



The Power to Question

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siRNA Gene Silencing Protocol



- In a six well tissue culture plate, seed 2×10^5 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 CO₂ 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 μ l of siRNA duplex (i.e., 0.25-1 μ g or 20-80 pmols siRNA) into 100 μ l siRNA Transfection Medium: [sc-36868](#).
 - Solution B: For each transfection, dilute 2-8 μ l of siRNA Transfection Reagent: [sc-29528](#) into 100 μ l siRNA Transfection Medium: [sc-36868](#). Peak activity should be at about 6 μ l 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 CO₂ 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.
- 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 μ l 1x Electrophoresis Sample Buffer: [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 Chomczynski and Sacchi (Anal Biochem. 1987 Apr;162(1):156-159. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Chomczynski P, Sacchi N.) or a commercially available RNA isolation kit.