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 proportionally 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 µl of siRNA duplex (i.e. 0.25-1 µg or 20-80 pmol/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: If a lower siRNA concentration is desired, dilute siRNA appropriately and protein and should be determined experimentally.

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.

NOTE: Fluorescein Conjugated Control siRNA should only be incubated for a total 5-7 hours at 37° C in a CO₂ 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 contains 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.


### siRNA SUPPORT REAGENTS

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>CAT. #</th>
<th>DESCRIPTION</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control siRNA A</td>
<td>sc-37007</td>
<td>Control siRNA 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</td>
<td>66 µl, 10 µM; 10-20 transfections</td>
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<tr>
<td>Control siRNA B</td>
<td>sc-44230</td>
<td>see description above</td>
<td>see above</td>
</tr>
<tr>
<td>Control siRNA C</td>
<td>sc-44231</td>
<td>see description above</td>
<td>see above</td>
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<tr>
<td>Control siRNA D</td>
<td>sc-44232</td>
<td>see description above</td>
<td>see above</td>
</tr>
<tr>
<td>Control siRNA E</td>
<td>sc-44233</td>
<td>see description above</td>
<td>see above</td>
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<tr>
<td>Control siRNA F</td>
<td>sc-44234</td>
<td>see description above</td>
<td>see above</td>
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<tr>
<td>Control siRNA G</td>
<td>sc-44235</td>
<td>see description above</td>
<td>see above</td>
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<tr>
<td>Control siRNA H</td>
<td>sc-44236</td>
<td>see description above</td>
<td>see above</td>
</tr>
<tr>
<td>Control siRNA I</td>
<td>sc-44237</td>
<td>see description above</td>
<td>see above</td>
</tr>
<tr>
<td>Control siRNA J</td>
<td>sc-44238</td>
<td>see description above</td>
<td>see above</td>
</tr>
<tr>
<td>Control siRNA (Fluorescein Conjugate) A</td>
<td>sc-36869</td>
<td>Control siRNA (Fluorescein Conjugates) 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</td>
<td>66 µl, 10 µM; 10-20 transfections</td>
</tr>
<tr>
<td>Control siRNA (Fluorescein Conjugate) B</td>
<td>sc-44239</td>
<td>see description above</td>
<td>see above</td>
</tr>
<tr>
<td>Control siRNA (Fluorescein Conjugate) C</td>
<td>sc-44240</td>
<td>see description above</td>
<td>see above</td>
</tr>
<tr>
<td>Control siRNA (Fluorescein Conjugate) D</td>
<td>sc-44241</td>
<td>see description above</td>
<td>see above</td>
</tr>
<tr>
<td>siRNA Dilution Buffer</td>
<td>sc-29527</td>
<td>TRIS-EDTA-based buffer prepared from RNase-free water suitable for storage and dilution of siRNA; pH 8</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>siRNA Transfection Reagent</td>
<td>sc-29528</td>
<td>Delivers siRNA into cells with minimal toxicity; enables highly efficient siRNA transfection in a variety of cell lines including HeLa, A549, Jurkat and NIH-3T3</td>
<td>0.3 ml; 50-100 transfections</td>
</tr>
<tr>
<td>siRNA Transfection Medium</td>
<td>sc-36888</td>
<td>Reduced serum medium suitable for addition to siRNA suspension and siRNA transfecion reagent immediately prior to cell transfection; modification of Eagle’s Minimal Essential Medium, buffered with HEPS and sodium bicarbonate, and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, growth factors and phenol red</td>
<td>20 ml</td>
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</tbody>
</table>

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.