siRNA Transfection Protocol

Santa Cruz Biotechnology, Inc.

siRNA TRANSFECTION PROTOCOL

▪ In a six well tissue culture plate, seed 2 x 10^5 cells per well in 2 ml 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.

▪ Incubate the cells at 37° C in a CO_2 incubator until the cells are 60-80% confluent. This will usually take 18-24 hours.

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

▪ Prepare the following solutions:

Solution A: For each transfection, dilute 2-8 µl of siRNA duplex (i.e. 0.25-1 µg or 20-80 pmols siRNA) into 100 µl siRNA Transfection Medium: sc-36868.

Solution B: For each transfection, dilute 2-8 µl of siRNA Transfection Reagent: sc-29528 into 100 µl siRNA Transfection Medium: sc-36868. Peak activity should be at about 6 µl siRNA Transfection Reagent.

NOTE: Do not add serum and antibiotics to the siRNA Transfection Medium: sc-36868.

NOTE: Optimal siRNA amount used for transfection may vary for each target protein and should be determined experimentally.

NOTE: If a lower siRNA concentration is desired, dilute siRNA appropriately with siRNA Dilution Buffer: sc-29527.

NOTE: Although highly efficient in a variety of cell lines, siRNA Transfection Reagent: sc-29528 may not be suitable for use with all cell lines.

▪ Add the siRNA duplex solution (Solution A) directly to the dilute 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 once with 2 ml of siRNA Transfection Medium: sc-36868 Aspirate the medium and proceed immediately to the next step.

▪ For each transfection, add 0.8 ml siRNA Transfection Medium to each tube containing the siRNA Transfection Reagent mixture (Solution A + Solution B). Mix gently and overlay the mixture onto the washed cells.

▪ Incubate the cells 5-7 hours at 37° C in a CO_2 incubator.

NOTE: Longer transfection times may be desirable depending on the cell line. However prolonged serum starvation may result in unwanted cell detachment or death.

NOTE: Fluorescein Conjugated Control siRNA should only be incubated for a total 5-7 hours at 37° C in a CO_2 incubator. At the end of incubation they are ready to be assayed by fluorescent microscopy.

▪ Add 1 ml of normal growth medium containing 2 times the normal serum and antibiotics concentration (2x normal growth medium) without removing the transfection mixture. If toxicity is a problem, remove the transfection mixture and replace with 1x normal growth medium.

▪ Incubate the cells for an additional 18-24 hours.

▪ Aspirate the medium 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 step above.

NOTE: Controls should always be included in siRNA experiments. Use either Control siRNAs: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 or sc-44238 or Control siRNA (Fluorescein Conjugates): sc-36869, sc-44239, sc-44240 or sc-44241. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

NOTE: For Western blot analysis prepare cell lysate as follows: Wash cells once with PBS. Lyse cells in 300 µl 1x electrophoresis sample buffer (sc-24945: Electrophoresis Sample Buffer, 2X) 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.

siRNA SUPPORT REAGENTS

PRODUCT	CAT. #	DESCRIPTION	AMOUNT
Control siRNA-A	sc-37007	Control siRNAs A–J are negative controls for experiments using targeted siRNA transfection; each consists of a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA	66 µl, 10 µM; 10-20 transfections
Control siRNA-B	sc-44230	see description above	see above
Control siRNA-C	sc-44231	see description above	see above
Control siRNA-D	sc-44232	see description above	see above
Control siRNA-E	sc-44233	see description above	see above
Control siRNA-F	sc-44234	see description above	see above
Control siRNA-G	sc-44235	see description above	see above
Control siRNA-H	sc-44236	see description above	see above
Control siRNA-I	sc-44237	see description above	see above
Control siRNA-J	sc-44238	see description above	see above
Control siRNA (Fluorescein Conjugate)-A	sc-36869	Control siRNA (Fluorescein Conujugates) A–D are controls to monitor transfection efficiency by fluorescence microscopy; each consists of a scrambled sequence conjugated to fluorescein that will not lead to the specific degradation of any cellular mRNA.	66 µl, 10 µM; 10-20 transfections
Control siRNA (Fluorescein Conjugate)-B	sc-44239	see description above	see above
Control siRNA (Fluorescein Conjugate)-C	sc-44240	see description above	see above
Control siRNA (Fluorescein Conjugate)-D	sc-44241	see description above	see above
siRNA Dilution Buffer	sc-29527	TRIS-EDTA based buffer prepared from RNase-free water suitable for storage and dilution of siRNA; pH 8.	1.5 ml
siRNA Transfection Reagent	sc-29528	Delivers siRNA into cells with minimalcell toxicity; enables highly efficient siRNA transfection in a variety of cell lines including HeLa, A549, Jurkat and NIH-3T3.	0.3 ml; 50-100 transfections
siRNA Transfection Medium	sc-36868	Reduced-serum medium suitable for addition to siRNA suspension and siRNA 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

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