AlphaLISA - a “no wash” high-throughput alternative to ELISA for PK, PD and immunogenicity measurement during drug development?

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Agenda

- Why did we evaluate AlphaLISA?
- Comparison to ELISA/MSD
- Introduction to AlphaLISA – Assay formats
- Assay validation - Assay performance
- Hook effect
- Conclusion

Why did we evaluate AlphaLISA?

- We are looking for:
  - High-throughput user friendly technology
  - Rapid assay development
  - Short assay protocol
  - Miniaturization of sample volume and reagents
  - Outsource-friendly
  - Improved sensitivity and dynamic range compared to ELISA

→ a „no wash“ high through-put alternative to ELISA for PK, PD and Immunogenicity assays?

Comparison AlphaLISA, ELISA and MSD

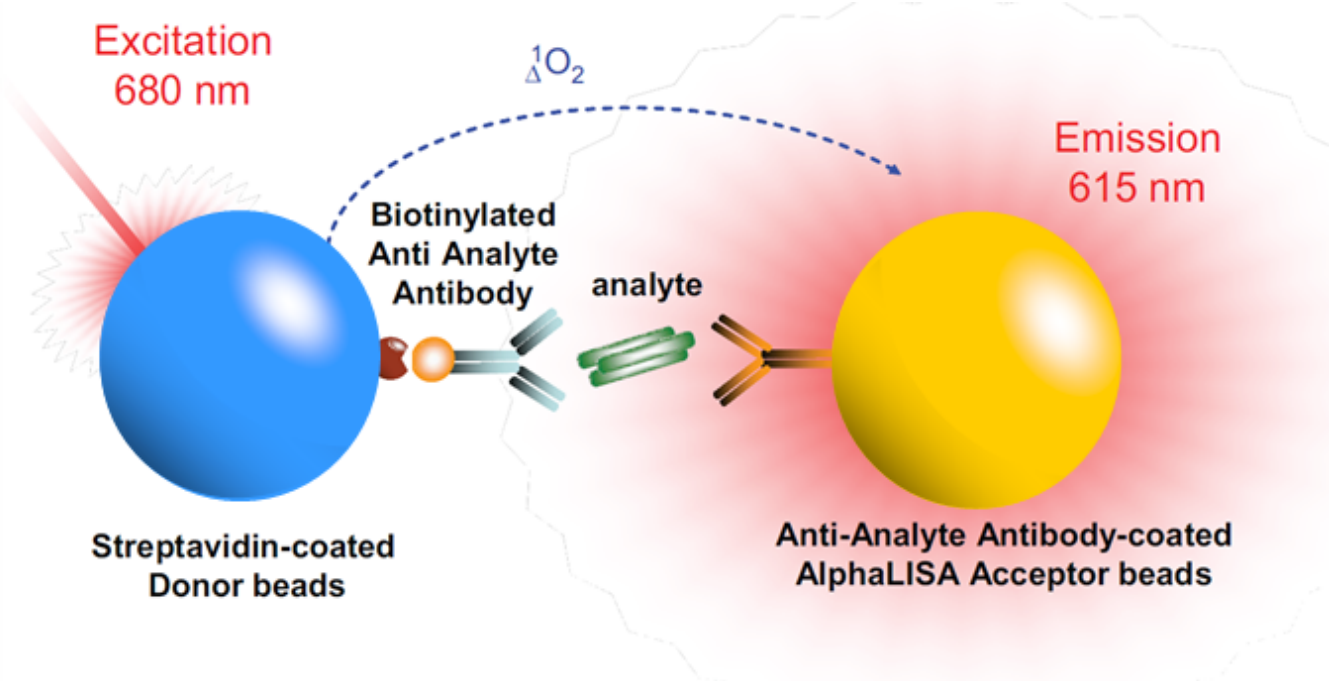
	AlphaLISA	ELISA	MSD
working range	1:150 - 1:20000	1:10 - 1:5000	1:10 - 1:80000
sample volume	1µl	5µl	5µl
time	3h	6h + coating time	4.5h
plate format	384well	96well	96well

- Fast protocol
- Lowest sample volume
- price beads/well: ~0.70CHF

- Low cost method

- Highest sensitivity
- Widest working range
- price /well:~1CHF

Technology



Source: 1: Bielefeld-Sevigny, Assay and Drug Development Technologies, Feb 2009, p90-92

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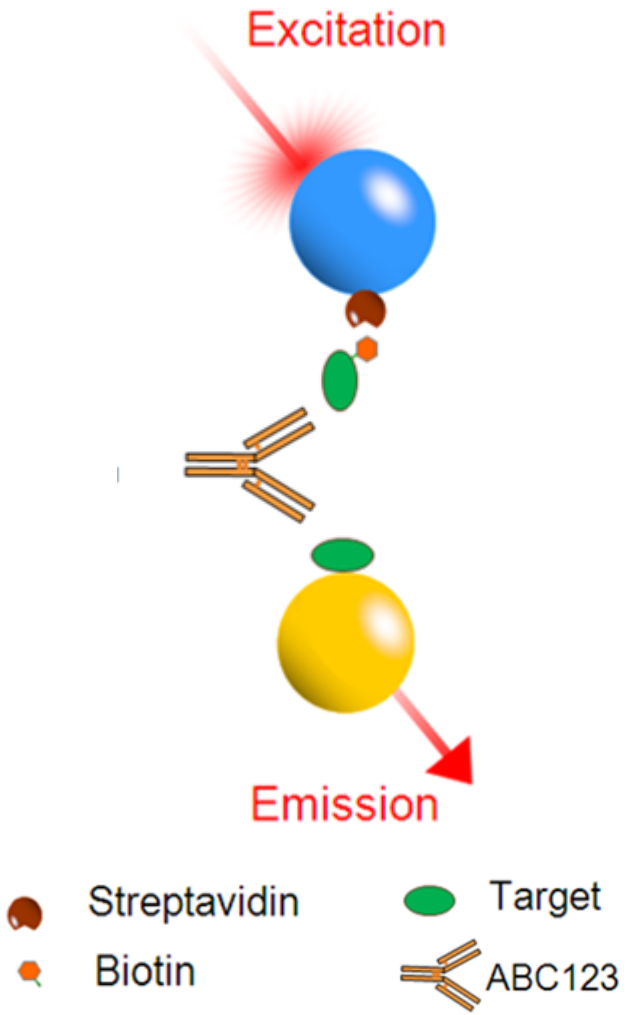
Assay performance – PK assay



Validation (384 well plate):

- Calibrator Accuracy: 100 % to 101 %
- Intra-run QC Accuracy: 77 % to 106 %
  Precision: 2 % to 22 %
- Inter-run QC Accuracy: 89 % to 94 %
  Precision: 0 % to 17 %
- LLOQ: 0.49 µg/mL
- ULOQ: 10.0 µg/mL

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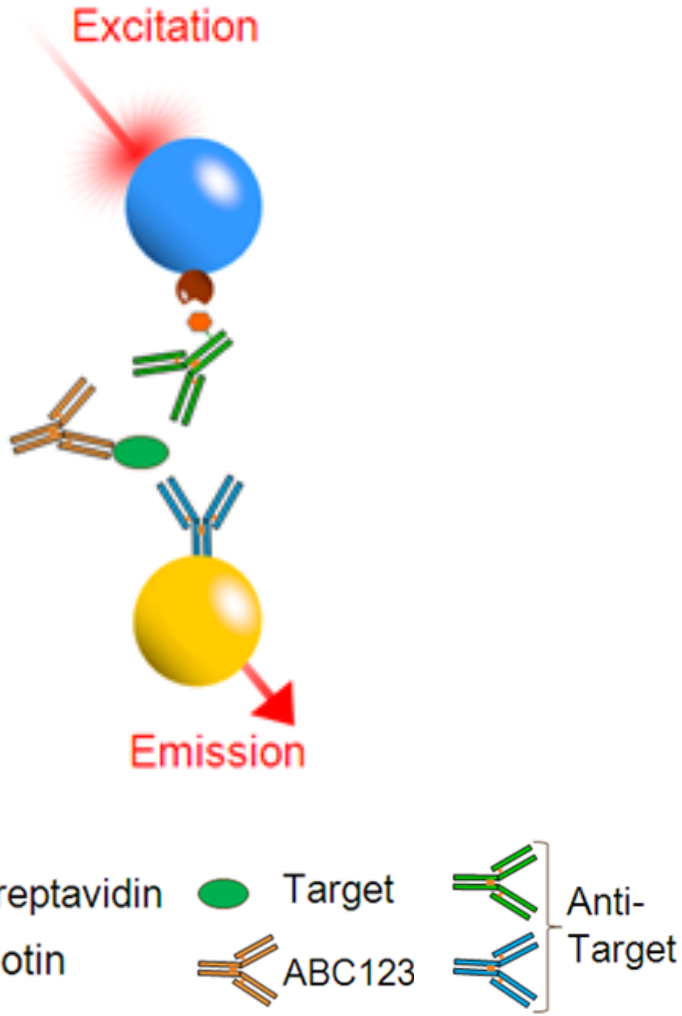
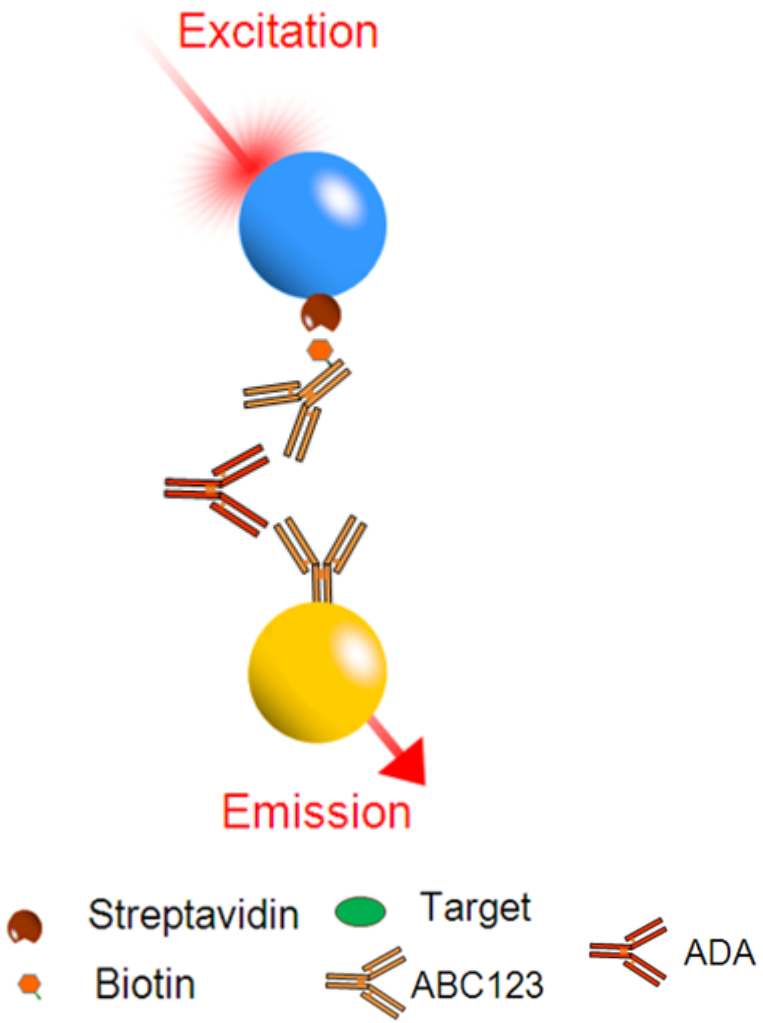
Assay performance – PD assay



Validation (384 well plate):

- Calibrator Accuracy: 87 % to 105 %
- Intra-run QC Accuracy: 78 % to 127 %
  Precision: 0 % to 8 %
- Inter-run QC Accuracy: 92 % to 102 %
  Precision: 10 % to 14 %
- LLOQ: 2.14 ng/mL
- ULOQ: 100 ng/mL

Assay performance – Immunogenicity assay



Validation (384 well plate) (beg. 2009):

- Calibrator Accuracy: 98 % to 108 %
- Intra/Inter-run QC Accuracy: 81 % to 104 %
  Precision: 5 % to 11 %
- Sensitivity: 150 ng/mL
- Drug tolerance without acid dissociation: LowQC: 1 µg/mL, DT: 0.21 µg/mL HighQC: 25 µg/mL, DT: 8.04 µg/mL
- Drug tolerance with acid dissociation: LowQC: 1 µg/mL, DT: 14.9 µg/mL HighQC 25 µg/mL, DT: 158 µg/mL µg/mL

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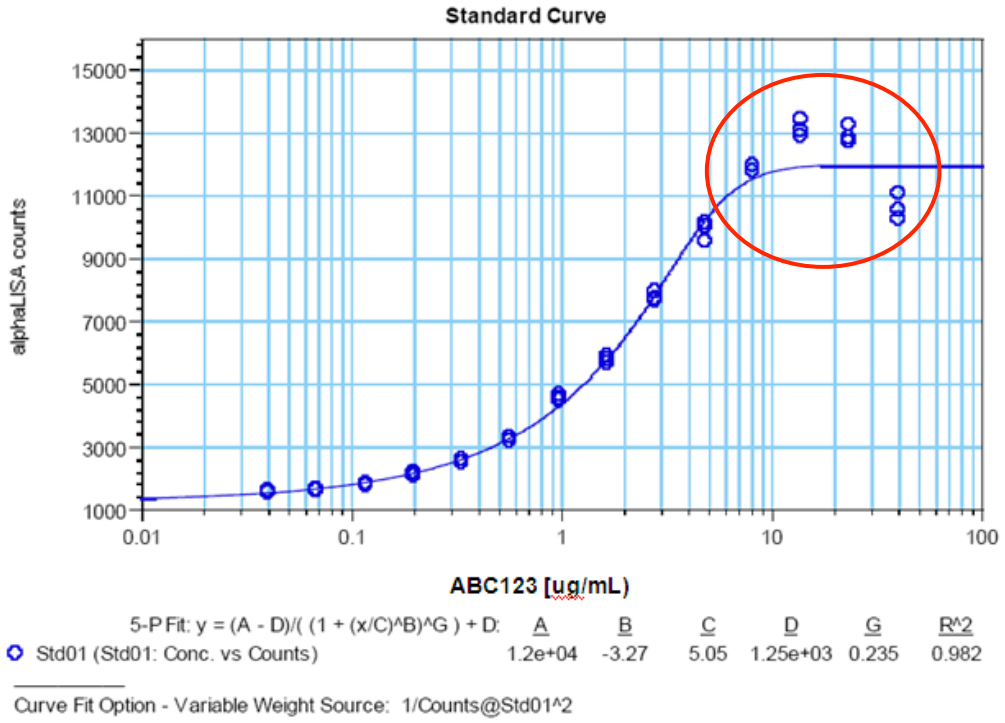
Advantage – Short generic protocol

- Standard 3-addition step protocol:
  - 1) Add serum/plasma sample
  - 2) Add biotinylated reagent and Acceptorbeads coated with second reagent (incubate 60 min at RT)
  - 3) Add Streptavidin Donor beads (incubate 30 min at RT, Read the plate)

Disadvantage – Hook effect

Example - PK assay

Hook Point: highest signal before the bead binding capacity is saturated



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Hook effect

Strategy for dealing with hook effect during PK/PD sample analysis

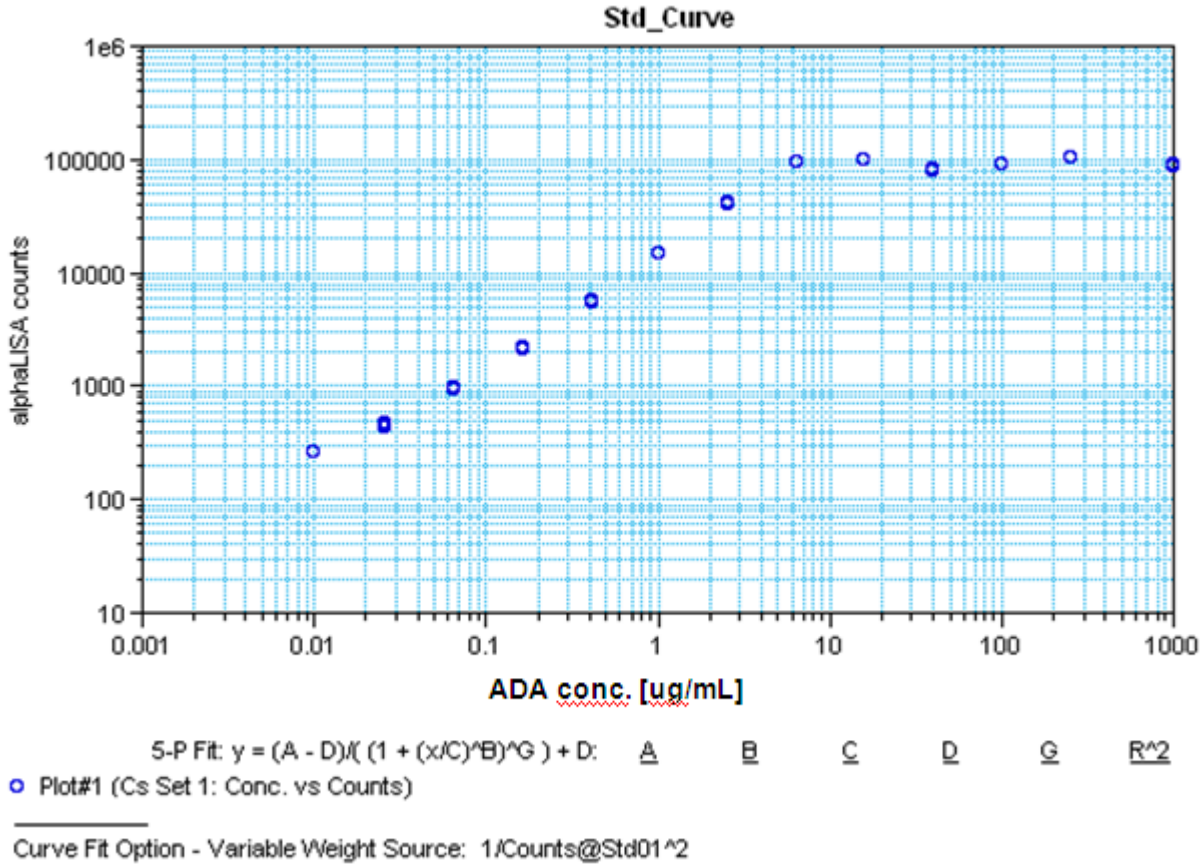
- Estimated dilutions can be taken according to the dose being studied and kinetic profile in method validation report
- Large dilution, aim to measure at low end of calibration curve
- Measure two dilutions for each sample:
  - If two measurements are linear (could be back-calculated within 20 %) the mean is used
  - If the two measurements are not linear, implying that the less diluted sample was subject to hook effect, the result of the sample with the larger dilution is taken.

Concerns:

- Hook effect could incorporate subjectivity in the sample analysis
- Maybe cost implications

Hook effect – not always observed

Example - Immunogenicity assay



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Conclusion 1

- We are looking for:
  - High-throughput screening friendly technology ✓
  - 384-well plate format ✓
  - Fast assay protocol ✓
  - Miniaturization of sample volume and reagents ✓
  - Assay development in short time ✓
  - Improved sensitivity and dynamic range compared to ELISA ✗

Conclusion 2

- Hook effect is an issue:
  - but maybe acceptable for immunogenicity assays if the assay has a wide working range – no quantification for ADA concentration
  - it is more complicated for PK and PD assays to deal with hook effect – quantification of drug and target concentrations
- AlphaLISA, a „no wash“ high through-put alternative to ELISA?
  - Yes – for immunogenicity assay
  - Yes – for PK and PD assays if hook effect issue could be solved
  - No – for PK and PD assays if there is a strong hook effect