WB-Validated TAF1C Lentiviral shRNA Knockdown Kit



Catalog #: V65610

Aliases

TAF1C; TATA-Box Binding Protein Associated Factor, RNA Polymerase I Subunit C; TAFI110; MGC:39976; TAFI95; SL1; TATA Box Binding Protein (TBP)-Associated Factor, RNA Polymerase I, C, 110kDa; TATA Box Binding Protein (TBP)-Associated Factor, RNA Polymerase I, C, 110kD; TATA Box-Binding Protein-Associated Factor RNA Polymerase I Subunit C; RNA Polymerase I-Specific TBP-Associated Factor 110 KDa; Transcription Initiation Factor SL1/TIF-IB Subunit C; TATA Box-Binding Protein-Associated Factor 1C; TBP-Associated Factor 1C; TATA-Box Binding Protein Associated Factor, RNA Polymerase I, C; Transcription Factor SL1; SL1, 110kD Subunit

Background

Gene Name: TAF1C NCBI Gene Entry: 9013

Storage

Store at -80 °C for one year.

Kit Components

- 1. WB-Validated TAF1C shRNA lentiviral particles (4 mL)
- 2. Non-Target shRNA lentiviral particles (4 mL)
- 3. Verification Tool: KD-Validated Anti-TAF1C Rabbit mAb#65610 (5 μL)

Tested Cell Line

HT-1080

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 HT-1080 cell as an example assuming your cell culture medium is DMEM-based.

1.Release 0.5 million HT-1080 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%

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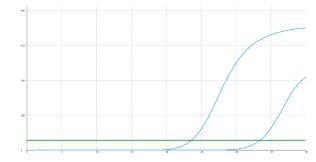
confluence the following day.

- 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.
- 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 $\mu g/mL$. 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 μ g/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 μ g/mL. Tip: To assess the efficacy of puromycin selection, culture a dish of wild-type HT-1080 cells as a negative control.
- 11.Allow puromycin selection for 48 h. Almost all wild-type HT-1080 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.

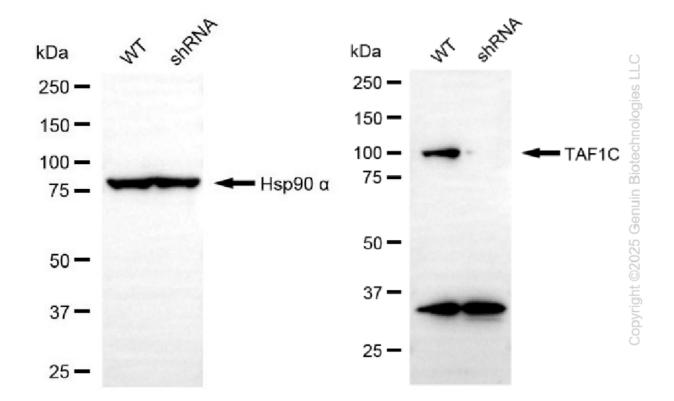
Validation Data



Genotype	Ct Value
Wild-Type	23.39
Knock-Down	32.36
∆Ct (Ct _{KD} -Ct _{WT})	8.97
% mRNA	
Reduction	99.8%

RT-qPCR analysis. HT-1080 cells were infected with TAF1C-specific shRNA lentiviral particles, total RNA was extracted from wild-type and knockdown cells, RT-qPCR was performed using gene-specific primers. Δ Ct (CtKD-CtWT) was used to calculate mRNA reduction (%) between wild-type and knockdown cells using the following formula: $(1-1/2\Delta$ Ct) x 100%.

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Western blotting analysis.TAF1C protein expression in wild-type (WT) and shRNA knockdown (KD) HT-1080 cells was detected using Western blotting. Hsp90 α served as a loading control. The blots were incubated with primary antibodies against TAF1C and Hsp90 α , respectively, followed by incubating with HRP-conjugated goat anti-rabbit secondary antibody. Images were developed using NaQTM ECL Substrate Kit.