







**Figure 5. Relationship Between siRNA Uptake and Biological Activity.** HeLa cells were transfected with 1 pm–80 nM siRNA targeting the UQCR mRNA. Cells were harvested after 48 hr, samples were prepared for real-time analysis using the TaqMan® Gene Expression Cells-to-C<sub>T</sub>™ Kit, siRNA quantitation was performed using the Custom TaqMan Small RNA Assay, and knockdown of UQCR mRNA was determined using a TaqMan Gene Expression Assay. To determine the number of siRNA copies delivered per cell, seven 5-fold serial dilutions starting from 20 pmol were separately spiked into nontransfected cell lysates harvested from the same number of cells used for the siRNA transfections. Comparison of C<sub>T</sub> values between transfected and spiked samples enabled the calculation of siRNA copies per cell.

estimate the number of siRNA molecules in cells, siRNA detection results were compared to a standard curve generated by adding known amounts of siRNA to untreated cell lysates and then immediately processing samples using the same cell lysis/real-time RT-PCR procedure. The results are shown in Figure 5.

shows target knockdown and siRNA detection results with a titration of siRNA concentrations used for transfection. Using a broad range of *Silencer Select* siRNA concentrations (0.5–80 nM), similar, high levels of knockdown (~90%) were obtained. Clear differences in the number of copies of siRNA detected in cells, however, corresponded to the amount

of siRNA used for transfection, varying from ~40,000 to 1,000,000 copies delivered per cell. With the lowest siRNA concentrations (<0.01 nM), fewer than 1,000 copies of siRNA per cell could be detected, and no target knockdown was observed. Similar results

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#### Sensitive siRNA Detection with Custom TaqMan® Small RNA Assays

In the Cells-to-C<sub>T</sub> lysate, the Custom TaqMan Small RNA Assay for UQCR siRNA exhibited higher nonspecific amplification than in buffer or purified RNA, with average C<sub>T</sub> values from negative control samples at about 30 cycles. Samples transfected with siRNA targeting UQCR, however, gave C<sub>T</sub> values of 15–20 cycles with the assay. Thus the  $\Delta C_T$  value (C<sub>T</sub> from UQCR siRNA-transfected cells – C<sub>T</sub> from nontargeting control siRNA-transfected cells) was typically 10–15 cycles, providing sensitive detection of the *Silencer Select* siRNA.

In this set of experiments, transfection of 5 nM siRNA resulted in ~100,000 siRNA molecules detected per cell. This level of siRNA induced strong knockdown of the target mRNA, UQCR (Figure 5). Interestingly, 100,000 siRNA molecules corresponds to <1% of the 5 nM siRNA used for transfection; suggesting that 99% of the siRNA/lipid complexes did not enter cells. To confirm that this was not due to siRNA degradation inside the cell, the experiment was repeated, but quantitation was performed only 6 hours post-transfection. Similar siRNA detection results were obtained; 1–2% of siRNA used for transfection detected in the cells, but only 50% target knockdown was observed with the shortened culture time. Figure 5 also

#### Tips from the Bench:

##### Avoiding siRNA Contamination in TaqMan® Small RNA Assays

When using Custom TaqMan Small RNA Assays designed to detect siRNAs, there is a risk of contamination if the siRNA transfections and TaqMan Assays are performed in the same laboratory environment. Research and Development Scientists at Applied Biosystems offer the following recommendations to minimize this risk:

- Designate an “siRNA-free” area of the laboratory that is used only for setting up real-time PCR assays
- Designate PCR pipettes that are never used for pipetting siRNAs
- Always include no-RT and no-template controls to monitor reagent contamination

##### TaqMan® Small RNA Assays: Other Applications

- Quantitation of siRNA delivery and persistence in animal tissues, organs, blood, or specific cells
- Investigation of naturally occurring siRNAs
- Monitoring siRNA levels expressed from plasmid or viral shRNA expression vectors
- Quantitation of novel small RNAs such as miRNAs, piwi-interacting RNAs (piRNAs), repeat-associated short interfering RNAs (rasiRNAs), and other uncharacterized small RNAs

##### *Silencer*® Select siRNAs Yield Cleaner, More Consistent Data

- Validated publication-quality data can be obtained faster, and with greater efficiency
- Novel chemical modifications reduce off-target effects by up to 90%
- Best knockdown performance in side-by-side comparisons
- Exceptional efficacy, specificity, and potency—have complete confidence in your reagents
- Cleaner, more consistent phenotypic data

were seen with other *Silencer* Select siRNAs and corresponding Custom TaqMan Small RNA Assays tested, and results from these experiments are in agreement with published reports [3].

These results demonstrate that Custom TaqMan Small RNA Assays can be used to successfully determine the number of siRNA copies per cell. They also illustrate the siRNA concentration range required to induce potent mRNA knockdown.

### Detect Any Small RNA Sequence with Custom TaqMan Small RNA Assays

Custom TaqMan Small RNA Assays can be designed for most target sequences. They are designed for detection and quantitation of miRNA, siRNA, shRNA, and any other small RNA species in either purified RNA samples or in cultured cell lysates prepared using Applied Biosystems Cells-to-Ct products.

Results of this study corroborate earlier findings [2] that real-time RT-PCR for small RNA targets using Applied Biosystems innovative stem-loop RT primers are specific, sensitive, and easy-to-use. Furthermore, it demonstrates the utility of these Custom TaqMan Small RNA Assays for in vitro quantitation of siRNA, even with the chemical modifications used in *Silencer* Select siRNA to reduce off-target effects. This strategy can be used to evaluate siRNA delivery efficiency to different cell lines, and for studying stability of siRNAs in cells after transfection.

ORDERING INFORMATION	P/N	SIZE	PRICE
<b>Custom TaqMan® Assays</b>			
Custom TaqMan® Small RNA Assays	Varies	Varies	Varies
Design your own assays at <a href="http://www4.appliedbiosystems.com/beta/smallrna">http://www4.appliedbiosystems.com/beta/smallrna</a> . For more information, and to obtain a quote, contact your local Applied Biosystems Sales Representative.			
<b><i>Silencer</i>® Select siRNAs</b>			
<i>Silencer</i> ® Select Pre-designed siRNA	Varies	Varies	Varies
<i>Silencer</i> ® Select Validated siRNA	Varies	Varies	Varies
<i>Silencer</i> ® Select Custom Designed siRNA	Varies	Varies	Inquire*
*To search for and order <i>Silencer</i> ® Select siRNAs, or to view the full list of options, visit <a href="http://www.appliedbiosystems.com/geneassist">www.appliedbiosystems.com/geneassist</a>			
<b>TaqMan® Real-Time PCR Reagents</b>			
TaqMan® Gene Expression Cells-to-Ct™ Kit	4399002	40 lysis rxns/200 PCRs	\$540
	AM1728	100 lysis rxns/500 PCRs	\$1,250
	AM1729	400 lysis rxns/2000 PCRs	\$4,000
TaqMan® MicroRNA Cells-to-Ct™ Kit	4391848	100 lysis rxns/500 PCRs	\$1,250
	4391996	400 lysis rxns/2000 PCRs	\$4,500
TaqMan® Gene Expression Assays (Inventoried)	4331182 <sup>§</sup>	250 rxns	\$150
TaqMan® Gene Expression Assays (Made-to-Order)	4351372 <sup>§</sup>	360 rxns	\$250

§Visit [www.allgenes.com](http://www.allgenes.com) to search for and order specific assays.

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#### REFERENCES

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