# Enhancing Robustness in Hybridization ECLIA for Sensitive Quantification of siRNA Therapeutics in Human Plasma

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## HYBRIDIZATION ECLIA

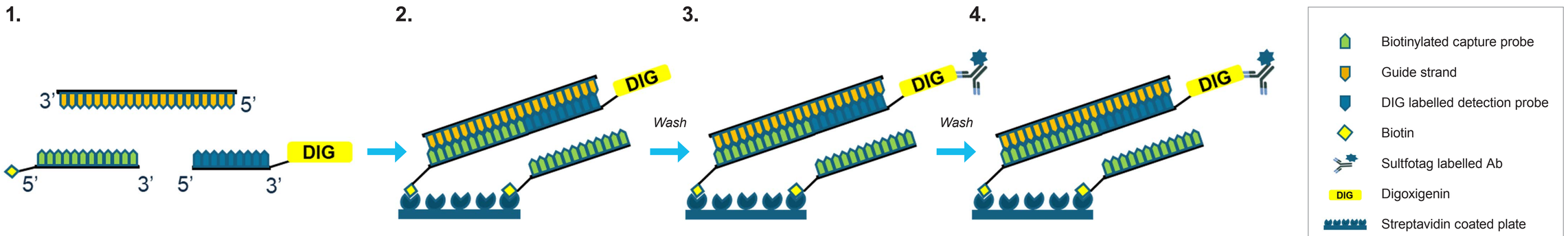


**Figure 1.** Visual workflow overview of the Hybridization ECLIA Assay (adapted by Søndergaard Galle from Thayer et al.).

## OBJECTIVES

- Transfer and implementation of a hybridization Electrochemiluminescence immunoassay (ECLIA) for quantification of small interfering RNA (siRNA) therapeutic in human plasma.
- Enhancement of assay robustness through targeted adaptations and procedural refinements.
- QC robustness assessment using the initial and optimized assay.

## ASSAY WORKFLOW

1. Denaturation of siRNA double strand and hybridization of the guide strand with a sequence-specific biotinylated capture probe and digoxigenin (DIG)-labeled detection probe.
2. Transfer of the hybridized sample to a pre-blocked MSD small-spot streptavidin-coated plate.
3. Addition of SulfoTag-conjugated anti-DIG detection antibody.
4. Measurement of chemiluminescence signal.

## ASSAY SPECIFICATIONS

| Assay Parameters | |
|---|---|
| Biological matrix | Human plasma |
| Assay volume | 20 µL |
| MRD | 30 |
| Assay format | Hybridization ECLIA |
| Analytical range | 0.500 – 300 ng/mL |
| QC samples | LLOQ 0.5 ng/mL |
| | LQC 1.5 ng/mL |
| | MQC 18 ng/mL |
| | HQC 225 ng/mL |
| | ULOQ 300 ng/mL |

**Table 2.** Final assay specifications (abbreviations: QC Quality Control, MRD Minimal Required Dilution, LLOQ Lower Limit of Quantification, LQC Low Quality Control, MQC Medium Quality Control, HQC High Quality Control, ULOQ Upper Limit of Quantification).

## ASSAY OPTIMIZATION

| Assay Procedure Step | | Tested Parameters | Initial Assay | | Optimized Assay |
|---|---|---|---|---|---|
| | Sample preparation | Assay buffer | Sponsor buffer | | Celerion buffer |
| | Sample pre-dilution at MRD | Stability at RT | Not assessed | → | 3h |
| | Sample dilution in probe solution | Stability at RT | Not assessed | | 2h |
| | Denaturation/hybridization | Reaction volume | 150 µL | → | 100 µL |
| | Hybridized sample | Stability | 12°C up to 30 min | | RT up to 30 min |
| | Blocking | Duration | 10–15 min | | 10–210 min |
| | Washing | Washing buffer | Sponsor buffer | | Celerion buffer |
| | Binding | Incubation duration | 60 min | → | 60–75 min |
| | Detection | Incubation duration | 30 min | | 30–60 min |
| | Detector SulfoTag-labelled anti-DIG Ab | Switching Ab | Initial Ab | | New Ab |
| | | Titration | 1:2000 | | 0.25 µg/mL (1:4000) |
| | Standard curve | Number of calibrators | 10 (incl. 1 anchor point) | → | 8 (no anchor point) |
| | Duplicate analysis | Singlicate | Duplicate | | Singlicate |

**Figure 3.** Assay optimization. Evaluated parameters for each assay step.

## QC — ROBUSTNESS

All tested procedural adjustments were incorporated into the assay protocol, resulting in a refined procedure. To evaluate the performance of the optimized assay, a QC robustness assessment was performed.

| | | Celerion QCs | | | Sponsor QCs | | |
|---|---|---|---|---|---|---|---|
| | Nominal Conc. | Conc. | | | Conc. | | |
| QC | [ng/mL] | RLU | [ng/mL] | %Bias | RLU | [ng/mL] | %Bias |
| Blank (1) | 0.00 | 98 | BLQ | N/AP | 457 | BLQ | N/AP |
| Blank (2) | 0.00 | | | | 471 | BLQ | N/AP |
| Blank (3) | 0.00 | | | | 457 | BLQ | N/AP |
| LQC (1) | 1.50 | 2523 | 1.62 | 7.9 | 2644 | 1.70 | 13.2 |
| LQC (2) | 1.50 | 2398 | 1.54 | 2.5 | 2685 | 1.73 | 15.0 |
| LQC (3) | 1.50 | 2335 | 1.50 | -0.3 | 2786 | 1.79 | 19.4 |
| MQC (1) | 18.0 | 26069 | 16.3 | -9.3 | 30132 | 18.8 | 4.7 |
| MQC (2) | 18.0 | 27131 | 17.0 | -5.7 | 29352 | 18.4 | 2.0 |
| MQC (3) | 18.0 | 28030 | 17.5 | -2.6 | 30132 | 18.8 | 4.7 |
| HQC (1) | 225 | 272837 | 206 | -8.4 | 291653 | 225 | 0.1 |
| HQC (2) | 225 | 267654 | 201 | -10.7 | 292634 | 226 | 0.6 |
| HQC (3) | 225 | 286027 | 219 | -2.5 | 288999 | 223 | -1.1 |

**Table 4.** QC robustness results. QC samples supplied by the Sponsor were tested alongside standards and QCs prepared by Celerion (3 QC levels, 3 replicates for each level). Sponsor supplied QC samples were previously tested successfully following the initial assay procedure (data not shown).

## CONCLUSIONS

Overall, the adaptations introduced to the assay procedure confirmed that its performance was equal to that of the original method, and led to

- A reduction in the volume/amount of critical reagents required
- Increased incubation time ranges

| Tested Parameter | Final Assay | Advantage |
|---|---|---|
| Assay buffer | Celerion buffer | Standardization |
| Reaction volume | 100 µL | Less Ref. material |
| Hybridized sample stability | RT up to 30 min | Thermal cycler not required |
| Blocking duration | 10–210 min | Increased flexibility |
| Washing buffer | Celerion buffer | Standardization |
| Binding — incubation time | 60–75 min | Increased flexibility |
| Detection — incubation time | 30–60 min | Increased flexibility |
| Detector | New Ab 0.25 µg/mL | Reduced amount |
| Standard curve | 8 calibrators | Increased throughput |
| Analysis | Singlicate | Increased throughput |

**Table 5.** Summary of the parameters tested and incorporated in the optimized procedure.

In summary, the introduced refinements confirmed the successful method transfer and resulted in a sensitive and reliable hybridization ECLIA, capable of quantifying the siRNA therapeutic in singlicate with a LLOQ of 0.5 ng/mL in human plasma.

## REFERENCES

Thayer, M.B., Humphreys, S.C., Chung, K.S. et al. POE Immunoassay: Plate-based oligonucleotide electroluminescent immunoassay for the quantification of nucleic acids in biological matrices. Sci Rep 10, 10425 (2020).