applied biosystems™
by life technologies™

Ambion® *In Vivo* siRNAs

As the field of RNA interference (RNAi) matures, researchers are becoming increasingly interested in discovering the *in vivo* effects of gene silencing. However, due to the presence of high levels of nucleases in blood and other biological fluids, *in vivo* RNAi experiments are more challenging than those performed *in vitro*. This increased challenge necessitates siRNAs that are specifically developed for *in vivo* applications, incorporating optimal sequence design features, appropriate chemical modifications, and the use of high-quality materials. Now, Applied Biosystems offers the most advanced siRNAs for *in vivo* applications—Ambion® *In Vivo* siRNAs.

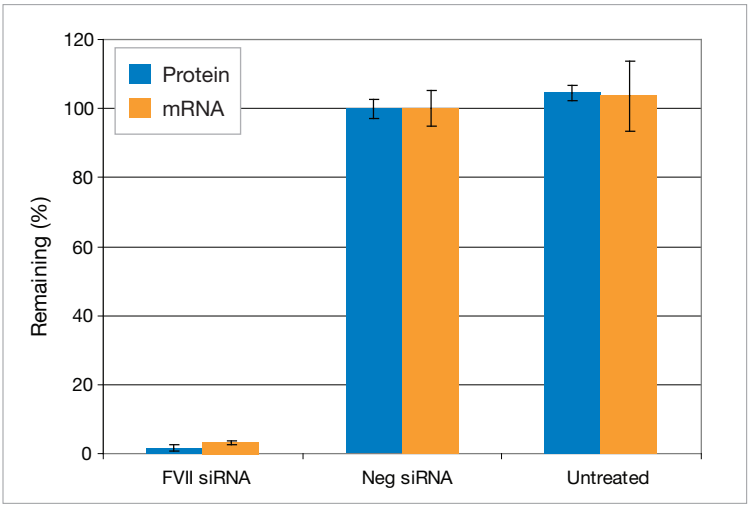

Figure 1. Ambion® *In Vivo* siRNA Produces Effective mRNA and Protein Knockdown. A single mouse tail-vein injection of Ambion® *In Vivo* siRNA (5 mg/kg of body weight) was administered using a suitable delivery reagent. Knockdown of Factor VII (FVII) mRNA in liver and FVII protein in blood after 24 hr is shown, as analyzed by real-time RT-PCR and chromogenic assay (Hyphen BioMed), respectively.

Ambion® *In Vivo* siRNAs, the new standard for *in vivo* RNAi applications, offer:

- **High stability against nucleases**—siRNAs reach their target intact and ready to initiate knockdown
- **No induction of the interferon response**—correlate phenotypes with knockdown activity instead of toxicity
- **Easy tracking of administered siRNAs**—measure biodistribution effectively using TaqMan® Assays
- **Combined knockdown effectiveness and stability**—no need to compromise one for the other

Ambion® *In Vivo* siRNA molecules are chemically modified, 21-mer double-stranded siRNAs that are recognized by the RNA-induced silencing complex (RISC) to mediate inhibition of a target gene. Proprietary chemical modifications allow Ambion® *In Vivo* siRNAs to overcome many *in vivo*–specific obstacles, ensuring their effectiveness and stability *in vivo*. Ambion® *In Vivo* siRNAs are at least 100x more stable in 90% mouse serum than unmodified siRNAs.

Specific and Effective Knockdown

Off-target effects in RNAi experiments should be kept to a minimum so that the observed phenotypes can be confidently attributed to target knockdown. Both strands in standard RNAi duplexes are able to participate in the knockdown reaction, increasing the chances of off-target effects. The sense strand of Ambion® *In Vivo* siRNAs is chemically modified and, therefore, unable to contribute to RNAi activity, minimizing off-target effects (Figures 1 and 2).

Reduced Immunogenicity and Toxicity

Immune reactions caused by siRNAs are due to an interferon response and can complicate analysis of knockdown because of associated nonspecific toxic effects. Induction of the interferon response can lead to severely altered global gene activity, making phenotypic assessment difficult. Standard siRNAs have the potential to activate this innate response, setting off defense mechanisms usually used to combat viruses. The chemical modifications introduced in Ambion® *In Vivo* siRNAs significantly reduce the immunostimulatory effect observed with some sequences (Figure 3), giving confidence that observed phenotypes are due to specific target knockdown and not to nonspecific toxicity.

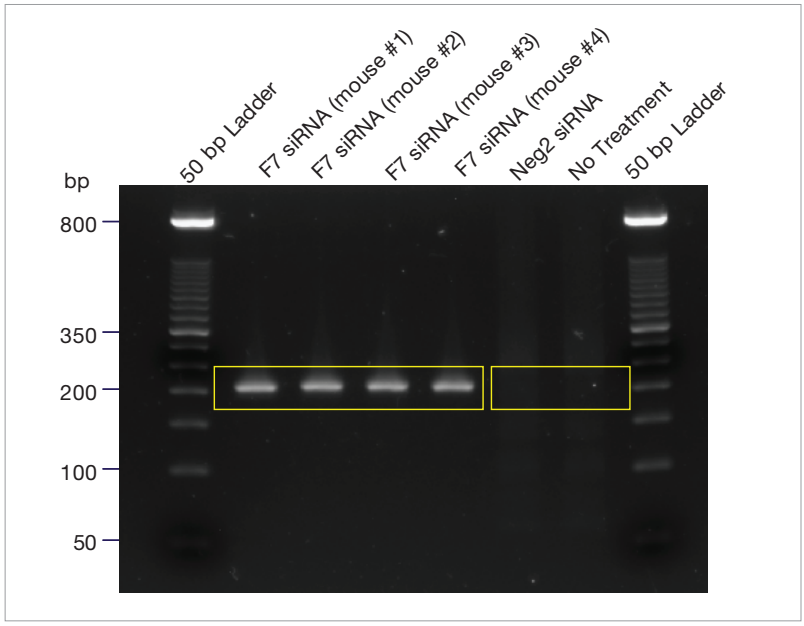

Figure 2. mRNA Cleavage Induced by Ambion® *In Vivo* siRNAs Is Confirmed by RACE.

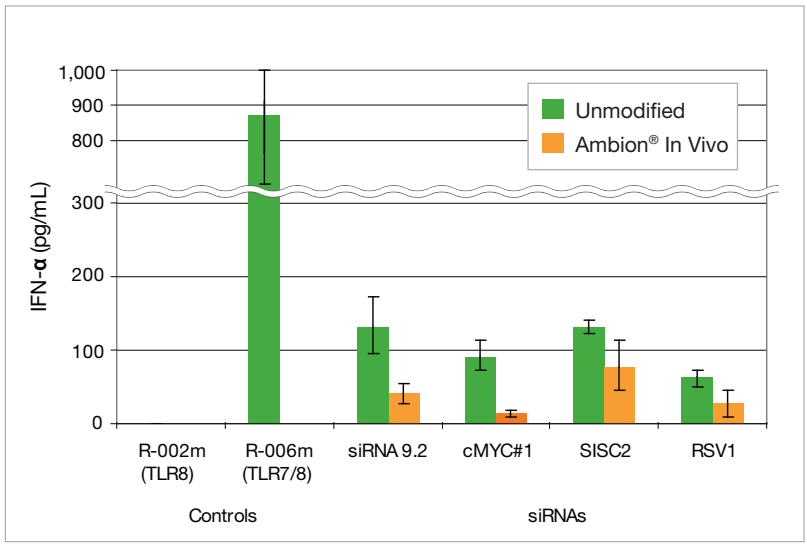

Figure 3. Chemical Modifications Attenuate Immunogenicity of Ambion® *In Vivo* siRNAs. IFN-α release (TLR7 activation) was measured in 5 x 10^5 PBMC cells after administration of 100 nM of selected unmodified siRNAs and corresponding Ambion® *In Vivo* siRNAs. For all siRNAs tested, Ambion® *In Vivo* siRNAs were less immunogenic than unmodified siRNAs. R-002m and R-006m are negative and positive controls, respectively, for TLR7 activation.

For more information or to order, visit www.appliedbiosystems.com/ambioninvivosirna.

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