Development of a hybridization ECLIA assay for the determination of the payload oligonucleotide – antibody conjugate

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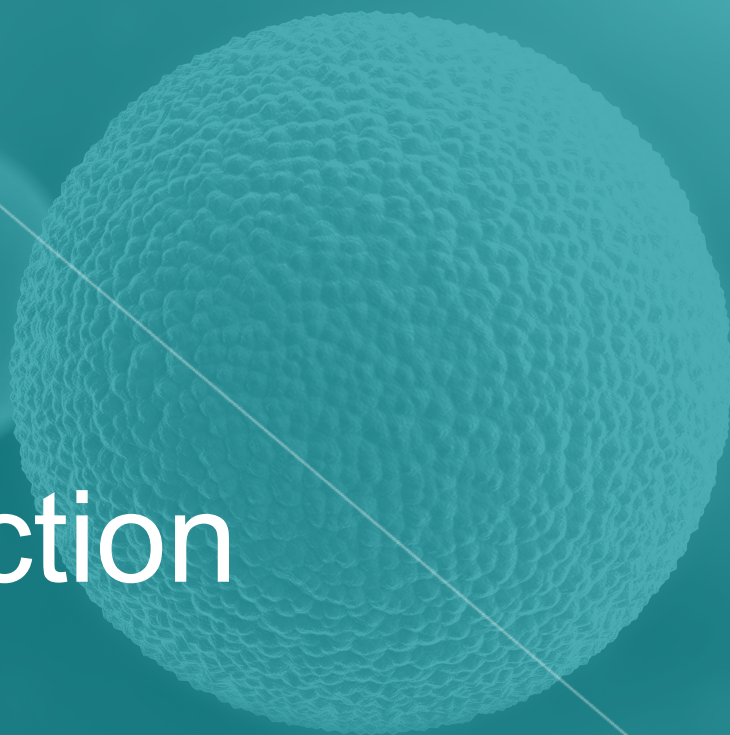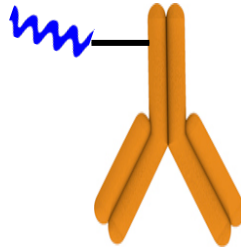
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Introduction

Introduction
ANTIBODY-OLIGONUCLEOTIDE CONJUGATE

▸ Anti-human IgG1 antibody + CpG Oligo 7-7

CpG Oligo 7-7

Anti-human IgG1 antibody

▸ Anti-human IgG1 antibody is selectively targeting a specific subset of cells

▸ CpG Oligo 7-7 is the payload inducing the intended response, free CpG oligo is unwanted
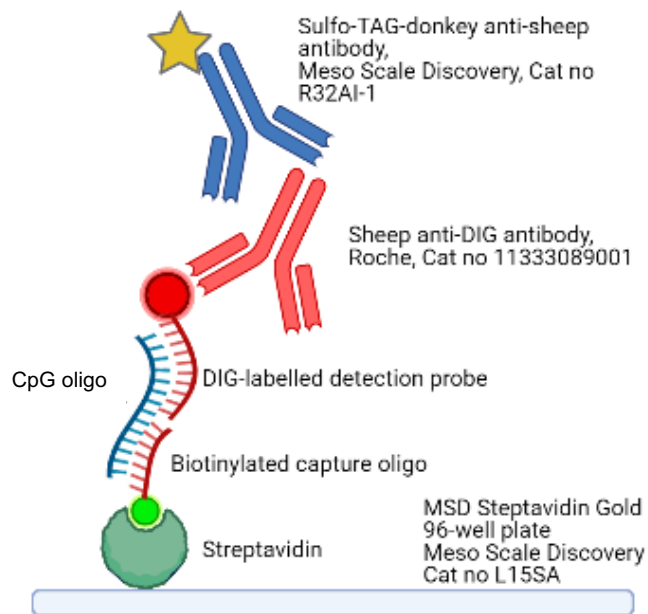
Introduction
ASSAY REQUEST

▸ Build a hybridization assay detecting free payload in monkey serum
- Quantitative
- As sensitive as possible
- No interference of antibody-bound CpG

Introduction
ASSAY SETUP

Development Strategy

▶ Build a hybridization assay
  – Probe design and selection
  – Optimization for sensitivity in presence of serum

▶ Removal excess Antibody-Oligo Conjugate
  – Option 1: Size exclusion using spin colums
  – Option 2: Solid Phase Extraction (SPE)

Assay Development

Assay Development
PROBE OPTIMIZATION

▸ Signal to background in assay buffer

	Capture 1	Capture 2	Capture 3
Detection 4	1.3	2.1	144.3
Detection 5	10.4	463	176.8
Detection 7	314	188.6	63.2

▸ Signal to background in 100% serum

	Capture 1	Capture 2
Detection 5		9.8
Detection 7	11.6	

Oligo combinations of capture 1/detection 7 and capture 2/detection 5 show best performance

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Assay Development
PROBE OPTIMIZATION

▶ Effect of serum on probes

Probe selection diluted serum sample

Capture 1 / Detection 7
Capture 2 / Detection 5
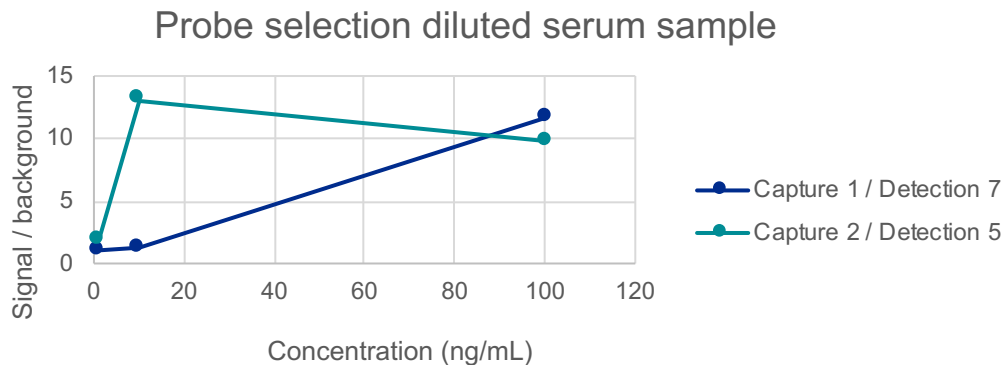
▶ Window 10% serum

Conc (ng/mL)	Capture 1/ Detection 7	Capture 2 / Dectection 5
3.84	1.3	2.1
7.69	10.4	463
15.4	314	188.6

Capture 2/detection 5 is selected

Assay Development
PROBE OPTIMIZATION

▸ Probe concentrations

CpG, 0.02uM vs 0.04uM probes

- 7-7-PEG in 10% matrix, 0.02uM probes
- 7-7-PEG in 10% matrix, 0.04uM probes
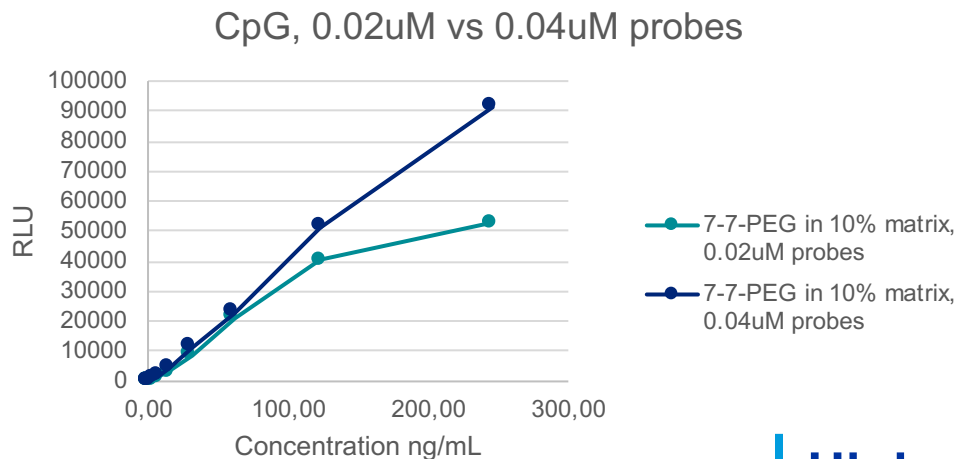
Higher concentration of probes improved the dynamic range on the higher end of the curve

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Assay Development
ASSAY OPTIMIZATION

▶ Addition of washing procedure

Conc (ng/mL)	No wash		Washed plate	
	ECL signal	Fold diff	ECL signal	Fold diff
6.25	4987	6.6	1298	18.7
3.13	2305	3.1	493	7.1
1.56	1171	1.6	223	3.2
0.78	871	1.2	138	2.0
0.00	750	1.0	70	1.0

A washing procedure was added to lower the background signal and improve the sensitivity on the lower end of the curve

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Assay Development
ASSAY EVALUATION

▶ Selectivity

	Unspiked	Spike at 8 ng/mL (Low)	
	conc (ng/mL)	Conc (ng/mL)	Recovery %
BM 1	0	6.82	85.2
BM 2	0	9.11	114
BM 3	0	6.97	87.1
BM 4	0	7.79	97.3
BM 5	0	7.15	89.4

The assay was suitable for comparing concentrations between different individuals

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Assay Development
ASSAY EVALUATION

▸ Interference of metabolite CpG oligo 9-7 (excluding linker) and antibody-oligo conjugate

Relative assay interference
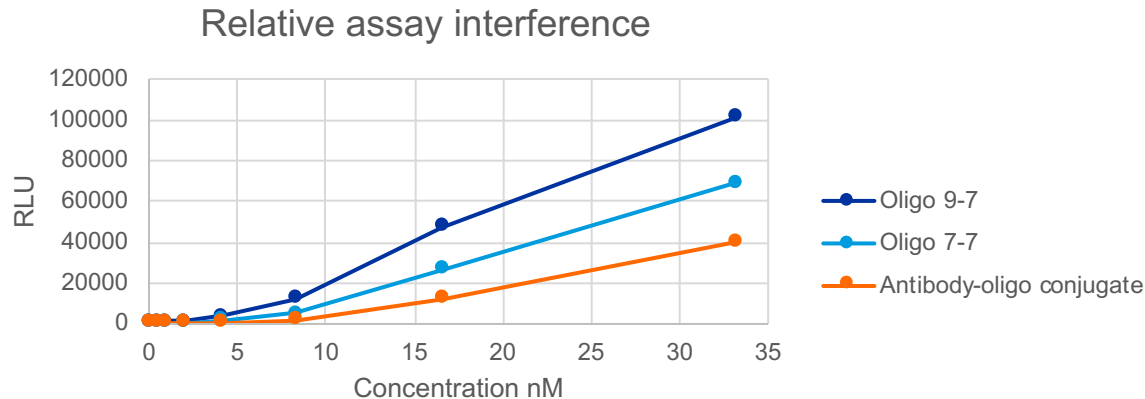
Oligo 9-7 is detected with higher affinity compared to oligo 7-7 → Oligo 7-7 is used as calibrator as a worst case (Free oligo's are unwanted)
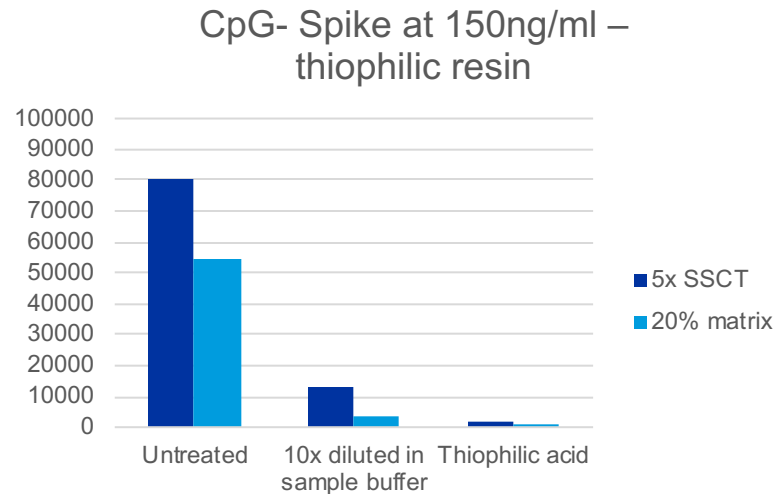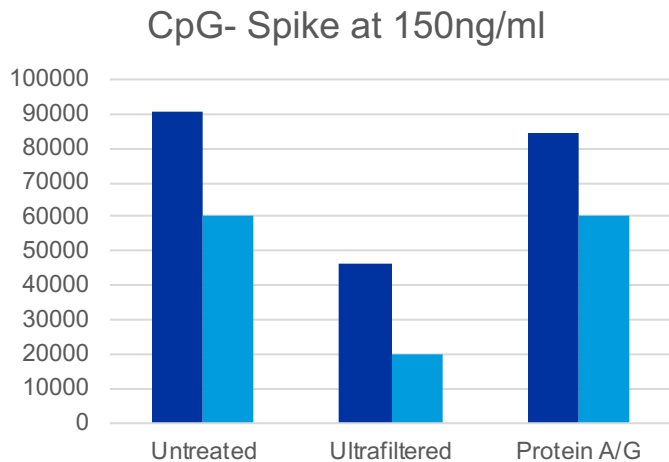
Oligo-antibody conjugate is detected in the assay
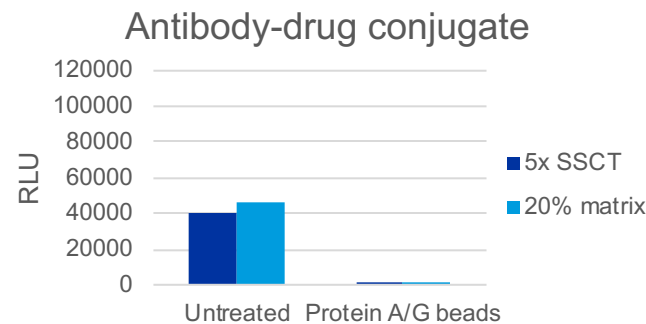
Purification Development

Assay Development
PURIFICATION

▸ Suitability of extraction methods

CpG- Spike at 150ng/ml

CpG- Spike at 150ng/ml – thiophilic resin

Protein A/G bead incubation allows good oligo recovery

Assay Development
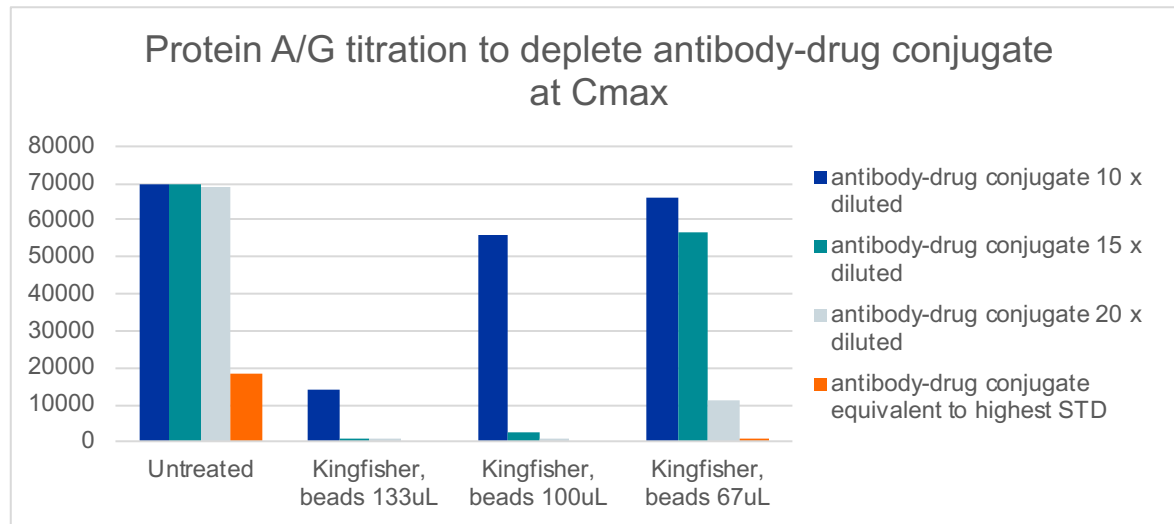## PURIFICATION

▸ Effect of Protein A/G beads on interfering factors
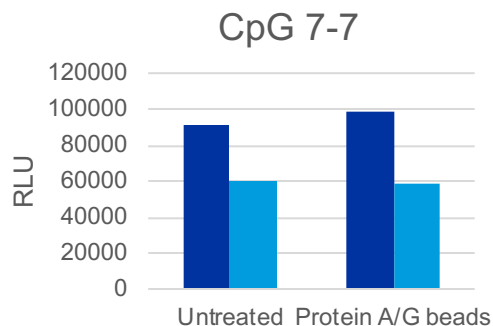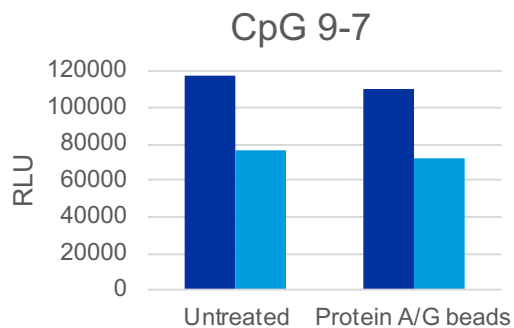
Protein A/G bead incubation reduces antibody-drug conjugate concentrations while not affecting oligo concentrations

Assay Development
PURIFICATION

▶ Titration of protein A/G beads

Protein A/G titration to deplete antibody-drug conjugate at Cmax

Categories: Untreated, Kingfisher, beads 133uL, Kingfisher, beads 100uL, Kingfisher, beads 67uL

Legend:
- antibody-drug conjugate 10 x diluted
- antibody-drug conjugate 15 x diluted
- antibody-drug conjugate 20 x diluted
- antibody-drug conjugate equivalent to highest STD

Y-axis: 0 to 80000

Antibody-drug conjugate is strongly captured with an increasing amount of beads at 15-fold sample dilution

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Assay Development
PURIFICATION

▸ Additional extraction cycle

### Antibody-drug conjugate 15x dilution
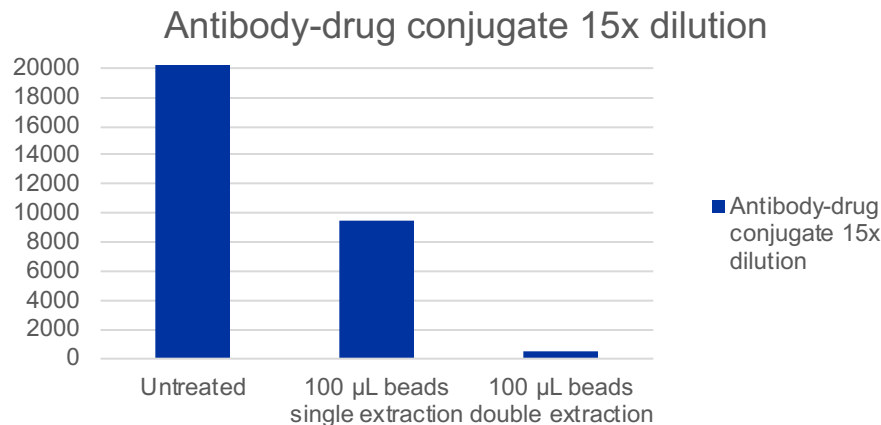
Double extraction reduces antibody-drug conjugate to 6 times background. Could not be further optimized, remaining signals affect sensitivity about 5-fold

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Assay Development
PURIFICATION

▸ Calibration curve spiked with antibody-drug conjugate with and without extraction

CpG 7-7 standard (co-spiked with antibody-drug conjugate) +/-extraction
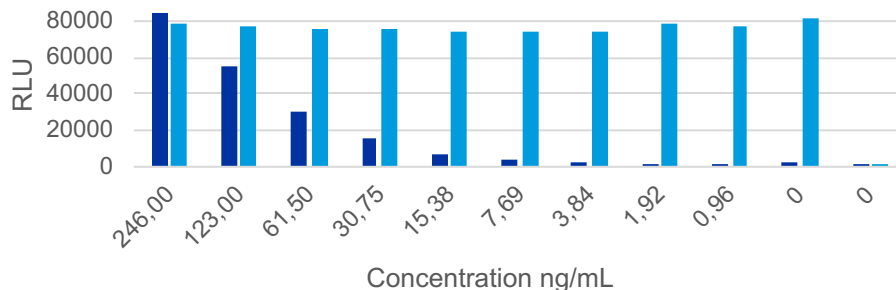
■ CpG 7-7 + antibody conjugate at CMax 15-fold dilution double extraction
■ CpG 7-7 + antibody conjugate at CMax 15-fold dilutionl not extracted

Purification with protein A/G beads results in an assay with an acceptable dynamic range

Assay Performance

Assay Development
ASSAY PERFORMANCE

▶ Precision

ng/mL	Recovery	Intra-run %CV	Inter-run %CV
15.4	113.5	7.5	15.1
184	103.0	8.2	13.6
246	98.1	15.1	16.9

Precision is acceptable

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Assay Development
ASSAY PERFORMANCE

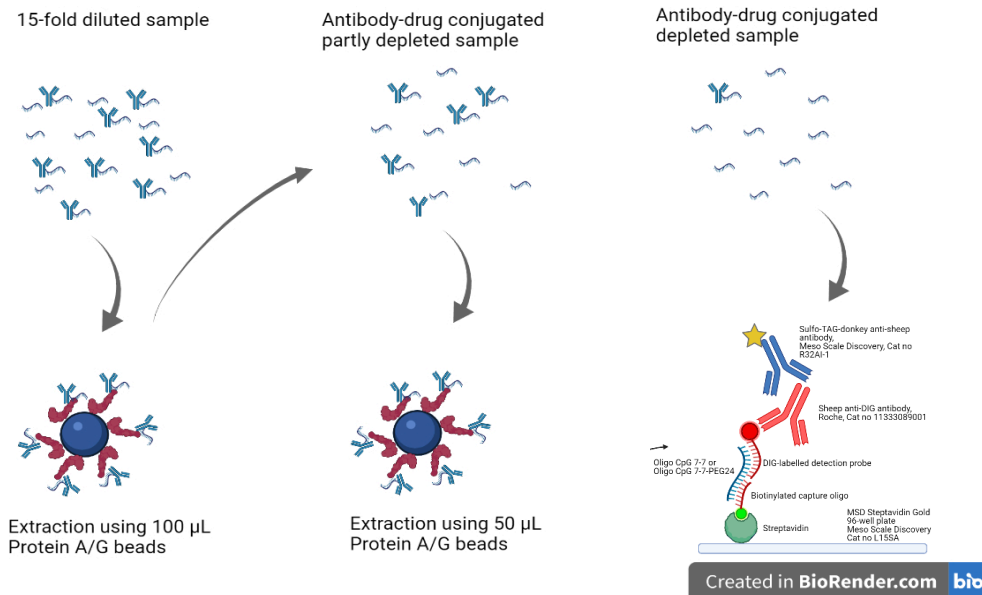▶ Currently final performance data is being generated. A validation will follow.

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Assay Development
FINAL ASSAY SETUP

▶ Conclusion

An assay to detect free oligo's in monkey serum to support preclinical studies was successfully developed

Thank You

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