shRNA Plasmid DNA Mediated Inhibition of Gene Expression

PROTOCOL

Santa Cruz Biotechnology, Inc.

NOTE: This protocol is recommended for a well from a 6 well tissue culture plate. Adjust cell and reagent amounts proportionately for wells or dishes of different sizes.

- In a six well tissue culture plate, grow cells to a 50–70% confluency in antibiotic-free normal growth medium supplemented with FBS.

NOTE: This protocol is recommended for a well from a 6 well tissue culture plate. Adjust cell and reagent amounts proportionately for wells or dishes of different sizes.

NOTE: Healthy and subconfluent cells are required for successful transfection experiments. It is recommended to ensure cell viability one day prior to transfection.

NOTE: The optimal shRNA Plasmid DNA:shRNA Plasmid Transfection Reagent ratio should be determined experimentally beginning with 1 µg of shRNA Plasmid DNA and between 1.0 and 6.0 µl of shRNA Plasmid Transfection Reagent as outlined below. Once the optimal shRNA Plasmid DNA:shRNA Plasmid Transfection Reagent ratio has been identified for a given cell type, the appropriate amount of shRNA Plasmid DNA/shRNA Plasmid Transfection Reagent complex used per well should be tested to determine which amount provides the highest level of transfection efficiency. For example, if the optimal shRNA Plasmid DNA:shRNA Plasmid Transfection Reagent ratio is 1 µg:1 µl, then amounts ranging from 0.5 µg/0.5 µl to 2.0 µg/2.0 µl should be tested.

- Prepare the following solutions:
  - **Solution A**: For each transfection, dilute 10 µl of resuspended shRNA Plasmid DNA (i.e. 1 µg shRNA Plasmid DNA) into 90 µl **shRNA Plasmid Transfection Medium** (sc-108062).
  - **Solution B**: For each transfection, dilute 1–6 µl of **shRNA Plasmid Transfection Reagent** (sc-108061) with enough **shRNA Plasmid Transfection Medium** (sc-108062) to bring final volume to 100 µl.

NOTE: Do not add antibiotics to the **shRNA Plasmid Transfection Medium** (sc-108062).

NOTE: Optimal results may be achieved by using siliconized microcentrifuge tubes.

NOTE: Although highly efficient in a variety of cell lines, **shRNA Plasmid Transfection Reagent** (sc-108061) may not be suitable for use with all cell lines.

- Add the shRNA Plasmid DNA solution (Solution A) directly to the dilute shRNA Plasmid Transfection Reagent (Solution B) using a pipette. Mix gently by pipetting the solution up and down and incubate the mixture 15–45 minutes at room temperature.
- Wash the cells twice with 2 ml of **shRNA Plasmid Transfection Medium** (sc-108062). Aspirate the medium and proceed immediately to the next step.

NOTE: Do not use PBS as the residual phosphate may compete with DNA and bind the shRNA Plasmid Transfection Reagent, thereby reducing the transfection efficiency.

- For each transfection, add 0.8 ml **shRNA Plasmid Transfection Medium** (sc-108062) to well.
- Add the 200 µl shRNA Plasmid DNA/shRNA Plasmid Transfection Reagent Complex (Solution A + Solution B) dropwise to well, covering the entire layer.
- Gently mix by swirling the plate to ensure that the entire cell layer is immersed in solution.
- Incubate the cells 5–7 hours at 37° C in a CO_2 incubator or under conditions normally used to culture the cells. Longer transfection times may be desirable depending on the cell line.
- Following incubation, add 1 ml of normal growth medium containing 2 times the normal serum and antibiotics concentration (2x normal growth medium).
- Incubate the cells for an additional 18–24 hours under conditions normally used to culture the cells.

OPTIONAL: For transient transfection, aspirate media and replace with fresh 1x normal growth medium. Assay the cells using the appropriate protocol 24–72 hours after the addition of fresh medium in the previous step.

- For selection of stably transfected cells, proceed with puromycin selection as follows:

  NOTE: The working puromycin concentration for mammalian cell lines ranges from 1-10 µg/ml. Prior to using the **puromycin antibiotic** (sc-108071), titrate the selection agent to determine the optimal concentration for target cell line. Use the lowest concentration that kills 100% of non-transfected cells in 3–5 days from the start of puromycin selection.

  - 48 hours post-transfection, aspirate the medium and replace with fresh medium containing puromycin at the appropriate concentration.
  - Approximately every 2–3 days, aspirate and replace with freshly prepared selective media.

  NOTE: Controls should always be included in shRNA experiments. Control shRNAs are available as 20 µg. Each encode a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. **Control shRNA Plasmids** include: sc-108060, sc-108065 and sc-108066.

  NOTE: For Western blot analysis prepare cell lysate as follows: Wash cells once with PBS. Lyse cells in 300 µl **Electrophoresis Sample Buffer, 2X** (sc-24945) by gently rocking the 6 well plate or by pipetting up and down. Sonicate the lysate on ice if necessary.

  NOTE: For RT-PCR analysis isolate RNA using the method described by P. Chomczynski and N. Sacchi (1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162: 156–159) or a commercially available RNA isolation kit.

RECOMMENDED SUPPORT PRODUCTS

PRODUCT	CAT. #	DESCRIPTION	AMOUNT
shRNA Plasmid Transfection Reagent	sc-108061	Delivers shRNA Plasmid DNA into cells with minimal cell 0.2 ml toxicity; enables highly efficient shRNA Plasmid DNA transfection in a variety of cell lines including CHO-K1, COS, LNCaP, NIH/3T3, 293, T24, C2C12, SF-9, primary human keratiocytes, primary aortic smoothmuscle, primary rabbit myoblasts, human bone marrow endothelial cells (HBMEC).	50-100 transfections
shRNA Plasmid Transfection Medium	sc-108062	Reduced-serum medium suitable for addition to shRNA suspension and shRNA Transfection Reagent immediately prior to cell transfection; modification of Eagle's Minimal Essential Medium, buffered with HEPES and sodium bicarbonate, and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, growth factors and phenol red.	20 ml
Control shRNA Plasmid-A	sc-108060	Control shRNA Plasmid-A, -B and -C are negative controls for experiments using targeted shRNA transfection, each encoding a unique, scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA.	20 µg 20 transfections
Control shRNA Plasmid-B	sc-108065		
Control shRNA Plasmid-C	sc-108066		

shRNA Plasmid support reagents are optimal for successful delivery of Santa Cruz Biotechnology, Inc.'s shRNA Gene Silencing Plasmids into mammalian cells. Amounts listed above are based on use of 6-well plates