ORIGENE

WESTERN BLOT PRE-VALIDATED

KD-Validated Lentiviral shRNA Knockdown Kit

Lentiviral particles for shRNA-mediated gene silencing — validated by Western blot for efficient, stable knockdown in diverse cell lines.

CAT. NO. TL9000XXV · LENTIVIRAL DELIVERY · **FOR RESEARCH USE ONLY**

WB-VALIDATED
Pre-tested knockdown confirmed by Western blot — guaranteed results

ALL-IN-ONE
Targeting + scrambled control lentiviral particles plus matched antibody

STABLE LINES
Puromycin selection enables long-term stable knockdown cell lines

HIGH TITER
$\geq 1\times10^7$ TU/mL for efficient transduction across cell types

KIT CONTENTS

shRNA Lentiviral Particles
0.5 mL, $\geq 1\times10^7$ TU/mL
Target-specific, KD-validated

-80°C

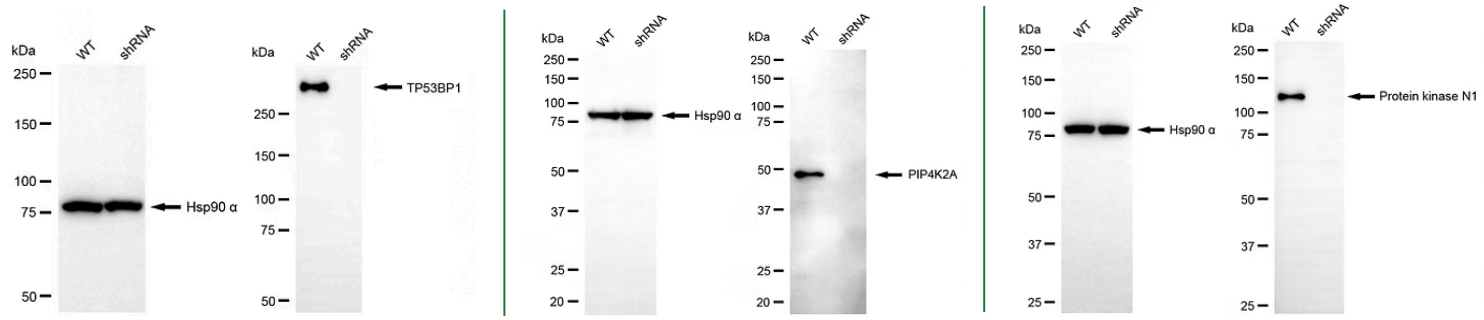
SCRAMBLED CONTROL PARTICLES
0.5 mL, $\geq 1\times10^7$ TU/mL
Scrambled negative control

-80°C

KD-VALIDATED RABBIT MAB
5 µL antibody Optimized for Western blot validation

-20°C

VALIDATED KNOCKDOWN



TRANSDUCTION PROTOCOL

Example Cell Line: HeLa · Culture Medium: DMEM + 10% FBS + 1% Pen/Strep

STEP-BY-STEP WORKFLOW

0 DAY — CELL SEEDING

Seed 0.5×10^6 HeLa cells in a 35 mm dish with 2 mL complete growth medium. Incubate overnight at 37°C, 5% CO_2. Target: ~50–60% confluence next day.

1 DAY — FIRST LENTIVIRAL TRANSDUCTION

Pre-warm lentiviral medium to 37°C. Remove 0.2 mL existing medium; add 0.2 mL lentiviral solution. Add polybrene to 5 µg/mL. Swirl gently; return to incubator.

Tip: Add virus along the dish wall to prevent splashing and ensure even distribution.

2 DAY — SECOND LENTIVIRAL BOOST

Do not remove existing medium. Add another 0.2 mL lentiviral medium plus polybrene (maintain 5 µg/mL). Total volume ~2.2 mL. Return to incubator.

3 DAY — EXPANSION & PUROMYCIN SELECTION

Trypsinize cells; transfer to a 60 mm dish with fresh medium. Add puromycin at 4 µg/mL to select transduced cells.

Tip: Include a non-infected wild-type control dish to confirm puromycin killing efficiency.

5 DAY — POST-SELECTION RECOVERY

Wild-type control cells should be dead; infected cells survive. Replace with regular growth medium. Maintain 2 µg/mL puromycin for selection pressure, or omit if cells show sensitivity. Allow recovery to confluence.

BEST PRACTICES

- ✓ Optimize MOI to balance transduction efficiency and cell viability
- ✓ Use healthy, low-passage cells at 50–70% confluence
- ✓ Include non-targeting and non-infected controls in every experiment
- ✓ Confirm knockdown at protein level (WB); qPCR validation recommended
- ✓ Aliquot virus on first thaw; avoid repeated freeze–thaw cycles
- ✓ Store all components immediately at indicated temperatures

⚠ IMPORTANT NOTES

- Lentiviral particles are replication-incompetent. Handle under BSL-2 conditions per institutional biosafety guidelines.
- Transduction efficiency and puromycin sensitivity vary by cell type — optimization may be required.
- Included rabbit mAb is optimized for Western blot. Dilution optimization may be necessary.
- Viral titer may decrease with improper storage or repeated freeze–thaw cycles
- All components stable for up to one year when stored as directed.

OriGene Headquarters
9620 Medical Center Dr Rockville, MD 20850
+1-301-340-3188
custsupport@origene.com
techsupport@origene.com
sales@origene.com

OriGene EU
SchillerstraBe 5
32052 Herford, Germany
+49-5221-346060-0
info-de@origene.com
www.origene.com

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