WB-Validated HDHD5 Lentiviral shRNA Knockdown Kit

Catalog #: V62225

Aliases

HDHD5; Haloacid Dehalogenase Like Hydrolase Domain Containing 5; CECR5; Haloacid Dehalogenase-Like Hydrolase Domain-Containing 5; Cat Eye Syndrome Chromosome Region, Candidate 5; Cat Eye Syndrome Critical Region Protein 5

Background

Gene Name: HDHD5
NCBI Gene Entry: 27440

Storage

Store at -80 °C for one year.

Kit Components

1. WB-Validated HDHD5 shRNA lentiviral particles (4 mL)
2 . Non-Target shRNA lentiviral particles (4 mL)
3. Verification Tool: KD-Validated Anti-HDHD5 Rabbit mAb #62225 (5 µL)

Tested Cell Line

HeLa

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.

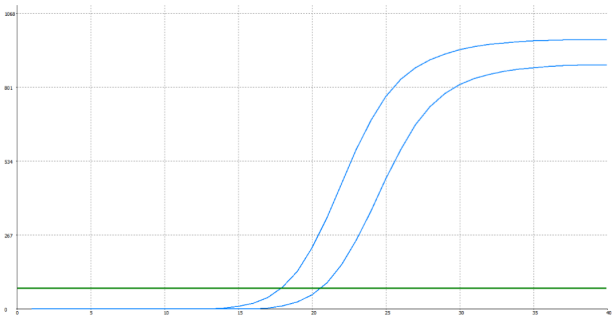
Instructions For Use

The following protocol uses HeLa cell as an example assuming your cell culture medium is DMEM-based.
1.Release 0.5 million HeLa 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 HeLa cells as a negative control.
11.Allow puromycin selection for 48 h. Almost all wild-type HeLa 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	17.75
Knock-Down	20.25
ΔCt (Ct_{KD}-Ct_{WT})	2.50
% mRNA Reduction	⬇ 82%

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RT-qPCR analysis. HeLa cells were infected with HDHD5-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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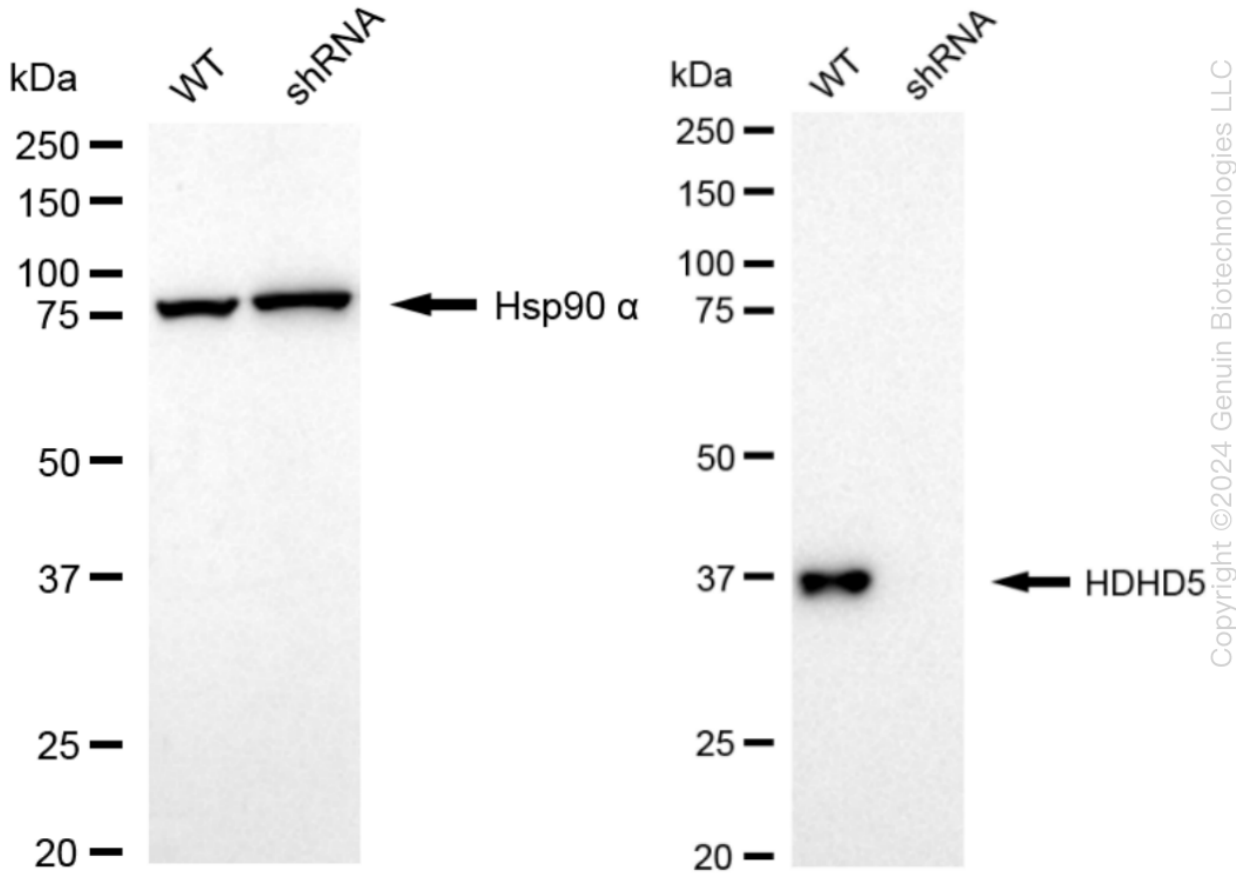

Western blotting analysis. HDHD5 protein expression in wild-type (WT) and shRNA knockdown (KD) HeLa cells was detected using Western blotting. Hsp90 α served as a loading control. The blots were incubated with primary antibodies (Cat#62225, 1:5,000) against HDHD5 and Hsp90 α, respectively, followed by incubating with HRP-conjugated goat anti-rabbit secondary antibody (Cat#201, 1:20,000). Images were developed using FeQ™ ECL Substrate Kit (Cat#226).