WB-Validated PSMA6 Knockdown Cell Lysate Kit



Catalog #: L64853

Aliases

PSMA6; Proteasome 20S Subunit Alpha 6; PROS27; P27K; IOTA; Proteasome (Prosome, Macropain) Subunit, Alpha Type, 6; Multicatalytic Endopeptidase Complex Iota Chain; Proteasome Subunit Alpha Type-6; Proteasome Subunit Alpha 6; 27 KDa Prosomal Protein; Proteasome Iota Chain; Macropain Iota Chain; MGC22756; MGC23846; MGC2333; PROS-27; Testicular Secretory Protein Li 44; Proteasome Subunit Alpha1; Proteasome Subunit Iota; Macropain Subunit Iota; Proteasome Subunit Al; Prosomal P27K Protein; EC 3.4.25.1; Proteasome Subunit Alpha-1; Alpha-1

Background

Gene Name: PSMA6 NCBI Gene Entry: 5687

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.

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Manufacturing Process

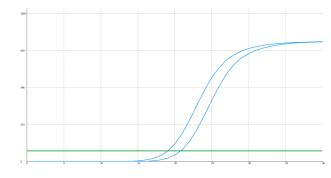
The following protocol was used to generate mRNA knockdown cell lysate:

- 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 $\mu g/mL$. 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 μ g/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 μ g/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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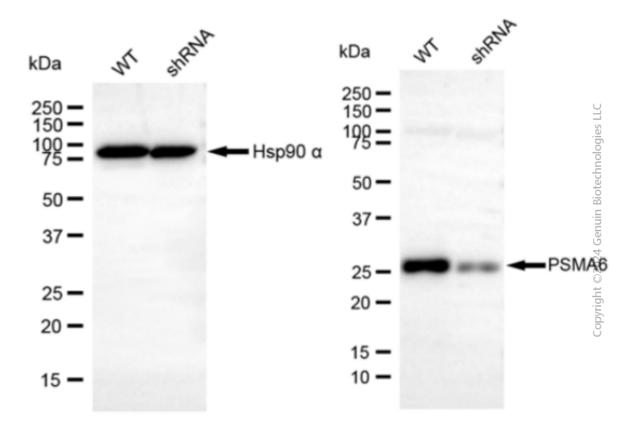
Validation Data



Genotype	Ct Value
Wild-Type	28.68
Knock-Down	20.41
∆Ct (Ct _{KD} -Ct _{WT})	1.73
% mRNA	
Reduction	₹ 70%

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RT-qPCR analysis. HeLa cells were infected with PSMA6-specific shRNA lentiviral particles, total RNA was extracted from wild-type and knockdown cells, RT-qPCR was performed using gene-specific primers. Δ Ct (CtKD-CtWT) was used to calculate mRNA reduction (%) between wild-type and knockdown cells using the following formula: $(1-1/2\Delta$ Ct) x 100%.



Western blotting analysis. PSMA6 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 against PSMA6 and Hsp90 α , respectively, followed by incubating with HRP-conjugated goat anti-mouse secondary antibody. Images were developed using FeQTM ECL Substrate Kit.