T1230-06-38

Performance of a Digoxigenin Antibody-Based Electrochemiluminescent Hybridization Assay: A HELISA Alternative for Low Femtomolar Detection of an Antisense Oligonucleotide

Annamaria Szabolcs, Isaac Shin, Timothy J. Break,
Seth B. Harkins, and Jacob N. Wohlstadter
Meso Scale Discovery, Rockville, MD
sharkins@meso-scale.com

aaps PharmSci 360

PURPOSE

With the rapid growth of antisense oligonucleotide (ASO) therapeutics, the demand for sensitive, specific, and reproducible oligonucleotide detection assays is increasing. Hybridization ELISA (HELISA) methods are popular choices for the development of pharmacokinetic (PK) assays for the detection of these ASO drugs. These assays typically use biotinylated capture probes bound to the surface of streptavidin-coated microplates and detection probes labeled with digoxigenin (DIG). Measurement of the analyte is then accomplished with an anti-DIG antibody labeled directly with a signaling molecule or with an enzyme that generates a signal during the processing of its substrate. Electrochemiluminescence (ECL)-based detection using SULFO-TAG labeled anti-DIG antibodies is an appealing alternative that may increase assay sensitivity and improve reproducibility and has been shown to be one of the most effective methods for ASO detection by multiple groups.

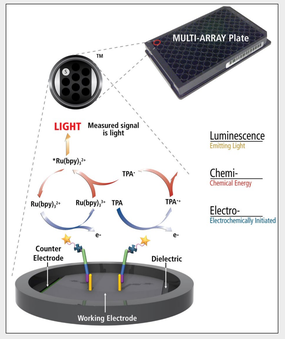
OBJECTIVES

Here, we introduce three ECL hybridization assay protocols that further improve assay sensitivity compared to standard methods. These methods do not require extraction and use a newly optimized plate and buffer system. We also developed a system to increase sensitivity of these protocols.

METHODS

Meso Scale Discovery's Electrochemiluminescence Technology

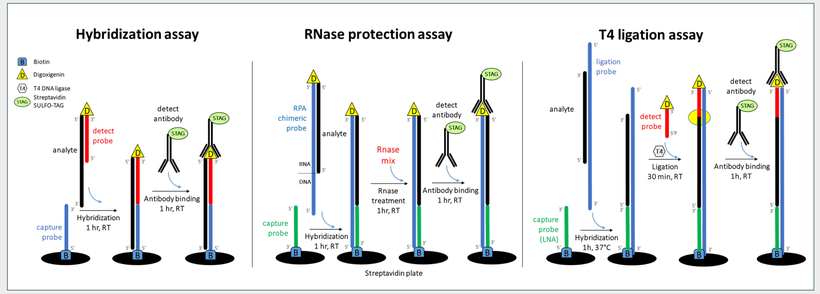
- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.



A new plate format was developed to support the development of PK assays for short oligonucleotide analytes.

Assay Design

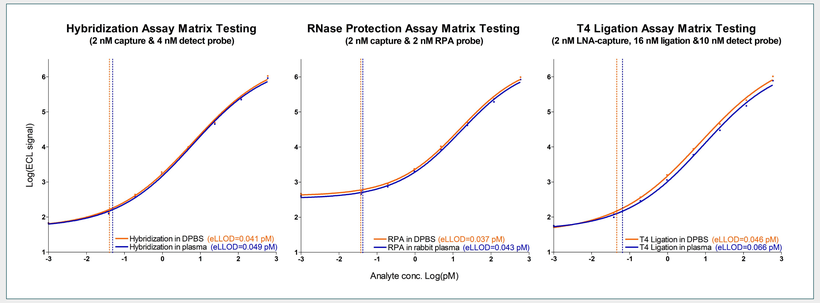
Depending on the characteristics of a given analyte (sequence, melting temperature, oligo modifications, etc.) and the need for metabolite detection, an ideal assay format can be selected, probe sequences can be designed and assay conditions can be adjusted for optimum performance and convenience.



Methods were developed for ECL-based detection of a 20-base long unmodified DNA analyte (sequence: GGCTAAATCGCTCCACCAAG) using the formats of dual-probe hybridization, a modified RNase protection assay, and ligation with T4 DNA ligase. To minimize free biotin interference from biological matrices, each assay format was designed to use a capture probe pre-coating step. Sample pre-treatment, hybridization conditions, probe designs and concentrations, as well as incubation times were refined for each of the three assay formats.

RESULTS

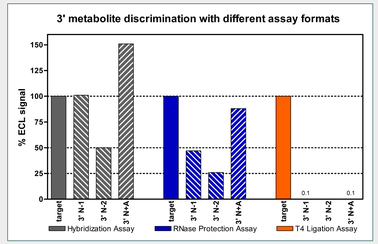
Minimal matrix effects observed in rabbit plasma



After optimization of assay conditions in diluent (Dulbecco's modified phosphate buffered saline without Ca & Mg; DPBS), the sensitivity of the three assay formats was tested in rabbit plasma. All assays showed excellent sensitivity with an estimated lower limit of detection (eLLOD) around 50 fM and Hill slopes between 0.99 and 1.03.

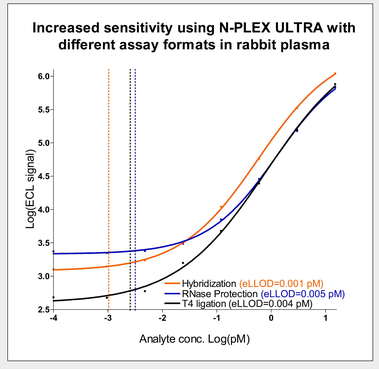
The T4 ligation assay provides excellent 3' discrimination

While the hybridization and RNase protection assays showed low specificity for 3' modifications in rabbit plasma, the T4 ligation assay was able to accurately discriminate between the target analyte and its metabolites shortened by one or two bases, as well as a 3' adenylated analog. With some modifications, the ligation assay can also be used for high sensitivity detection of 5' phosphorylation of oligonucleotide analytes



N-PLEX ULTRA provides sensitivity boost to all three assay formats

The N-PLEX ULTRA™ signal amplification approach can conveniently be coupled with all three assay formats described here. The method requires two additional incubation steps that increase the assay time by a total of 90 minutes. In our model systems, the N-PLEX ULTRA add-on provided a 10- to 50-fold increase in assay sensitivity and decreased estimated LLOD values to the low femtomolar range when tested in rabbit plasma. Additional strategic adjustments of the protocols can be implemented in order to further decrease assay backgrounds, thus improving signal-to-noise ratios and assay sensitivity.



CONCLUSION

ECL hybridization assay methods offer simple, highly sensitive, extraction-free, and high-throughput workflows for ASO PK assay development. The versatility of hybridization and enzymatic reaction-based approaches allow for the development of custom assays for the detection of many short oligonucleotide drugs, including DNA, RNA, or siRNA molecules. Further, multiple assay formats are adaptable to detect oligonucleotide drugs containing chemically modified bases. Lastly, the improvement in sensitivity with the N-PLEX ULTRA system sets a new standard for ultrasensitive oligonucleotide PK assay development.

Meso Scale Discovery®
A division of Meso Scale Diagnostics, LLC
www.mesoscale.com®

MSD® products are for research use only. Not for use in diagnostic procedures.

Download Poster