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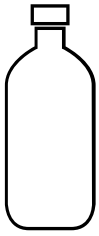
KD-Validated (via WB) Lentiviral shRNA Knockdown Kit



1 Kit (cat. no. TL9000xxV)

Lentiviral particles for shRNA-mediated gene knockdown that are pre-validated by Western Blot to ensure efficient and stable silencing of target genes in various cell lines.

RUO

For Research Use Only

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REF

TL9000xxV

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Product Overview

This kit provides ready-to-use shRNA lentiviral particles validated by Western blot, to ensure success in generating stable cell lines with knockdown of your target. The kit includes targeting and non-targeting lentiviral particles, as well as the corresponding antibody, so results can be validated.

Component Description

The kit is shipped on dry ice. Once received, store the components as indicated below.

Table 1. Storage Conditions for the Components

Component	Storage
KD-validated (via WB) shRNA lentiviral particles (0.5mL, $\geq$ 1×10^7 TU/mL)	-80°C
Scrambled negative control shRNA lentiviral particles (0.5mL, $\geq$ 1×10^7 TU/mL)	-80°C
KD-Validated Rabbit mAb (5 µL)	-20°C

Note: Store all components immediately at the indicated temperatures upon receipt. When stored as directed, the components are stable for up to one year.

Recommendations:

- Optimize MOI to balance transduction efficiency and viability.
- Use healthy, low-passage cells at 50–70% confluence for best results.
- Include non-targeting and non-infected controls to assess specificity and selection efficiency.
- Confirm knockdown at protein level (Western blot); qPCR validation is recommended.
- Aliquot virus upon first thaw and avoid repeated freeze–thaw cycles.

Note:

- Lentiviral particles are replication-incompetent and must be handled under BSL-2 conditions in accordance with institutional biosafety guidelines.
- Transduction efficiency and puromycin sensitivity vary by cell type; optimization may be required.
- The included KD-validated rabbit mAb is optimized for Western blot; dilution optimization may be necessary.
- Viral titer may decrease with improper storage or repeated freeze–thaw cycles.

Lentivirus shRNA Transduction Protocol

Example cell line: HeLa
Culture medium: DMEM + 10% FBS + 1% Pen/Strep

Day 0 - Cell Seeding:

1. Seed **0.5 × 10^6 HeLa cells** into a **35 mm tissue culture dish** containing **2 mL complete growth medium**.
2. Incubate overnight at 37 °C, 5% CO_2.
    - Cells should reach **~50–60% confluence** the next day.

Day 1 (24 h post-seeding) – First Lentiviral Transduction

1. Pre-warm the **shRNA lentiviral medium** to **37 °C**.
2. Carefully **remove 0.2 mL** of the existing growth medium from the dish.
3. Using a serological pipette, **gently mix the lentiviral solution 3 times**.
4. Slowly add **0.2 mL of lentiviral solution** to the dish.
   Tip: Add along the wall of the dish to prevent splashing.
5. Add **polybrene** to a **final concentration of 5 µg/mL**.
6. Gently swirl the dish to ensure even mixing.
7. Return cells to the incubator.

Day 2 (48 h post-seeding) – Second Lentiviral Boost

1. **Do not remove** the existing medium.
2. Add **0.2 mL of lentiviral medium** directly to the dish.
3. Add additional polybrene to maintain a **final concentration of 5 µg/mL**.
    - Total volume in dish should now be **22 mL**.
4. Return cells to the incubator.

Day 3 (72 h post-seeding) – Expansion & Selection

1. Cells may be near confluence.
2. **Trypsinize** cells from the 35 mm dish.
3. Transfer cells into a **60 mm dish** with fresh growth medium.
4. Add puromycin to a **final concentration of 4 µg/mL**.
    - *Tip: Include a wild-type (non-infected) HeLa control dish to verify puromycin effectiveness.*

Day 5 – Post-selection Recovery

1. After **48 h of puromycin selection**:
   - Nearly all wild-type control cells should be dead.
   - Lentivirus-infected cells should have surviving populations.
2. Replace medium with **regular growth medium**.
   Note: To maintain strong selection pressure and ensure a pure population with complete knockdown, puromycin may be continuously included in the growth medium at 2 µg/mL. If the cells are sensitive to puromycin (e.g., significant cell death or markedly reduced growth), puromycin should be omitted.
3. Allow cells to recover and grow to confluence.

Downstream Applications

After stable selection and recovery, cells may be used for:

- Western blot validation of target knockdown
- qPCR analysis of transcript reduction
- Functional assays (proliferation, apoptosis, migration, reporter assays)
- Drug response or pathway studies
- Long-term stable cell line generation

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