# WB-Validated SMARCC1 Lentiviral shRNA Knockdown Kit



**Catalog #: V61706** 

#### **Aliases**

SMARCC1; SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin Subfamily C Member 1; BAF155; CRACC1; SRG3; SWI/SNF-Related Matrix-Associated Actin-Dependent Regulator Of Chromatin Subfamily C Member 1; SWI/SNF Complex Subunit SMARCC1; SWI/SNF Complex 155 KDa Subunit; BRG1-Associated Factor 155; Rsc8; SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of; Chromatin, Subfamily C, Member 1; Mammalian Chromatin Remodeling Complex BRG1-Associated Factor 155; Chromatin Remodeling Complex BAF155 Subunit; HYC5; SWI3; RSC8

### **Background**

Gene Name: SMARCC1 NCBI Gene Entry: 6599

### **Storage**

Store at -80 °C for one year.

### **Kit Components**

- 1. WB-Validated SMARCC1 shRNA lentiviral particles (4 mL)
- 2. Non-Target shRNA lentiviral particles (4 mL)
- 3. Verification Tool: KD-Validated Anti-SMARCC1 Rabbit mAb #61706 (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.

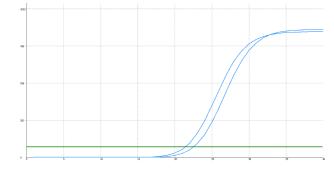
## WB-Validated SMARCC1 Lentiviral shRNA Knockdown Kit

- 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  $\mu$ 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  $\mu$ 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.

**Note:** 1. This product is for research use only.

- 2. This product is only supplied to end users.
- 3. Do not use this product for commercial.

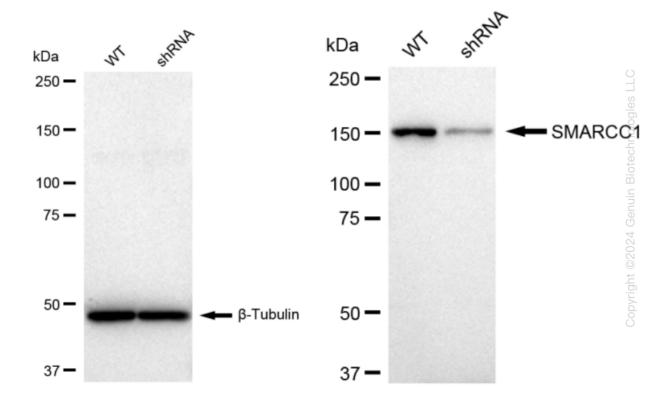
#### Validation Data



Genotype	Ct Value
Wild-Type	21.16
Knock-Down	22.29
$\Delta$ Ct (Ct <sub>KD</sub> -Ct <sub>WT</sub> )	1.13
% mRNA Reduction	<b>↓</b> 54%

RT-qPCR analysis. HeLa cells were infected with SMARCC1-specific shRNA lentiviral particles, total RNA was extracted from wild-type and knockdown cells, RT-qPCR was performed using gene-specific primers.  $\Delta$ 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%.

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Western blotting analysis. SMARCC1 protein expression in wild-type (WT) and shRNA knockdown (KD) HeLa cells was detected using Western blotting.  $\beta$ -Tubulin served as a loading control. The blots were incubated with primary antibodies against SMARCC1 and  $\beta$ -Tubulin, respectively, followed by incubating with HRP-conjugated goat anti-rabbit secondary antibody. Images were developed using FeQ<sup>TM</sup> ECL Substrate Kit.