable nanoparticle-based in vitro small RNA (e. g., siRNA, miRNA mimics and miRNA inhibitor) delivery tool, which provides a robust transfection efficiency in a variety of commonly used and hard-to-transfect mammalian cells, such as adherent and suspension cells, as well as primary cells, but with less cytotoxicity in comparison with other lipid-base transfection reagent as known in the market.

Advantages:

❖ Robust transfection efficiency and low cytotoxicity.

❖ Excellent for some primary cell lines, such primary fibroblast cells, primary neuron cells, macrophage cells, T cells and stem cells.

❖ Compatible with serum and antibiotics in growth medium.

Table 1: Product Package & Storage

Cat #	Product Name	Volume	Storage
T012	Chemi -Trans™ RNAiBor siRNA Transfection Reagent	200 μL	2 ~ 8 °C, stable for up to 12 months when stored appropriately. (DO NOT FREEZE.)
T013		1.0 mL	

Important Guidelines for Transfection:

(1) For a robust transfection efficiency, the siRNA (PAGE or HPLC purified grade) is recommended. Never fail to use siRNA preserved in water or desalted and lyophilized condition.

(2) It is strictly prohibited to dilute both the Chemi - Trans™ RNAiBor siRNA Transfection Reagent and the siRNA solution by Serum-free medium or dilution buffer in preparation of the transfection complexes, just to mix the Chemi - Trans™ Reagent and siRNA solution directly as the ratio of 1:1.

Standard Protocol for siRNA Transfection of Adherent Cells

Step Ⅰ. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to 60% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30 ~ 60 min before transfection.

Step Ⅱ. siRNA Transfection Protocol

Use the following procedure to transfect siRNA into mammalian cells in a 24-well format. For other formats, please refer to ***A Guideline for siRNA transfection (Table 2)***. All amounts and volumes are given on a per well basis. For each transfection sample, prepare complexes as follows:

a. Thaw a certain amount of well-reconstituted siRNA solution and Chemi - Trans™ RNAiBor Reagent based on the experimental. Vortex to mix each solution gently but thoroughly.

b. Add 1.5 μL Chemi - Trans™ RNAiBor Reagent to 1.5 μL wellreconstituted siRNA solution directly, without any dilution before use, **as the ratio of 1:1** at once, mix by pipetting up and down for 15 times. Incubate for 10 minutes at room temperature to let transfection complex form well and ensure that there is no residual droplet on the tube-wall.
c. Add the well-prepared siRNA transfection complex (**at Step b**) to 0.3 mL complete growth medium containing cells, serum and antibiotics each well. Mix gently by rocking the plate back and forth.
d. Incubate the transfected cells at 37°C in a CO_2 incubator for 24 to 72 hours. Then, to measure the gene silencing by qRT-PCR and Western Blotting respectively.

Table 2: A Guideline for siRNA transfection per cell culture vessel

Culture Vessel	Growth Medium (mL)	Amount of Plating Cells	siRNA (μL)	Chemi - TransTM RNAiBor Reagent (μL)
	Volume used per well			
v96-well	0.1	1 ~ 4 × 10^4	0.75	0.75
24-well	0.3	0.5 ~ 2 × 10^5	1.5	1.5
12-well	0.6	1 ~ 4 × 10^5	3.0	3.0
6-well	1.2	0.25 ~ 1 × 10^6	6.0	6.0

*[1] We kindly suggest reconstituting siRNA stock solution at 20 μM, and the volume of siRNA used per well, shown the above **Table 2,** is calculated based on this concentration.
[2] For optimal Transfection efficiency, we recommend using 1.5 ~ 5.0 μL siRNA. As a starting point, we recommend using 1.5 μL siRNA stock solution which usually gives satisfactory transfection result for most adherent cell lines and primary cells. For hard-to transfection cells, we recommend using a final siRNA volume of 2.0 ~ 5.0 μL, and keep the 1:1 ratio of the well-reconstituted siRNA solution and Chemi - TransTM RNAiBor Reagent. The above conditions are given per well in a 24-well plate.
[3] You may perform a rapid 96-well plate transfections by plating cells directly into the transfection complexes. Prepare complexes in the plate and directly add cells drop wise at twice the cell density as in the basic protocol in the complete growth medium. Cells will adhere as usual in the presence of complexes.