WB-Validated P4HB Knockdown Cell Lysate Kit

Catalog #: L61460

Aliases

P4HB; Prolyl 4-Hydroxylase Subunit Beta; PDIA1; PDI; P4Hbeta; ERBA2L; PROHB; PO4HB; PO4DB; DSI; GIT; Procollagen-Proline, 2-Oxoglutarate 4-Dioxygenase (Proline 4-Hydroxylase), Beta Polypeptide; Protein Disulfide Isomerase Family A, Member 1; Protein Disulfide Isomerase-Associated 1; Cellular Thyroid Hormone-Binding Protein; Prolyl 4-Hydroxylase, Beta Polypeptide; Collagen Prolyl 4-Hydroxylase Beta; Protein Disulfide-Isomerase; EC 5.3.4.1; P55; Procollagen-Proline, 2-Oxoglutarate 4-Dioxygenase (Proline 4-Hydroxylase), Beta Polypeptide (Protein Disulfide Isomerase; Thyroid Hormone Binding Protein P55) ; Procollagen-Proline, 2-Oxoglutarate 4-Dioxygenase (Proline 4-Hydroxylase), Beta Polypeptide (Protein Disulfide Isomerase-Associated 1); Protein Disulfide Isomerase/Oxidoreductase; Glutathione-Insulin Transhydrogenase; Thyroid Hormone-Binding Protein P55; Testicular Secretory Protein Li 32; Protocollagen Hydroxylase; CLCRP1; PHDB

Background

Gene Name: P4HB
NCBI Gene Entry: 5034

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 HT-1080 cell lysate for use. For

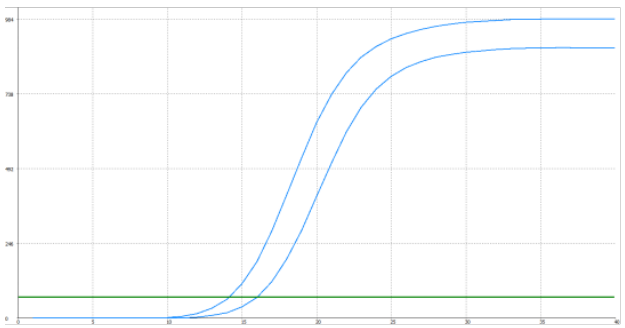
Western blotting, we recommend running wild-type and knockdown lysates on the same gel, and loading each well with equal volume and equal amount of total proteins.

Manufacturing Process

The following protocol was used to generate mRNA knockdown cell lysate:
1.Release 0.5 million 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.
Cells were lysed with IntactProtein™ cell/tissue lysis kit (Cat#415) and stored in -20°C.

Note: This product is for research use only.

Validation Data



Genotype	Ct Value
Wild-Type	14.16
Knock-Down	15.84
ΔCt (CtKD-CtWT)	1.68
% mRNA Reduction	69%

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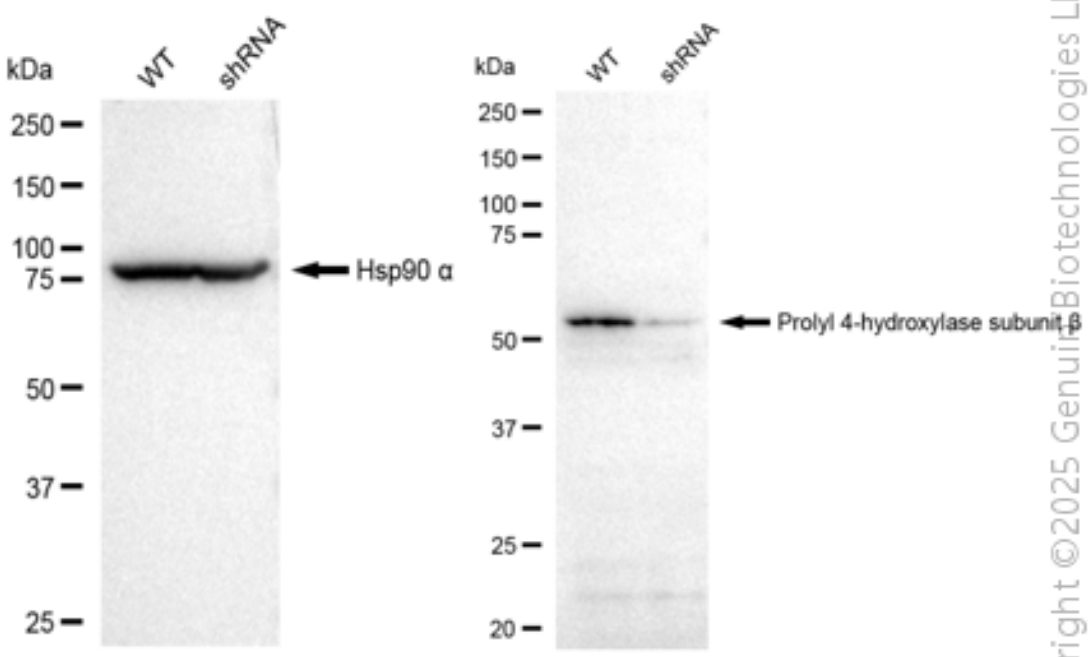
RT-qPCR analysis. HT-1080 cells were infected with P4HB-specific shRNA lentiviral particles, total RNA was extracted from wild-type and knockdown cells, RT-qPCR was performed using gene-specific primers. ΔCt (CtKD-CtWT) was used to calculate mRNA reduction (%) between wild-type and knockdown cells using the following formula: (1-1/2ΔCt) x 100%.



Western blotting analysis. P4HB 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 P4HB and Hsp90 α, respectively, followed by incubating with HRP-conjugated goat anti-rabbit secondary antibody. Images were developed using FeQ™ ECL Substrate Kit.