

WB-Validated PIK3C3 Lentiviral shRNA Knockdown Kit



Catalog #: V62502

Aliases

Phosphatidylinositol 3-Kinase Catalytic Subunit Type 3; HVps34; Vps34; Phosphatidylinositol 3-Kinase P100 Subunit; Vacuolar Protein Sorting 34 Homolog; Phosphoinositide-3-Kinase, Class 3; PtdIns-3-Kinase Type 3; PI3-Kinase Type 3; EC 2.7.1.137; PI3K Type 3; Phosphatidylinositol 3-Kinase, Catalytic Subunit Type 3; Phosphoinositide-3-Kinase Class 3; EC 2.7.1; VPS34

Background

Gene Name: PIK3C3

NCBI Gene Entry: [5289](#)

Storage

Store at -80 °C for one year.

Kit Components

1. WB-Validated PIK3C3 shRNA lentiviral particles (4 mL)
2. Non-Target shRNA lentiviral particles (4 mL)
3. Verification Tool: KD-Validated Anti-PIK3C3 Rabbit mAb #62502 (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.

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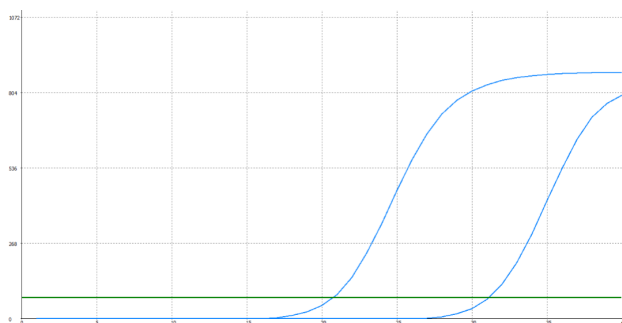
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- Carefully add 1 mL of the lentiviral solution to the well. Tip: To prevent splashing, add the solution to the dish along the wall.
- Add a polybrene stock solution to the culture medium at a final concentration of 5 µg/mL. Gently swirl the dish to mix.
- 48 h after cell release, without discarding the original medium, add another 1 mL of lentiviral medium directly into the dish.
- 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.
- 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.
- 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.
- 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.
- 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.
 - This product is only supplied to end users.
 - Do not use this product for commercial.

Validation Data

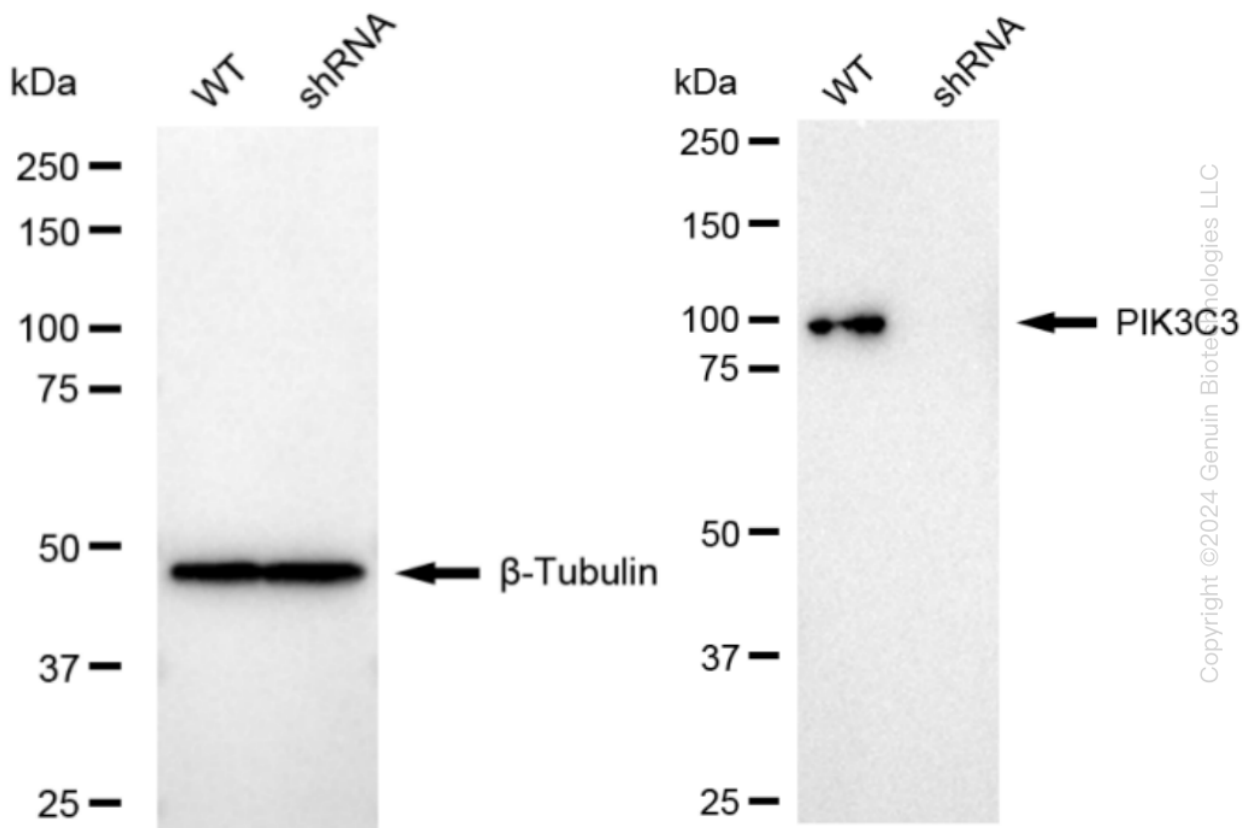


Genotype	Ct Value
Wild-Type	20.40
Knock-Down	30.61
$\Delta Ct (Ct_{KD}-Ct_{WT})$	10.21
% mRNA Reduction	↓ 99.9%

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RT-qPCR analysis. HeLa cells were infected with PIK3C3-specific shRNA lentiviral particles, total RNA was extracted from wild-type and knockdown cells, RT-qPCR was performed using gene-specific primers. $\Delta Ct (Ct_{KD}-Ct_{WT})$ was used to calculate mRNA reduction (%) between wild-type and knockdown cells using the following formula: $(1-1/2^{\Delta Ct}) \times 100\%$.

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Western blotting analysis. PIK3C3 protein expression in wild-type (WT) and shRNA knockdown (KD) HeLa cells was detected using Western blotting. β -Tubulin served as a loading control. The blots were incubated with primary antibodies against PIK3C3 and β -Tubulin, respectively, followed by incubating with HRP-conjugated goat anti-rabbit secondary antibody. Images were developed using FeQ™ ECL Substrate Kit.

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