This protocol is optimized for transfection of RKO, an adherent cell line that can be obtained from ATCC. It has also been used successfully to transfect other common lines, including 293t, H292, CHO, and Caco-2. I order siRNA from Invitrogen. I chose to use "Stealth" siRNA, a chemically modified RNA, because it has certain advantages, mainly that it results in longer knock-down than normal siRNA. The stock solution of siRNA is 20 μM. I use 10 μl per transfection.

Here is the general protocol that I use for the transfection of siRNA:

you will need: Lipofectamine 2000
OPTI-MEM I Media
siRNA (I have about 800 μl, enough for 80 transfections)
other normal tissue culture supplies (trypsin, plates, etc)

Plate cells in 10 cm dishes the night before transfection at approximately 30-40% confluent in your normal, antibiotic-free media (supplemented with 10% FBS).

The following morning **transfect the cells:**

wash cells:
--wash the cells with 5 ml OPTI-MEM media, aspirate, then add another 4 ml OPTI-MEM to each dish

make transfection 'complexes':
--in one 1.5 ml eppendorf, dilute 10 μl Lipofectamine 2000 into 500 μl OPTI-MEM
--in a second 1.5 ml eppendorf, dilute 10 μl of 20 μM siRNA solution into 500 μl OPTI-MEM
--mix both tubes gently, and incubate 5 min at room temp
--combine the contents of both tubes in a single eppendorf (approx. 1.0 ml final volume)
--incubate at room temperature for 20 min
--add entire volume (1 ml) gently to cells on 10 cm dish (already containing 4 ml OPTI-MEM)

When the cells are near confluence (usually the following day), split the cells approximately 1:4 into 10 cm plates. Split into normal culture media, with 10% FBS.

The following day, assuming the cells are 30-40% confluent, transfect the cells a second time, using the same procedure as above.

When the cells are near confluence, split into whatever cell containers you need to do your experiment in (10 cm plates, 6-well plates, 96-well plates, etc.). Assuming knock-down is effective, it should remain suppressed for several cell passages (at least 3-4 days). Then do the experiment as you normally would.