WB-Validated FOXO4 Lentiviral shRNA Knockdown Kit

Catalog #: V2584

Aliases

FOXO4; Forkhead Box O4; AFX1; MLLT7; Myeloid/Lymphoid Or Mixed-Lineage Leukemia (Trithorax Homolog, Drosophila); Translocated To, 7 2 3; Fork Head Domain Transcription Factor AFX1; Forkhead Box Protein O4; AFX; Myeloid/Lymphoid Or Mixed-Lineage Leukemia (Trithorax (Drosophila) Homolog); Translocated To, 7

Background

Gene Name: FOXO4
NCBI Gene Entry: 4303

Storage

Store at -80 °C for one year.

Kit Components

1. WB-Validated FOXO4 shRNA lentiviral particles (4 mL)
2 . Non-Target shRNA lentiviral particles (4 mL)
3. Verification Tool: KD-Validated Anti-Forkhead box O4 Rabbit mAb #62585 (5 µL)

Tested Cell Line

293T

Validation Methods

RT-qPCR; Western Blotting (WB)

Shipping

Shipped with dry ice. Immediately store the product in a standard freezer at -80°C upon receipt.

Instructions For Use

The following protocol uses 293T cell as an example assuming your cell culture medium is DMEM-based.
1.Release 0.5 million 293T cells into a 35 mm tissue culture dish in 2 mL of the growth medium (DMEM containing 10% FBS and 1% pen/strep). Cell density should reach 50-60% confluence the following day.
2.24 h after cell release, pre-warm the shRNA lentiviral medium to 37°C.
3.Discard 1 mL of the original growth medium of the 35 mm dish.
4.Using a serological pipette, gently mix the lentiviral solution 3 times.

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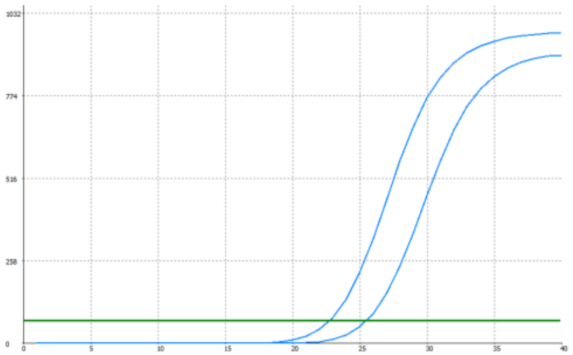
5.Carefully add 1 mL of the lentiviral solution to the well. Tip: To prevent splashing, add the solution to the dish along the wall.
6.Add a polybrene stock solution to the culture medium at a final concentration of 5 µg/mL. Gently swirl the dish to mix.
7.48 h after cell release, without discarding the original medium, add another 1 mL of lentiviral medium directly into the dish.
8.Add an additional polybrene stock solution into the dish to obtain a final concentration of 5 µg/mL. Tip: Now, the medium in the dish should be a total of 3 mL.
9.72 h after cell release, cells may reach confluence. Trypsinize the cells off the 35 mm dish and culture those cells in a 60 mm dish.
10.Add puromycin to the dish at a final concentration of 4 µg/mL. Tip: To assess the efficacy of puromycin selection, culture a dish of wild-type 293T cells as a negative control.
11.Allow puromycin selection for 48 h. Almost all wild-type 293T cells should die, while the dish infected with lentiviruses should have some remaining cells.
12.Replace the medium with regular growth medium without puromycin and allow the cells to grow to confluence before harvesting or staining.

Note: 1. This product is for research use only.
2. This product is only supplied to end users.
3. Do not use this product for commercial.

Validation Data



Genotype	Ct Value
Wild-Type	22.69
Knock-Down	25.31
ΔCt (Ct_{KD}-Ct_{WT})	2.62
% mRNA Reduction	⬇ 84%

RT-qPCR analysis. 293T cells were infected with FOXO4-specific shRNA lentiviral particles, total RNA was extracted from wild-type and knockdown cells, RT-qPCR was performed using gene-specific primers. ΔCt (CtKD-CtWT) was used to calculate mRNA reduction (%) between wild-type and knockdown cells using the following formula: (1-1/2ΔCt) x 100%.

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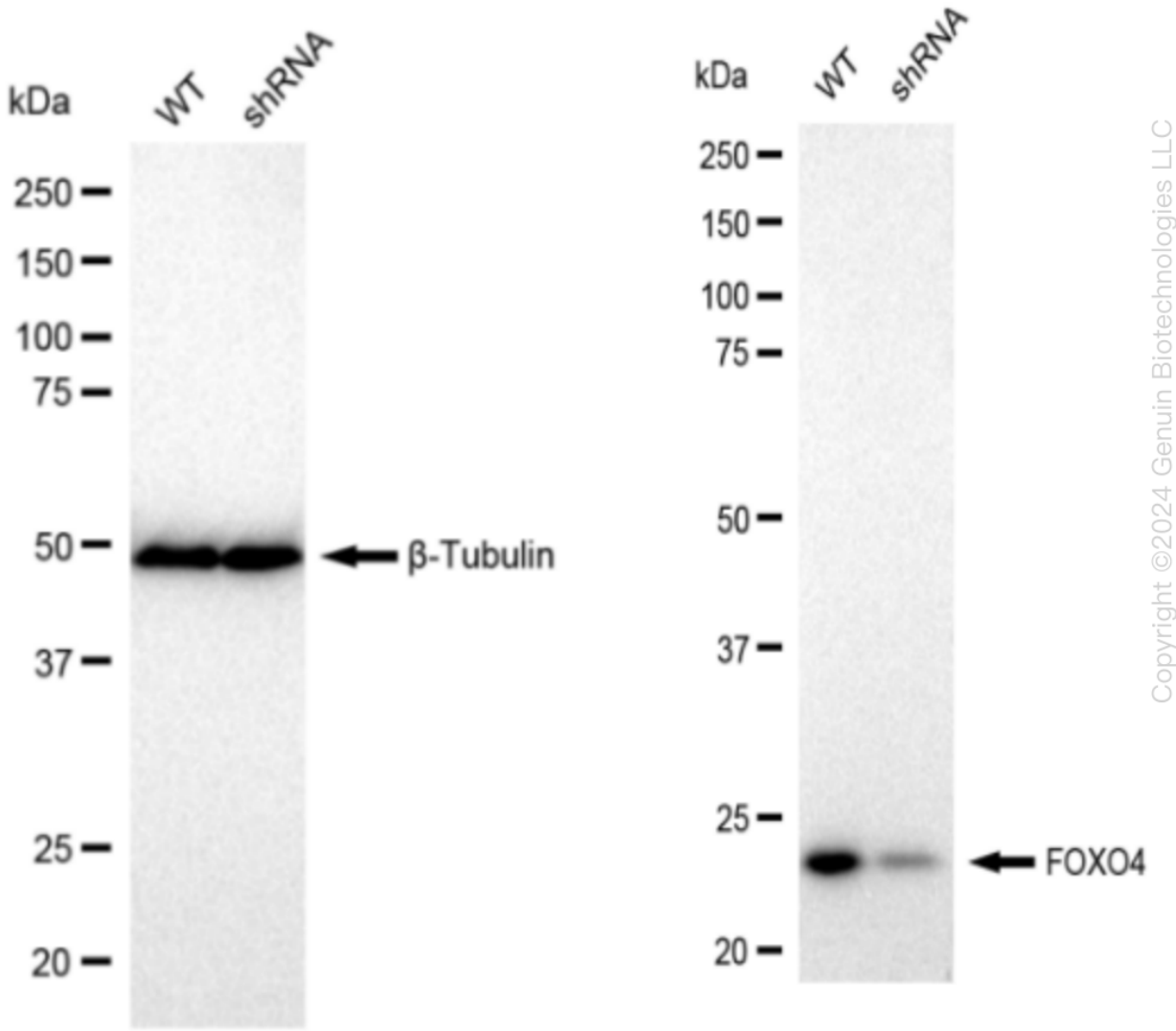

Western blotting analysis. FOXO4 protein expression in wild-type (WT) and shRNA knockdown (KD) 293T cells was detected using Western blotting. β-Tubulin served as a loading control. The blots were incubated with primary antibodies (Cat#62585, 1:5,000) against FOXO4 and β-Tubulin, respectively, followed by incubating with HRP-conjugated goat anti-rabbit secondary antibody (Cat#201, 1:20,000). Images were developed using FeQ™ ECL Substrate Kit (Cat#226).