

A robust ELISA-like hybridization assay for the detection of therapeutic oligonucleotides

Nicholas Rice

ADViRNA, Worcester, MA, USA, Nicholas Rice, Sean McCauley, Kyle Foster, Parixit Oza, Hannah McCauley, Sarah Goudreau, Alexey Wolfson

ADViRNA 17 Briden Street Worcester, MA 01605

Background: Oligonucleotide therapeutics are rapidly becoming a major class of drugs, with several compounds already approved, tens more in the late stages of clinical development, and hundreds more in preclinical stage. The ability to efficiently measure therapeutic oligonucleotide concentration in blood and target tissues/organs is essential for PK/PD studies. The currently available methods such as HPLC-based hybridization assay or mass-spectrometry are either low throughput or require sophisticated equipment and substantial optimization in setting up detection protocols. Several attempts have been made to develop ELISA-like hybridization detection protocols, but none have been widely implemented due to the requirement for non-standard reagents, extensive assay optimization or limitations in efficacy due to the nature of oligonucleotides (i.e. single-stranded vs double stranded, chemical modifications, etc).

Methods: By optimizing the hybridization conditions, the enzymatic ligation and standardizing the chemical nature of the capture probe we have developed a robust ELISA-like assay based on a hybridization-ligation approach. The assay design utilizes commonly commercially available reagents and can be easily customized for any oligonucleotide analyte with a simple sequence modification of the capture probe.

Results: The detection range of the assay ranges from 1 to 1000 fmol in plasma and tissue extracts. We describe applications of the assay for the measurement of fully modified siRNAs concentrations in animal tissue samples with mutiple siRNAs.

Conclusion: Implementation of this assay can significantly improve and facilitate PK/PD studies of therapeutic oligonucleotides while substantially reducing costs.