WB-Validated CRYAB Lentiviral shRNA Knockdown Kit

Catalog #: V61176

Aliases

CRYAB; Crystallin Alpha B; HSPB5; CRYA2; Heat Shock Protein Family B Member 5; Renal Carcinoma Antigen NY-REN-27; Heat Shock Protein Beta-5; Rosenthal Fiber Component; Alpha-Crystallin B Chain; Epididymis Secretory Protein Li 101; Heat-Shock 20 KD Like-Protein; Crystallin, Alpha B; Alpha(B)-Crystallin; HEL-S-101; CTRCT16; CMD1II; CTPP2; HspB5; MFM2

Background

Gene Name: CRYAB
NCBI Gene Entry: 1410

Storage

Store at -80 °C for one year.

Kit Components

1. WB-Validated CRYAB shRNA lentiviral particles (4 mL) 2. Non-Target shRNA lentiviral particles (4 mL) 3. Verification Tool: KD-Validated Anti-Crystallin alpha B Rabbit mAb #61176 (5 µL)

Tested Cell Line

H9C2

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.

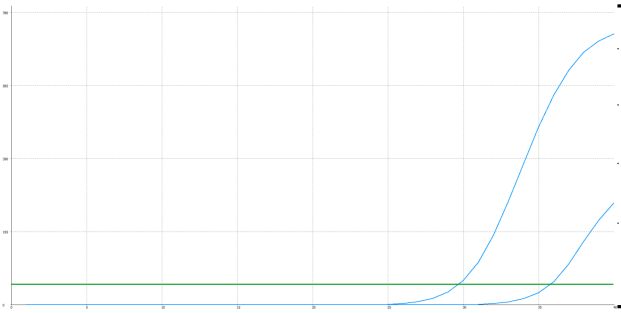
Instructions For Use

The following protocol uses H9C2 cell as an example assuming your cell culture medium is DMEM-based. 1.Release 0.5 million H9C2 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 H9C2 cells as a negative control. 11.Allow puromycin selection for 48 h. Almost all wild-type H9C2 cells should die, while the dish infected with lentiviruses should have some remaining cells. 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.
4. The shRNA sequence was designed based on the human gene sequence. Since there was no antibody available with species reactivity of Human, the murine H9C2 cell line was chosen to validate the gene knockdown effectiveness using an antibody product with species reactivity of Rat.

Validation Data



Genotype	Ct Value
Wild-Type	29.56
Knock-Down	34.19
ΔCt (Ct_{KD}-Ct_{WT})	4.63
% mRNA Reduction	⬇ 96%

RT-qPCR analysis. H9C2 cells were infected with CRYAB-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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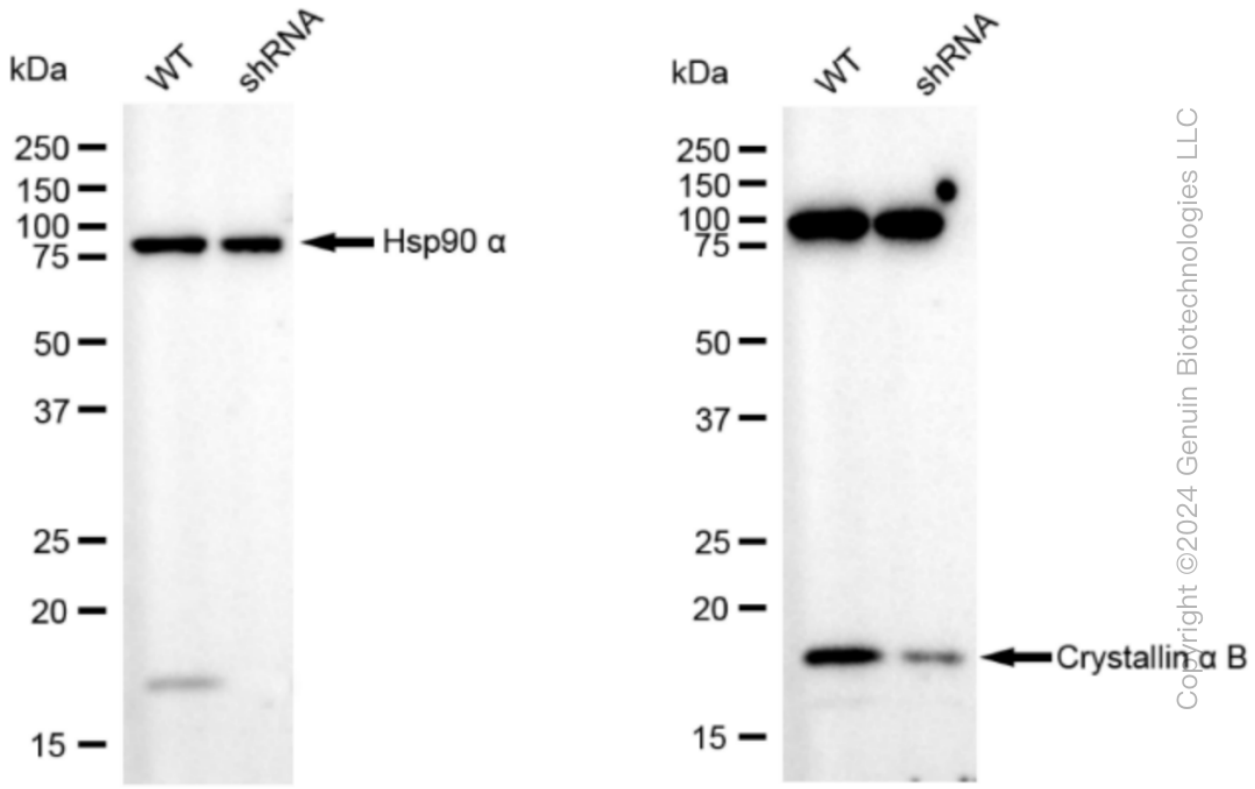

Western blotting analysis. CRYAB protein expression in wild-type (WT) and shRNA knockdown (KD) H9C2 cells was detected using Western blotting. Hsp90 α served as a loading control. The blots were incubated with primary antibodies against CRYAB and Hsp90 α, respectively, followed by incubating with HRP-conjugated goat anti-rabbit secondary antibody. Images were developed using FeQ™ ECL Substrate Kit.