SMAD7 Human Pre-designed siRNA Set

S1476644

Storage -20℃. FAM-labeled siRNA Negative Control (HPLC) store in the dark.
Shipping Ultra-low temperature transportation.

Introduction

SMAD7 Human Pre-designed siRNA Set contains three designed siRNAs for SMAD7 gene (Human), as well as a negative control, a positive control, and a FAM-labeled negative control. The full name of SMAD7 is SMAD family member 7, with a Gene ID of 4092.

Chemically synthesized siRNA is a ready-to-use double-stranded RNA of 21~25 bp. After purification and annealing, it is formulated into a working concentration solution for direct use in cell transfection. The product is supplied as a lyophilized powder with stable performance, and its dosage is strictly calculated and labeled in moles. This product does not guarantee knockdown of target gene protein levels. (Oligo synthesis parameters: rA molecular weight: 329.21, rG molecular weight: 345.21, rC molecular weight: 305.18, rU molecular weight: 306.17)

siRNA (nmol)	2.5	5	10	50	Final Concentration
Volume of Water Added (μL)	125	250	500	2500	20 μM
Volume of Water Added (μL)	50	100	200	1000	50 μM
Volume of Water Added (μL)	25	50	100	500	100 μM

Formula for siRNA resuspension buffer:siRNA (nmol) / Volume (μL) = 10^3 μM (μmol/L)

Kit Contents

S1476644	Component	1 set	Storage
S1476644A	SMAD7 siRNA-1 sense and antisense (HPLC)*2	1 OD*2 (2.5 nmol*2)	-20℃.
S1476644B	SMAD7 siRNA-2 sense and antisense (HPLC)*2	1 OD*2 (2.5 nmol*2)	-20℃.
S1476644C	SMAD7 siRNA-3 sense and antisense (HPLC)*2	1 OD*2 (2.5 nmol*2)	-20℃.
S1476644D	siRNA Negative Control (HPLC)*2	1 OD*2 (2.5 nmol*2)	-20℃.
S1476644E	FAM-labeled siRNA Negative Control (HPLC)*4	0.5 OD*4 (1.25 nmol*4)	-20℃. Store in the dark.
S1476644F	GAPDH siRNA Positive Control (HPLC)*2	1 OD*2 (2.5 nmol*2)	-20℃.
S1476644G	RNase-free water	1 mL	-20℃.

Precautions

1. The product is shipped at room temperature as a lyophilized powder. It is recommended to store it below -20°C. Avoid repeated freeze-thaw cycles to prevent RNA degradation; aliquot for storage. Briefly centrifuge before use, then reconstitute with RNase-free Water or sterile ddH_2O to prepare a 20~100 μM stock solution. The average molecular weight of siRNA is 13,300. The lyophilized powder adheres to the tube wall and is easily lost when opened. Therefore, centrifuge the tube before opening the cap, then carefully remove the cap. After dissolution, cap the tube and vortex to mix.
2. Strictly follow RNA handling protocols and use RNase-free laboratory supplies for RNAi experiments to prevent RNA degradation. It is best to keep the product on ice during use.
3. Fluorescently labeled RNA is packaged in brown centrifuge tubes. Since fluorescent labels (e.g., FAM, CY3, CY5) are light-sensitive, they must be stored in the dark.

Control Instructions

1. Negative control

An siRNA with no homology to the target cells, used to exclude non-siRNA interference effects on target gene expression in experiments. The value of this group is defined as 1 when calculating interference efficiency. A negative control is essential for a rigorous RNAi experiment.

2. Positive Control (GAPDH siRNA)

An siRNA that defaults to 90% interference efficiency against GAPDH, used to verify the reliability of each step in the experimental process.

3. FAM-Labeled Negative Control (FAM-negative control)

A negative control with a fluorescent group. This group is set in parallel to detect transfection efficiency (note: do not co-transfect with other groups). Perform operations in the dark, and observe transfection efficiency via fluorescence microscopy or flow cytometry 6~12 hours later. The transfection efficiency of other parallel experimental groups is theoretically consistent with this group.

Experimental Cell Instructions

For transfection experiments, set up at least 3 replicate wells per transfection sample. When seeding cells, ensure the number of cells per well is as consistent as possible and that cells are evenly distributed across the well surface to reduce inter-well variability and ensure experimental reliability and reproducibility. (The following is a reference for transfection with Lipo2000 reagent; for other transfection reagents, refer to their respective instructions.)

1. Determine Transfection Concentration

1.1. Perform gradient experiments to determine the optimal siRNA dosage for your cell line. For example, test several concentrations of Lipo2000 or vary siRNA concentrations within the 20~100 nM range to identify conditions that achieve the best gene knockdown. High siRNA concentrations may be cytotoxic.

1.2. Allow longer intervals between analyses to reduce cell viability damage caused by

excessive cell growth. Transfecting cells at higher density may be more suitable for optimizing transfection conditions.

1.3. Fluorescently labeled siRNA can be used as an indicator of transfection efficiency to optimize conditions and troubleshoot experimental issues promptly.

2. Transfection of Cells

2.1. Adherent Cells: 24 hours before transfection, seed 0.5~2×10⁵ cells in 400 µL of antibiotic-free medium. Transfect when cell confluency reaches 60~80%. Ensure cells are completely digested and mixed thoroughly during plating to avoid clumping.

2.2. Suspension Cells: 24 hours before transfection, seed 0.5~2×10⁵ cells in 400 µL of antibiotic-free medium. Transfect when the cell number reaches 4~8×10⁵ per well.

3. Transfection Procedure

The following example describes transfection of siRNA at a final concentration of 50 nM in a 24-well plate. For other plate formats, refer to the table below.

3.1. Dilute siRNA in 50 µL Opti-MEM, gently pipette 3~5 times to mix.

3.2. Invert the transfection reagent to mix, dilute 1.0 µL of reagent in 50 µL Opti-MEM, gently pipette 3~5 times to mix, and incubate at room temperature for 5 minutes.

3.3. Combine the siRNA and transfection reagent dilutions, gently pipette 3~5 times to mix, and incubate at room temperature for 20 minutes.

3.4. Add the transfection complex to the 24-well plate at 100 µL per well, ensuring even distribution.

3.5. Incubate the plate at 37°C in a 5% CO_2 incubator for 18~48 hours. Fresh medium can be replaced 4~6 hours after transfection.

Culture Plate Format	Total Volume per Well	Culture Medium	Opti-MEM (for siRNA dilution)	Opti-MEM (for transfection reagent dilution)	Final Concentration	siRNA Product* (20 µM stock)	Transfection Reagent
96-well	100 µL	50 µL	25 µL	25 µL	100 nM	0.5 µL	0.25 µL
96-well	100 µL	50 µL	25 µL	25 µL	50 nM	0.25 µL	0.25 µL
96-well	100 µL	50 µL	25 µL	25 µL	30 nM	0.15 µL	0.25 µL
96-well	100 µL	50 µL	25 µL	25 µL	20 nM	0.1 µL	0.25 µL
96-well	100 µL	50 µL	25 µL	25 µL	10 nM	0.05 µL	0.25 µL
24-well	500 µL	400 µL	50 µL	50 µL	100 nM	2.5 µL	1 µL
24-well	500 µL	400 µL	50 µL	50 µL	50 nM	1.25 µL	1 µL
24-well	500 µL	400 µL	50 µL	50 µL	30 nM	0.75 µL	1 µL
24-well	500 µL	400 µL	50 µL	50 µL	20 nM	0.5 µL	1 µL
24-well	500 µL	400 µL	50 µL	50 µL	10 nM	0.25 µL	1 µL
12-well	1 mL	800 µL	100 µL	100 µL	100 nM	5 µL	2 µL
12-well	1 mL	800 µL	100 µL	100 µL	50 nM	2.5 µL	2 µL
12-well	1 mL	800 µL	100 µL	100 µL	30 nM	1.5 µL	2 µL
12-well	1 mL	800 µL	100 µL	100 µL	20 nM	1.0 µL	2 µL

Culture Plate Format	Total Volume per Well	Culture Medium	Opti-MEM (for siRNA dilution)	Opti-MEM (for transfection reagent dilution)	Final Concentration	siRNA Product* (20 μM stock)	Transfection Reagent
12-well	1 mL	800 μL	100 μL	100 μL	10 nM	0.5 μL	2 μL
6-well	2 mL	1500 μL	250 μL	250 μL	100 nM	10 μL	5 μL
6-well	2 mL	1500 μL	250 μL	250 μL	50 nM	5 μL	5 μL
6-well	2 mL	1500 μL	250 μL	250 μL	30 nM	3 μL	5 μL
6-well	2 mL	1500 μL	250 μL	250 μL	20 nM	2 μL	5 μL
6-well	2 mL	1500 μL	250 μL	250 μL	10 nM	1 μL	5 μL

Note: The data in the table is for reference only. The dosage of transfection reagent may need further optimization for some cell types. *The volume of siRNA product sampled is based on a 20 μM stock concentration.

Efficacy Detection

Perform expression level detection 24~72 hours after transfection. The optimal detection time depends on cell type, transfection reagent, and experimental objectives.

1. RNA Level Detection: Use qPCR. A significant reduction in mRNA expression can be detected 24~72 hours after siRNA transfection.
2. Protein Level Detection: Primarily performed via Western Blot. The detection time is influenced by intracellular protein expression levels and half-life, generally occurring 48~96 hours post-transfection.
3. Functional Screening: Use methods such as EdU cell proliferation and EdUTP cell apoptosis assays for cell function screening.

In Vivo Experiments

It is recommended to use siRNA modified with Chol, OMe, PS, etc., to prolong efficacy.

Local Administration: The most direct delivery method, offering high siRNA delivery efficiency and low dosage, with rapid absorption. Suitable for superficial organs and tissues, including the eye, muscle, and subcutaneous tissue.

Systemic Administration: For target sites inaccessible via local administration, such as internal organs and diffusely distributed targets (e.g., lymphocytes, metastatic tumor cells), systemic injection can be used, providing widespread tissue distribution including the heart, liver, spleen, lungs, and kidneys.

Key Experimental Considerations

1. Maintain an RNase-Free Environment: The environment and air contain abundant RNases, and even trace amounts can cause severe RNA degradation. Always dilute RNA products with DEPC-treated water or RNase-free water. Avoid contact between RNA products and human skin or non-RNase-free laboratory supplies. Since exhaled air and droplets contain high levels of RNases, experiments must be performed in a laminar flow hood while

wearing a mask.

2. Optimize Transfection Conditions: This is extremely important. For different cells and experiments, optimize the concentrations of siRNA and transfection reagent. If transfection efficiency is poor, try alternative transfection reagents, as protocols can vary significantly and must be followed strictly. If possible, use more efficient transfection methods such as electroporation or viral transfection.
3. Validate Phenotypes with Multiple siRNAs: Use two or more siRNAs to validate phenotypes, to rule out false positives caused by off-target effects. An increasing number of journals now require this practice.
4. Use Antibiotic-Free Medium: Use antibiotic-free medium for 6~8 hours after transfection, as antibiotics are toxic to cells and can interfere with siRNA delivery.
5. Ensure Healthy Cell Condition: Use cells with fewer than 50 passages, as transfection efficiency declines in highly passaged cells. Cells must be in a healthy state before transfection; poor cell condition typically results in low transfection efficiency and significantly impacts interference efficiency detection.
6. Include Negative, Positive, and Fluorescent Controls: Use scrambled siRNA as a negative control to exclude off-target effects on experimental data. Use a positive control (we provide GAPDH siRNA as a gift) to validate the experimental workflow and quickly identify potential issues. Use a fluorescent control to analyze siRNA transfection efficiency and facilitate optimization of experimental conditions.

Frequently Asked Questions and Troubleshooting

1. Low Cell Transfection Efficiency?

1.1. Low siRNA purity: Avoid RNase contamination during resuspension. Use DEPC-treated supplies and reagents.

1.2. Low concentration of siRNA-transfection reagent complex: Optimize transfection efficiency by using an appropriate dose of transfection reagent.

1.3. Serum interference: Do not include serum in the mixture when preparing complexes. Serum can be present during culture, but antibiotics should be avoided.

1.4. Improper storage of liposomal transfection reagent: The reagent may have expired. Store it at 4℃.

2. Massive Cell Death After Transfection?

2.1. Poor cell condition: Improve cell growth status; cells in a healthy growth phase generally tolerate transfection reagents better.

2.2. Excessively high concentration of siRNA or transfection reagent.

2.3. High toxicity of the transfection reagent: Switch to an alternative reagent.

2.4. Low siRNA purity: Impurities are affecting cell viability.

2.5. Prolonged transfection time: Failure to replace medium with fresh medium in a timely manner after transfection.

3. No Fluorescence Observed After Transfection?

3.1. Typically, FAM-labeled siRNA exhibits green fluorescence (excitation wavelength 495 nm), and CY3-labeled siRNA exhibits red fluorescence (excitation wavelength 550 nm). First, confirm that the correct fluorescence excitation wavelength is used.

3.2. Wash cells immediately after transfection for fluorescence observation to avoid delays.

3.3. Improper product storage: siRNA degradation or FAM quenching. FAM is prone to photodegradation, so store in the dark.

3.4. Perform transfection in the dark to avoid prolonged exposure of FAM to white light.

3.5. Prolonged transfection time: Failure to replace medium with fresh medium in a timely manner after transfection.

4. Target Gene Interference Efficiency is Only ~60%. Is the Kit Considered Ineffective?

Typically, our kit products guarantee an interference efficiency of ~70%. An efficiency of ~60% is still considered meaningful, and if significant phenotypic and functional changes are observed in cells, this is generally accepted in the literature. Interference efficiency can often be improved by increasing transfection efficiency and optimizing the experimental system.

5. Why is mRNA Level Detection Emphasized? Can Protein and Function Be Detected Directly?

siRNA acts directly on mRNA, making mRNA level detection the most direct indicator. While many assume mRNA degradation directly leads to reduced protein levels, protein detection can also be used as a validity indicator. However, mRNA and protein levels often do not correlate perfectly, due to:

5.1. The timing of detection: mRNA reduction may not yet be reflected in protein levels or may not have reached a detectable threshold. Thus, protein and functional detection should generally be performed later.

5.2. Protein expression dynamics: The mRNA translation process is complex. Cellular gene expression maintains homeostasis, and once certain protein levels are sufficient for function, expression may be temporarily "shut off," with some transcribed mRNA not participating in translation. Thus, mRNA downregulation does not always correlate linearly with protein downregulation.

5.3. More complex underlying mechanisms may also be involved.

6. How Long Does siRNA Act in Cells? When is the Best Time for Detection?

siRNA-mediated RNAi is a transient phenomenon and cannot be stably passaged, typically lasting no longer than 3~4 days post-transfection. The optimal detection time varies by cell type and target gene, generally occurring 24~48 hours post-transfection. It is recommended to detect mRNA levels at 24~48 hours and protein levels at 48~72 hours.

7. Is Cell Transfection Efficiency Related to siRNA Sequence?

Transfection efficiency depends on the cell type and transfection method, and is not directly related to the siRNA sequence. Thus, siRNA transfection efficiency may vary across different cell types.

8. Why is the Same siRNA Effective in Cell A but Not in Cell B?

Differences in transfection efficiency and gene expression levels between cell types affect siRNA efficacy, so consistent interference efficiency across different cells cannot be guaranteed.

After-Sales Service

If no objections are raised within one month of receiving the siRNA product, it will be deemed to be of good quality, and no further complaints will be accepted. If a quality issue is confirmed, we will arrange for free re-synthesis or compensation not exceeding the value of the siRNA product itself.

Precautions

When the transfection efficiency reaches over 90%, we guarantee the validity of at least one pair of primers for mRNA level detection via qPCR (if invalid for the first time, two additional pairs will be designed free of charge). No guarantee is provided for the validity of protein level detection. For siRNA experiments, the FAM-NC included in the kit must be used to conduct transfection efficiency evaluation assays, and the bright-field and fluorescence images should be retained for record.