WB-Validated PAFAH1B1 Knockdown Cell Lysate Kit

Catalog #: L61494

Aliases

PAFAH1B1; Platelet Activating Factor Acetylhydrolase 1b Regulatory Subunit 1; LIS1; PAFAH; MDCR; MDS; Platelet-Activating Factor Acetylhydrolase 1b, Regulatory Subunit 1 (45kDa); Platelet-Activating Factor Acetylhydrolase IB Subunit Beta; NudF; Platelet-Activating Factor Acetylhydrolase, Isoform Ib, Alpha Subunit (45kD); Platelet-Activating Factor Acetylhydrolase, Isoform Ib, Alpha Subunit 45kDa; Platelet-Activating Factor Acetylhydrolase, Isoform Ib, Subunit 1 (45kDa); Miller-Dieker Syndrome Chromosome Region; PAF Acetylhydrolase 45 KDa Subunit; Lissencephaly 1 Protein; Lissencephaly-1 Protein; PAF-AH 45 KDa Subunit; Lissencephaly-1; PAF-AH Alpha; PAFAH Alpha; PAFAHA; LIS-1; LIS2; NUDF

Background

Gene Name: PAFAH1B1
NCBI Gene Entry: 5048

Storage

Stored at -20°C for 2 years.

Kit Components

1. 100 µg WT cell lysate
2. 100 µg KD cell lysate

Tested Cell Line

HeLa

Validation Methods

RT-qPCR; Western Blotting (WB)

Shipping

Shipped with gel ice packs. Immediately store the product in a standard freezer at -20°C upon receipt.

Instructions For Use

This knockdown cell lysate should be paired with wild-type HeLa cell lysate for use. For Western blotting, we recommend running wild-type and knockdown lysates on the same gel, and loading each well with equal volume and equal amount of total proteins.

Manufacturing Process

The following protocol was used to generate mRNA knockdown cell lysate:
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.
13.Cells were lysed with IntactProtein™ cell/tissue lysis kit (Cat#415) and stored in -20°C.

Note: This product is for research use only.

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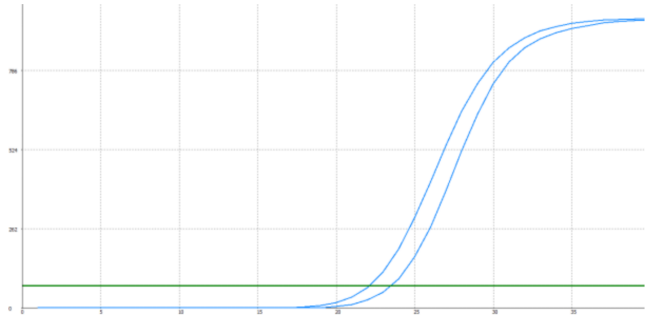
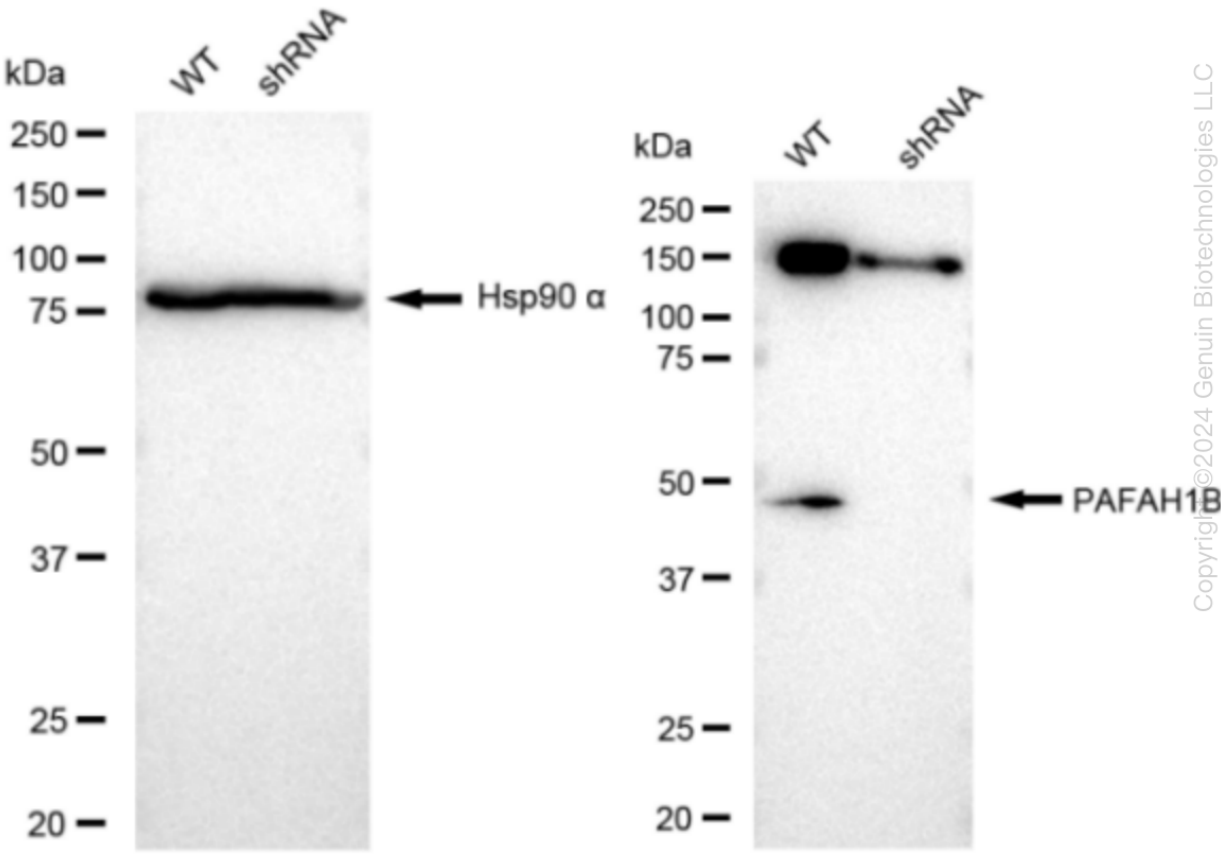
Validation Data



Genotype	Ct Value
Wild-Type	22.00
Knock-Down	23.34
ΔCt (Ct_{KD}-Ct_{WT})	1.34
% mRNA Reduction	⬇ 61%

RT-qPCR analysis. HeLa cells were infected with PAFAH1B1-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%.



Western blotting analysis. PAFAH1B1 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#61494, 1:5,000) against PAFAH1B1 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).