APPLICATION GUIDE

ROCKLAND

# Methods for Analysis of siRNA

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We have reported the distribution, localization, quantification, and trafficking of antisense oligonucleotides (ASOs) using ModDetect™ antibodies.[1,2] These panels of monoclonal antibodies (mAbs) are specific for chemical modifications, independent of nucleic acid sequence, and recognize commonly used modifications intended to stabilize ASO drugs (PS, 2-MOE, 2'-OMe and 2'-F). While our previous activities focused on the analysis of ASOs, here we supplement available methods by reporting an optimized protocol for the detection and quantification of small interfering RNA (siRNA).

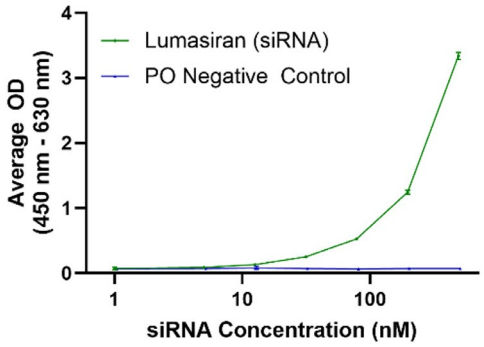


**Figure 1. Quantification of siRNA Lumasiran using anti-PS antibodies configured in a sandwich ELISA.** PO negative control demonstrates specificity.

siRNAs are a relatively new class of therapeutics that use the cell's RNA interference pathway to silence disease-causing genes. With several FDA-approved drugs, siRNAs show strong potential across genetic disorders, infections, and more. Their success depends on effective delivery and reliable methods for detection and quantification of these molecules in biological systems. siRNA can be detected and quantified by ELISA using ModDetect mAbs specific for these modifications (see page 2 for ordering information).

A sandwich ELISA[3] was configured using a cocktail of unconjugated ModDetect mAbs for capture of siRNA and biotinylated forms of these mAbs for detection. mAbs bind phosphorothioate (PS) present as non-overlapping epitopes within the siRNA. We used the FDA-approved siRNA drug Lumasiran[4] for validation. Lumasiran[5] is a GalNAc-siRNA conjugate of conventional design consisting of a double-stranded siRNA core flanked with a 2-nucleotide 3' overhang of the antisense strand. Only the two nucleotides at the 5' end of the sense and both the 3' and 5' ends of the antisense strands contain PS linkages. It is likely that because this siRNA contains fewer PS modifications relative to a fully PS-modified ASO, higher concentrations of drug are required in an optimized protocol to achieve signal (see Figures 1 and 2). The following protocol details conditions we used to obtain these results. Further optimization may be required based on the chemical composition of the siRNA to be quantified.

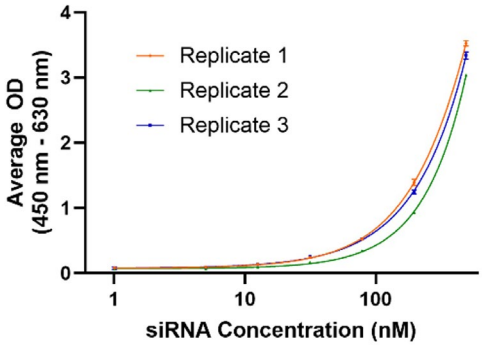


**Figure 2. Reproducibility of replicate experiments run in triplicate.**

## Protocol

Synthesize siRNA with more than one PS bond. The PS modifications must not be presented as overlapping epitopes, e.g., to minimize steric hindrance, to allow capture and detection by anti-PS antibodies. Ideally, PS modifications should be present at both the 5' and 3' ends of the siRNA on the sense and/or antisense strands. Signal may be proportional to the total number of PS bonds present within the siRNA. Coat 96-well plates with a mixture of ModDetect antibody clones PS04 and PS09 *(Rockland, PS04: #200-301-MV0; PS09: #200-301-MW3)* at 2 µg/mL each in 0.1M sodium bicarbonate pH 9.5 and incubate overnight at 4°C. Wash three times with PBS-T *(Rockland, #MB-075-1000)* and then add 300 µL of Blocking Buffer for Fluorescent Western Blotting *(Rockland, #MB-070)* for 2 hours at room temperature. Dispense blocking buffer from wells and pat dry. Prepare a series of siRNA in sample buffer *(Rockland, #MB-070)* over the range of 5 nM (0.087 µg/mL) to 500 nM (8.5 µg/mL)

concentrations by 2.5X serial dilution. Further dilute a portion of the 5nM sample to 1 nM by performing a 5X dilution to establish the LOD sample. Add 100 µL to each well in triplicates, followed by incubation for 2 hours at room temperature with agitation at 450 RPM. After three PBS-T washes, add 100 µL of a biotinylated version of the same combined ModDetect antibodies *(Rockland, PS04: #200-306-MV0; PS09: #200-306-MW3)* prepared at 0.5 µg/mL each and added to the appropriate wells for 1 hour at room temperature with agitation at 450 RPM. After three further PBS-T washes, add 100 µL of streptavidin-HRP *(Rockland, #S000-03)* at 0.125 µg/mL in sample buffer to each well for 30 minutes at room temperature protected from light with agitation at 450 RPM. After three additional PBS-T washes, add 100 µL of High Definition 3,3',5,5'-tetramethylbenzidine *(Rockland, #TMBE-100)* and incubate for 30 minutes at room temperature in the dark. Stop the reaction by adding 100 µL of 1N HCl to each well and read the absorbance at 450 nm minus reference at 630 nm within 5 minutes.

| Assay Parameters | Result |
|---|---|
| Range | 5 – 500 nM (0.087 – 8.5 pg/mL) |
| Lower Limit of Quantification (LLOQ) | 5.0 nM (0.087 pg/mL) |
| Upper Limit of Quantification (ULOQ) | 491 nM (8.5 pg/mL) |
| Limit of Detection (LOD) | ≤ 1.0 nM (≤ 0.017 pg/mL) |
| Coefficient of Determination ($r^2$) | ≥ 0.98 |
| Assay CV% | ≤ 20% |

## ModDetect™ Ordering Information

**ModDetect™ Anti-PS (Phosphorothioate) Clones**

| Product | Unconjugated | Biotin |
|---|---|---|
| Clone PS04 | 200-301-MV0 | 200-306-MV0 |
| Clone PS09 | 200-301-MW3 | 200-306-MW3 |
| | **DL488** | **DL549** |
| DyLight™ Clone PS04 | 200-341-MV0 | 200-342-MV0 |
| DyLight™ Clone PS09 | 200-341-MW3 | 200-342-MW3 |

**ModDetect™ Panels**

| Product | Format | Item No. |
|---|---|---|
| PS Panel | Unconjugated | KNA-100 |
| PS Biotinylated Panel | Biotin | KNA-101 |
| 2'MOE Panel | Unconjugated | KNA-200 |
| 2'MOE Biotinylated Panel | Biotin | KNA-201 |
| 2'OMe Panel | Unconjugated | KNA-300 |

## References


1. Chimento DP, Anderson AL, Fial I, et al. Bioanalytical assays for oligonucleotide therapeutics: Adding antibody-based immunoassays to the toolbox as an orthogonal approach to LC-MS/MS and ligand binding assays. Nucleic Acid Ther 2025;35:6–15; doi: 10.1089/nat.2024.0065
2. Fial I, Farrier SA, Chimento DP, Ascoli CA, Wan X, Oliver PL. Characterizing Antibodies Targeting Antisense Oligonucleotide Phosphorothioate and 2'-O-Methoxyethyl Modifications for Intracellular Trafficking and Biodistribution Studies. Nucleic Acid Ther. 2025 Aug;35(4):168-181. doi: 10.1177/21593337251361396
3. Kohl TO, Ascoli CA. Immunometric Antibody Sandwich Enzyme-Linked Immunosorbent Assay. Cold Spring Harb Protoc. 2017 Jun 1;2017(6):pdb.prot093716. doi: 10.1101/pdb.prot093716. PMID: 28572187
4. FDA. (2020). Oxlumo(lumasiran) injection, for subcutaneous use: Prescribing information[Prescribing information]. U.S. Food and Drug Administration.
5. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Lumasiran. [Updated 2025 Mar 5].



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