Human ACTR3 Knockdown Cell Line (WB-Validated)

Catalog #: C81176

Aliases

ACTR3; Actin Related Protein 3; ARP3; ARP3 Actin Related Protein 3 Homolog; Actin-Related Protein 3; Actin-Like Protein 3; ARP3 (Actin-Related Protein 3, Yeast) Homolog; ARP3 Actin-Related Protein 3 Homolog (Yeast)

Background

Gene Name: ACTR3
NCBI Gene Entry: 10096

Storage

Store at liquid nitrogen for 1 year.

Kit Components

1. Human ACTR3 Knockdown Cell Line (Wb-Validated)
2. Wild-type cell line

Parental Cell Line

Human cell line supplied by the client

Validation Methods

RT-qPCR, Western blotting (WB)

Shipping

Shipped on Dry Ice.

Instructions For Use

This knockdown cell line should be paired with wild-type cell line for use.

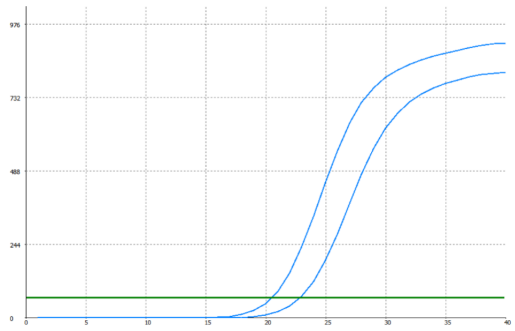
Manufacturing Process

The following protocol was used to generate mRNA knockdown cells:
1.Release 0.5 million 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 cells as a negative control.
11.Allow puromycin selection for 48 h. Almost all wild-type cells should die, while the dish infected with lentiviruses should have some remaining cells.
606.Replace the medium with regular growth medium without puromycin and allow the cells to grow to confluence before harvesting or staining.

Note: This product is for research use only.

Validation Data



Genotype	Ct Value
Wild-Type	20.43
Knock-Down	22.59
ΔCt (Ct_{KD}-Ct_{WT})	2.16
% mRNA Reduction	⬇ 78%

RT-qPCR analysis. HeLa cells were infected with ACTR3-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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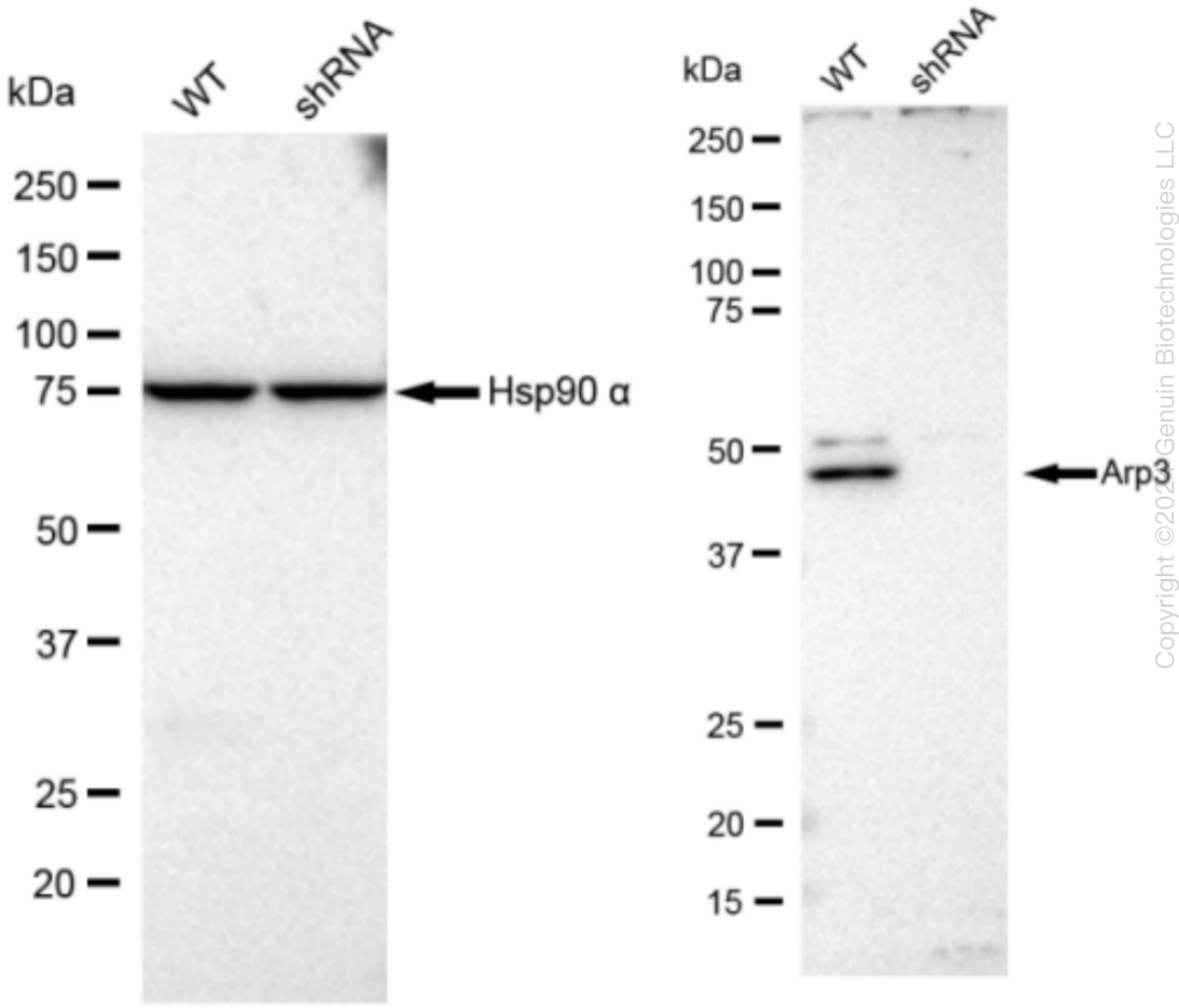

Western blotting analysis. ACTR3 protein expression in wild-type (WT) and shRNA knockdown (KD) HeLa cells was detected using Western blotting. HSP90-Alpha served as a loading control. The blots were incubated with primary antibodies (Cat#69173, 1:5,000) against ACTR3 and HSP90-Alpha, respectively, followed 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).