Sensitive, Rapid, and Cost-Effective Quantification of siRNA in Tissue and Plasma

Know Where Your siRNA Goes - and How Long It Stays

In vivo quantification of formulated and chemically modified small interfering RNA by heating-in-Triton quantitative reverse transcription polymerase chain reaction (HIT qRT-PCR)

Oligonucleotide drug developers need a rapid, sensitive way to determine how much drug reaches each tissue, how long it persists, and how exposure relates to target engagement. HIT qRT-PCR will be performed as described by Landesman et al. (Silence, 2010). The method addresses two major causes of under-quantification: incomplete release of an oligonucleotide from its formulation or protein-bound matrix, and rapid reannealing of high-melting-temperature siRNA duplexes before reverse transcription. Triton X-100 liberates the oligonucleotide, controlled heating separates the strands, and immediate sequence-specific stem-loop reverse transcription preserves accurate measurement of the selected sense or antisense strand. Direct analysis of plasma or tissue lysate avoids conventional RNA purification, reducing sample handling, turnaround time, and cost while retaining high sensitivity and broad quantitative range.

THE BIOANALYTICAL CHALLENGE

Chemically modified oligonucleotides may have high melting temperatures, bind proteins, remain associated with delivery particles, and persist at very low concentrations in tissues after plasma levels decline. Without efficient release and maintained strand separation, standard stem-loop qRT-PCR can substantially underestimate duplex concentration. Hybridization assays may also be labor-intensive or lack the sensitivity and range needed to connect tissue exposure with pharmacodynamic activity.

THE HIT qRT-PCR ADVANTAGE

The Landesman et al. workflow combines detergent-mediated liberation, controlled thermal denaturation, and sequence-specific stem-loop qRT-PCR. It measures the selected strand directly from plasma or powdered tissue, without RNA purification. Matrix-matched standards account for matrix effects, while sequence specificity supports strand-selective quantification, rapid assay adaptation, high throughput, and direct PK/PD analysis.

PUBLISHED PERFORMANCE

~70 pg
LLOQ per mL plasma or g tissue

4-5 logs
quantitative dynamic range

Strand-specific
sense or antisense detection

PK/PD
direct exposure-response linkage

SCIENTIFIC SCOPE: siRNA, ASO AND AOC PROGRAMS

The published validation directly supports chemically modified siRNA duplexes, including naked and LNP-formulated material. The same analytical concept can be adapted to RNA ASOs and oligonucleotide payloads from AOCs, but each new chemistry and conjugate requires feasibility testing and matrix-specific validation. For AOCs, HIT qRT-PCR measures the selected oligonucleotide payload after release; orthogonal assays are needed when the study must separately quantify intact conjugate, free payload, antibody, or linker species. The workflow has been tested with mouse, rat, and nonhuman-primate plasma and tissue matrices. Because detection primers recognize the therapeutic oligonucleotide sequence rather than a host gene, the analytical principle is species-agnostic; nevertheless, every new species, tissue, and plasma matrix will be qualified before study execution.

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From Sample to Decision-Ready Data

METHOD & STUDY OUTPUTS

HOW THE METHOD WORKS

1	2	3	4	5
Sample receipt 50 mg frozen powdered tissue or 50 µL plasma, maintained on dry ice	**Triton release** 0.25% Triton X-100 disrupts formulation and releases matrix-bound oligonucleotide	**Controlled heating** 95°C denatures duplex and minimizes reannealing before RT	**Stem-loop qRT-PCR** Drug-sequence and strand-specific reverse transcription and amplification	**PK/PD interpretation** Biodistribution, persistence, clearance, and exposure-response

WHAT A STUDY CAN DELIVER

- Tissue and plasma biodistribution across dose levels and time points
- Terminal persistence, clearance, and comparative formulation performance
- Strand-specific analysis and chemistry-specific metabolite sensitivity
- Exposure-response relationships with target engagement or PD readouts
- Assay development, qualification, and study-specific interpretation

REQUIRED STUDY SAMPLES

For each analysis, provide **50 mg of frozen powdered tissue** or **50 µL of blood plasma**. Samples must remain frozen and be preserved and shipped on dry ice. Matrix-matched untreated control material is also required for calibration standards and matrix qualification.

SPECIES APPLICABILITY

The method has been tested in tissue and plasma from mice, rats, and nonhuman primates. Because detection is driven by the oligonucleotide sequence rather than the species, the platform is species-agnostic, with matrix-specific qualification performed for each study.

FIGURE 1

Correlation between tissue siRNA concentration and target knockdown in rat liver

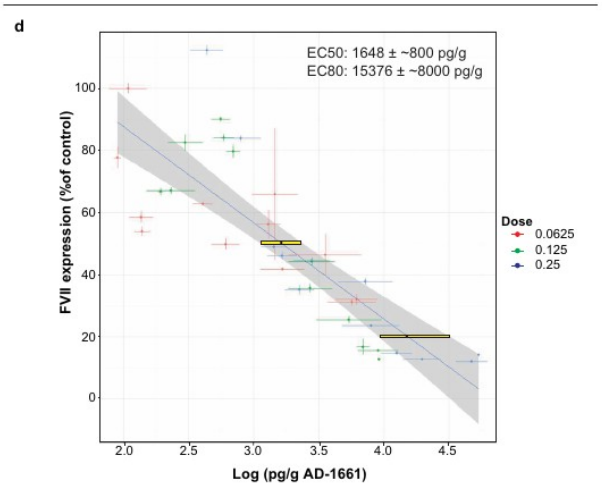

Figure 1. Correlation of liver concentration of the chemically modified, LNP-formulated FVII siRNA AD1661 with FVII target expression after single intravenous doses of 0.0625, 0.125, or 0.25 mg/kg in rats. Data from all doses and time points are shown together. The blue line is the linear regression, the gray region is the 95% confidence interval, and the yellow bars indicate the estimated EC50 and EC80 exposure levels. Adapted from Figure 7d in Landesman et al., Silence 2010, 1:16.

ENGAGEMENT MODEL

Assays are executed by SBH Sciences under the scientific guidance of Dr. Yosef Landesman, lead author of the original HIT qRT-PCR publication. The assay will be performed using the method described by Landesman et al., with chemistry- and matrix-specific adaptations documented during qualification. Services include study design, chemistry-specific assay adaptation, matrix qualification, execution, and integrated PK/PD interpretation.

FIGURE 2

Schematic workflow for HIT qRT-PCR standard-curve and sample preparation

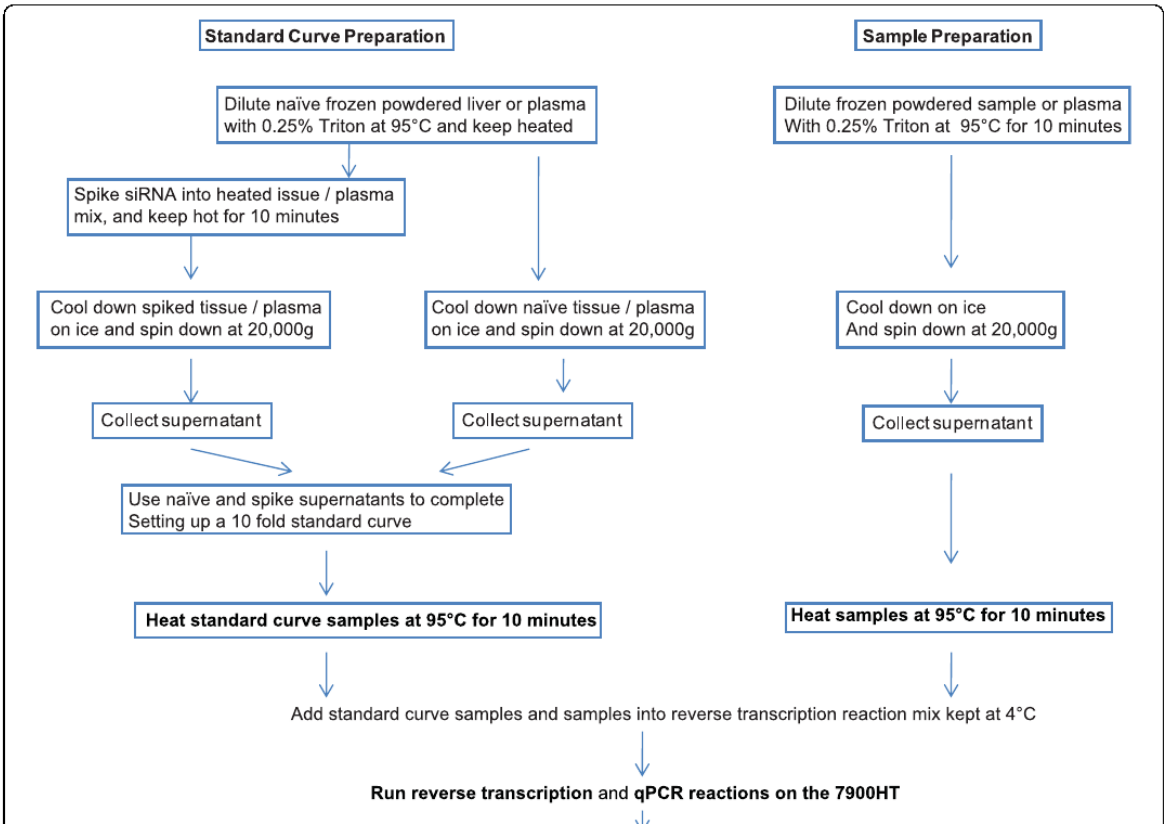

Figure 2. Standard-curve and test-sample preparation for HIT qRT-PCR. Naïve frozen powdered tissue or plasma is diluted in 0.25% Triton X-100 and heated to 95°C. Matrix standards are prepared by spiking the oligonucleotide into heated naïve matrix, followed by cooling, centrifugation, and collection of supernatant. Test samples are processed in parallel. Standards and test samples are then reheated at 95°C immediately before addition to the reverse-transcription mixture maintained at 4°C, followed by stem-loop reverse transcription and qPCR analysis. Adapted from Figure 9 in Landesman et al., Silence 2010, 1:16.