WB-Validated ATP6V1A Lentiviral shRNA Knockdown Kit

Catalog #: V63693

Aliases

ATP6V1A; ATPase H+ Transporting V1 Subunit A; V-ATPase Subunit A; ATP6V1A1; ATP6A1; Vma1; VA68; VPP2; ATPase, H+ Transporting, Lysosomal 70kDa, V1 Subunit A; V-Type Proton ATPase (V-ATPase) Catalytic Subunit A; V-Type Proton ATPase Catalytic Subunit A; Vacuolar Proton Pump Subunit Alpha; ATPase, H+ Transporting, Lysosomal (Vacuolar Proton Pump), Alpha Polypeptide, 70kD, Isoform 1; H+-Transporting ATPase Chain A, Vacuolar (VA68 Type); ATPase, H+ Transporting, Lysosomal, Subunit A1; H(+)-Transporting Two-Sector ATPase, Subunit A; Vacuolar Proton Pump Alpha Subunit 1; Vacuolar ATPase Isoform VA68; V-ATPase 69 KDa Subunit 1; Vacuolar-Type H(+)-ATPase; V-ATPase 69 KDa Subunit; V-ATPase A Subunit 1; EC 3.6.3.14; EC 7.1.2.2; EC 3.6.3; ARCL2D; IECEE3; DEE93; HO68

Background

Gene Name: ATP6V1A
NCBI Gene Entry: 523

Storage

Store at -80 °C for one year.

Kit Components

1. WB-Validated ATP6V1A shRNA lentiviral particles (4 mL)
2. Non-Target shRNA lentiviral particles (4 mL)
3. Verification Tool: KD-Validated Anti-ATP6V1A Rabbit mAb #63693 (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.

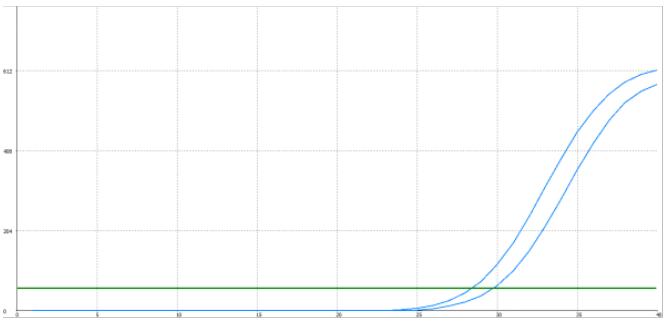
Instructions For Use

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% 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 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 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.

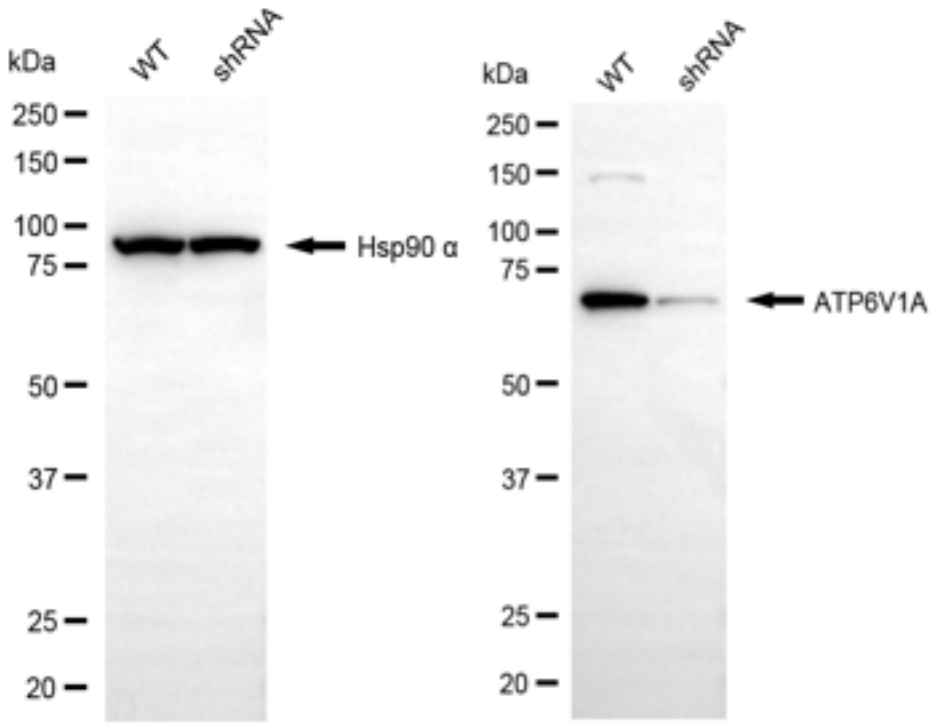
Validation Data



Genotype	Ct Value
Wild-Type	27.91
Knock-Down	29.10
ΔCt (CtKD-CtWT)	1.19
% mRNA Reduction	56%

RT-qPCR analysis. HT-1080 cells were infected with ATP6V1A-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%.

WB-Validated ATP6V1A Lentiviral shRNA Knockdown Kit



Western blotting analysis. ATP6V1A 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 ATP6V1A and Hsp90 α, respectively, followed by incubating with HRP-conjugated goat anti-rabbit secondary antibody. Images were developed using FeQ™ ECL Substrate Kit.