



Forte Bio

Ligand Binding Assays on the Octet  
Platform for Bioprocess Contaminants:  
**Residual Protein A and HCP**

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# Agenda

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1. Introduction to the Octet Platform
2. Sensitivity Requirements for Bioprocess Contaminant Assays
3. Modulating Sensitivity on Octet Platform
4. Assay Methods
5. Specific Applications:
  - 1) Human IgG Quantitation Assay (Streptavidin and Protein A biosensors)
  - 2) Residual Protein A contamination Detection Kit
  - 3) Residual CHO HCP Contamination Detection Kit
6. Conclusions

Message:

The flexible Octet platform provides various assay formats to modulate the **dynamic range** and the **sensitivity**, enabling many high sensitive contamination assays on a single platform simplifying user's assay design and saving time.

# Information Provided By Octet Systems

- Quantification
  - 1) Quantitative: Active Concentration in solution
  - 2) Qualitative: Yes/No binding confirmation (specificity, epitope mapping)
- Kinetics
  - 1) Affinity Constants,  $K_D$
  - 2) Association rate constants,  $k_a$
  - 3) Dissociation rate constants,  $k_d$
  - 4) Steady State Affinity Constants
  - 5) Relative affinity ranking

# Biosensor Chemistry Options

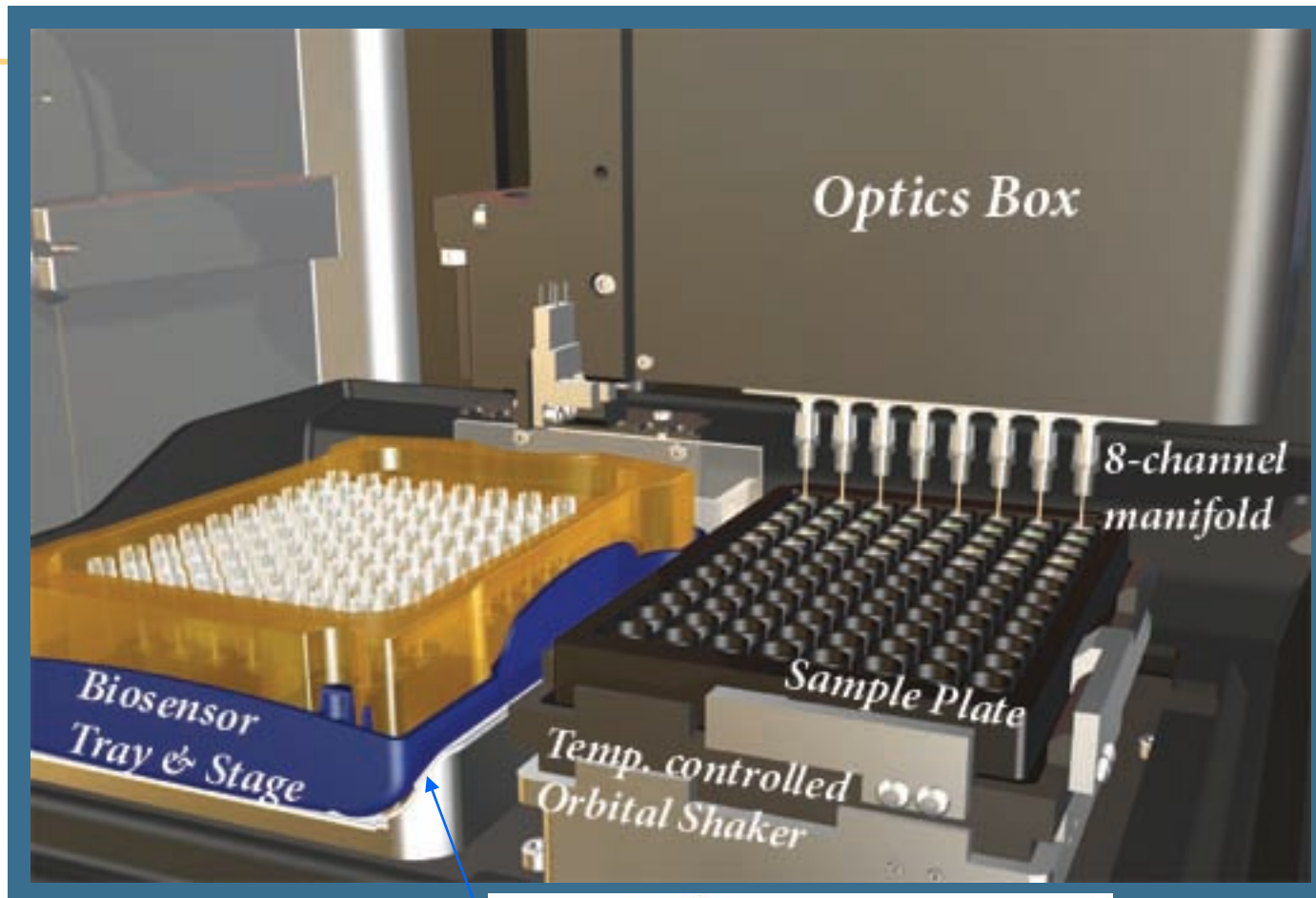
Application	Sensor Type
Quantitation	Anti-Human IgG Fc Anti-Murine IgG (Fab') <sub>2</sub> Protein A Streptavidin (SA) Anti-Penta HIS
Kinetics	Streptavidin (SA) Super Streptavidin (SSA) Amine Reactive (AR) Aminopropylsilane (APS) Anti-hIgG Fc Capture Surface (AHC)

Other biosensor chemistries available through custom services program.

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# Inside the Octet RED, QK<sup>e</sup> and QK Systems

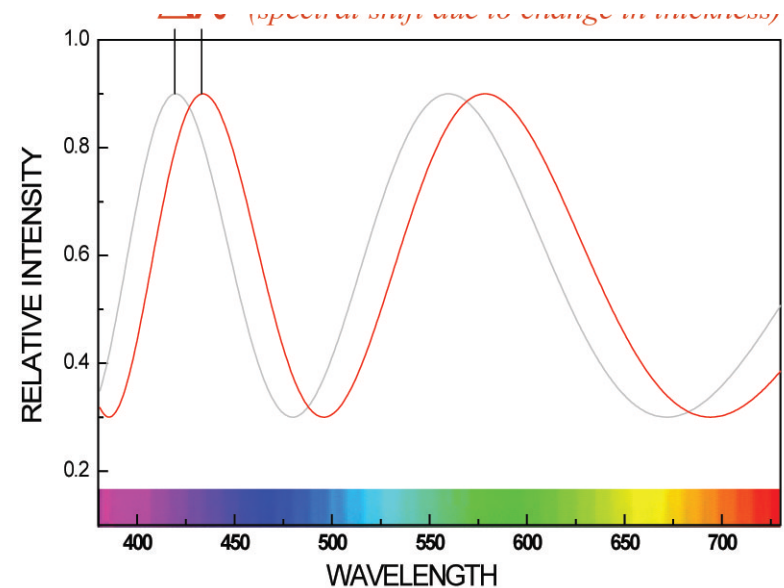
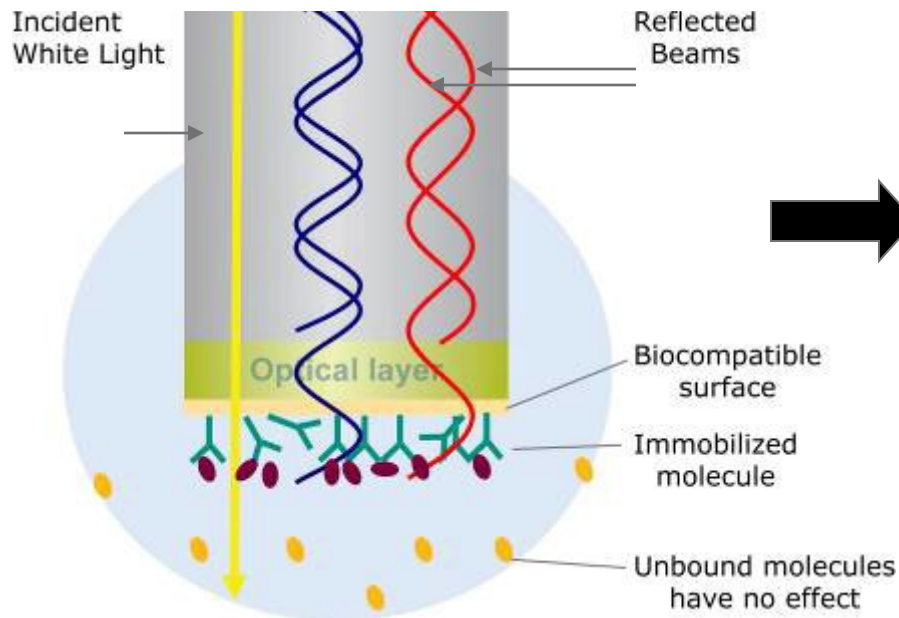


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# Bio-Layer Interferometry (BLI)

- Any change in the number of molecules bound causes a measured shift in the pattern
- Binding is monitored in real-time, so rate constants are measured



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# Improving Sensitivity in Quantitation Assays on the Octet Platform

- Basic Principle
  - ❑ **Signal** is directly proportional to the **amount** and **density** of the binding mass
- Amplification Mechanism
  - ❑ Increase mixing speed
  - ❑ Increase analyte incubation time
  - ❑ Increase binding mass (sandwich, cross-linked tag)
  - ❑ Increase density of binding mass (precipitating enzyme substrate)

# Contamination Assays Require Excellent Sensitivity

- **FDA recommends** assessment of all therapeutics by biological means.
  - ❑ HCP less than 10 ppm (10 ng/mL in 1 mg/mL of drug product)
  - ❑ Residual Pro-A less than 5 ppm (2.5 ng/mL in 0.5 mg/mL of Human IgG)
- **Optimization of purification process** requires:
  - ❑ Greater sensitivity than what FDA recommends
  - ❑ A wide dynamic range to allow interrogating the contaminant concentration from very high to very low level



# Assay Formats for Increasing Sensitivity

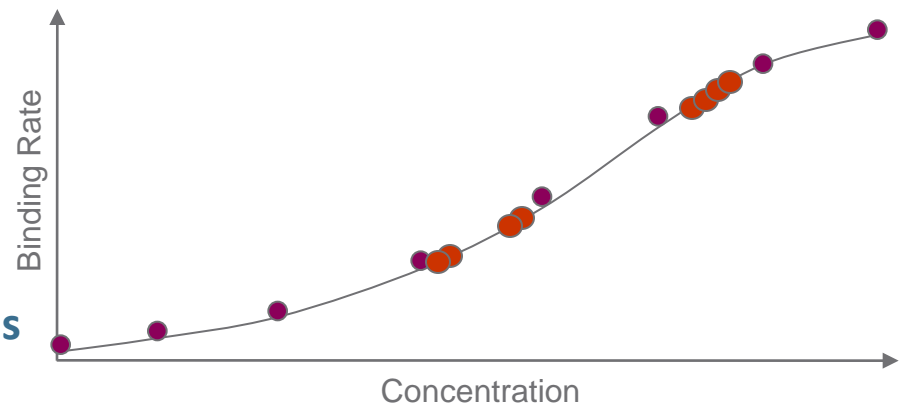
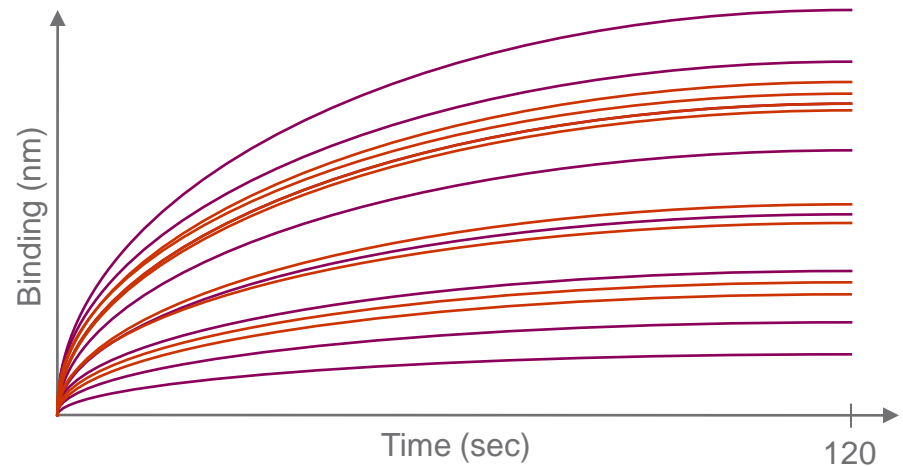
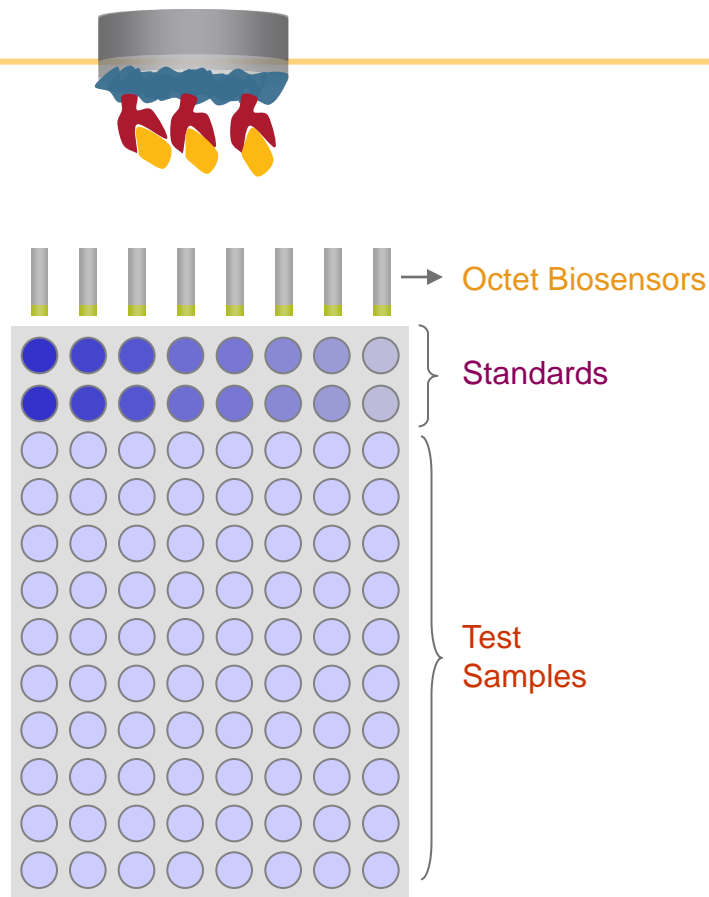
		<u>Amplification</u>
1-Step	sensor   -Capture : <u>Analyte</u>	{ High Speed Mixing Longer Incubation
2-Step	sensor   -Capture : <u>Analyte</u> : <u>Tag</u>	{ High Speed Mixing Longer Incubation <b>2nd reagent</b>
3-Step	sensor   -Capture : <u>Analyte</u> : <u>Tag-Enz.</u> : 1) <u>Substrate</u> 2) anti-T- <u>Mass</u>	{ High Speed Mixing Longer Incubation <b>2nd reagent</b> <b>PPT Substrate</b>

1<sup>st</sup> and 2<sup>nd</sup> steps can be done off-line for higher throughput

# Quantitation Capabilities of the Octet Platform

- Basic (Direct) Quantitation
  - ❑ 1-step assay using a biosensor to capture analyte directly
  - ❑ Regeneration is possible with some assays
- Advanced (Amplified) Quantitation
  - ❑ Sandwich-style assay
    - 2-step assay using a biosensor to capture analyte followed by use of a second antibody to sandwich the analyte
  - ❑ Enzyme-linked assay
    - 3-step assay using a biosensor to capture analyte bound by two separately labeled capture molecules followed by binding an HRP-conjugated antibody to the complex followed by precipitation of substrate directly onto the biosensor surface

# Octet Automated Workflow for Direct (1-step) Quantitation



- Data is taken for 2 minutes per 8 wells
- 1 step and no washing

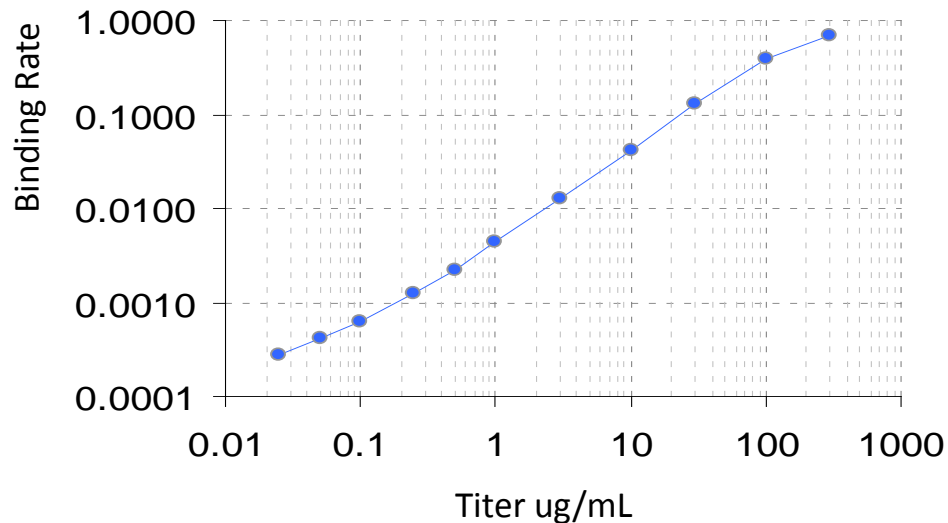
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# 1-Step Example: HIgG quantification

## High Sensitivity Analysis Allows Early Clone Selection

Calibration curve shown covers 25 ng/mL to 300 µg/mL range

1000 rpm, 5 minutes per column read time



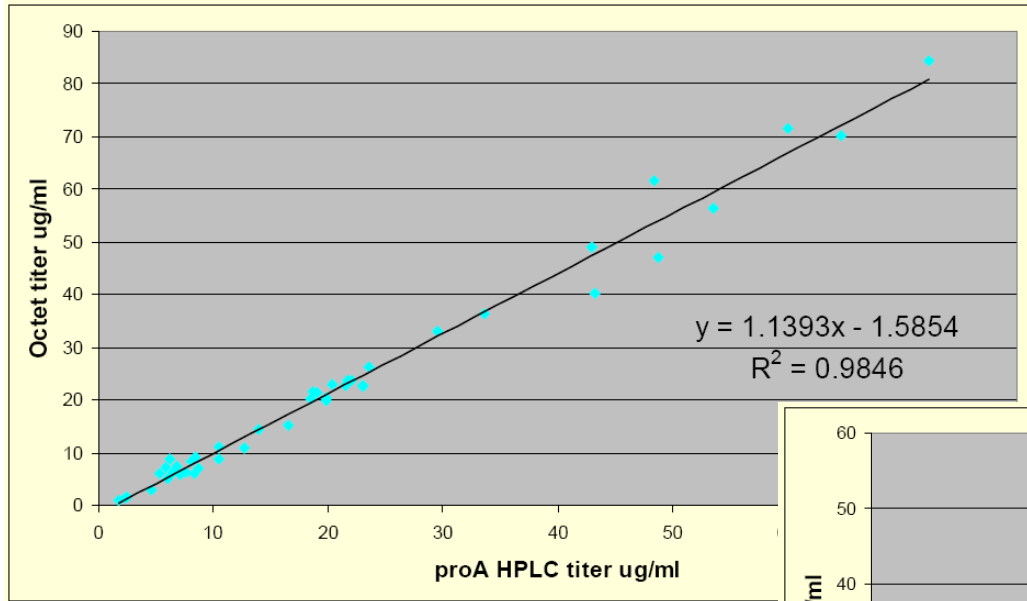
Unknown Sample (nominal µg/mL)	Conc (µg/mL)	CV%
0.025	0.026	9.7%
0.05	0.04	9.9%
0.1	0.09	9.7%
0.5	0.50	4.8%
1	1.07	6.9%
10	10.3	6.6%
50	57.4	5.1%

Higher sensitivity can be achieved with longer incubation.

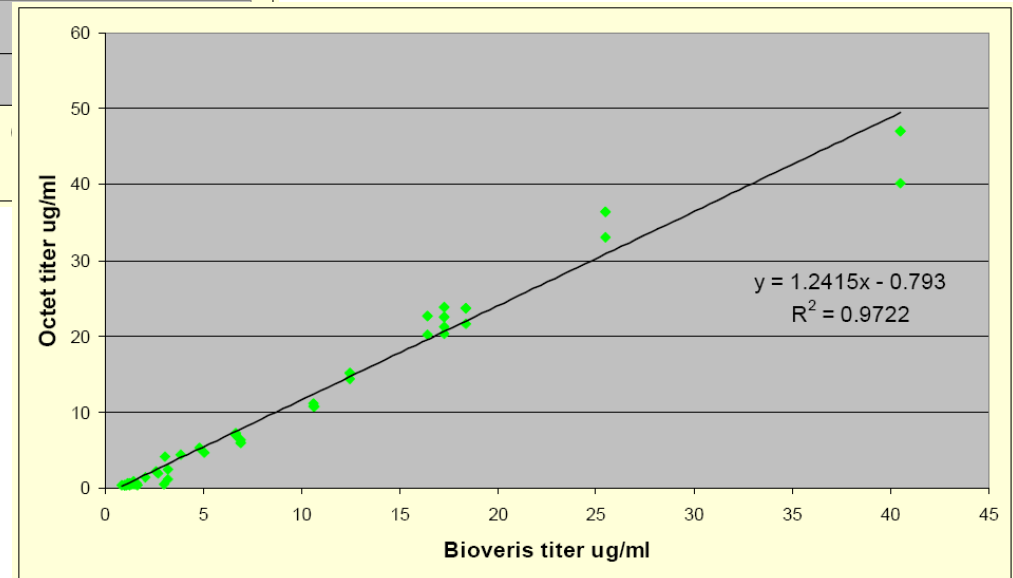
Wide dynamic range allows less dilutions.

# 1-Step Example: Protein-A Biosensor

Accuracy Correlates (Octet vs. HPLC and ECL)



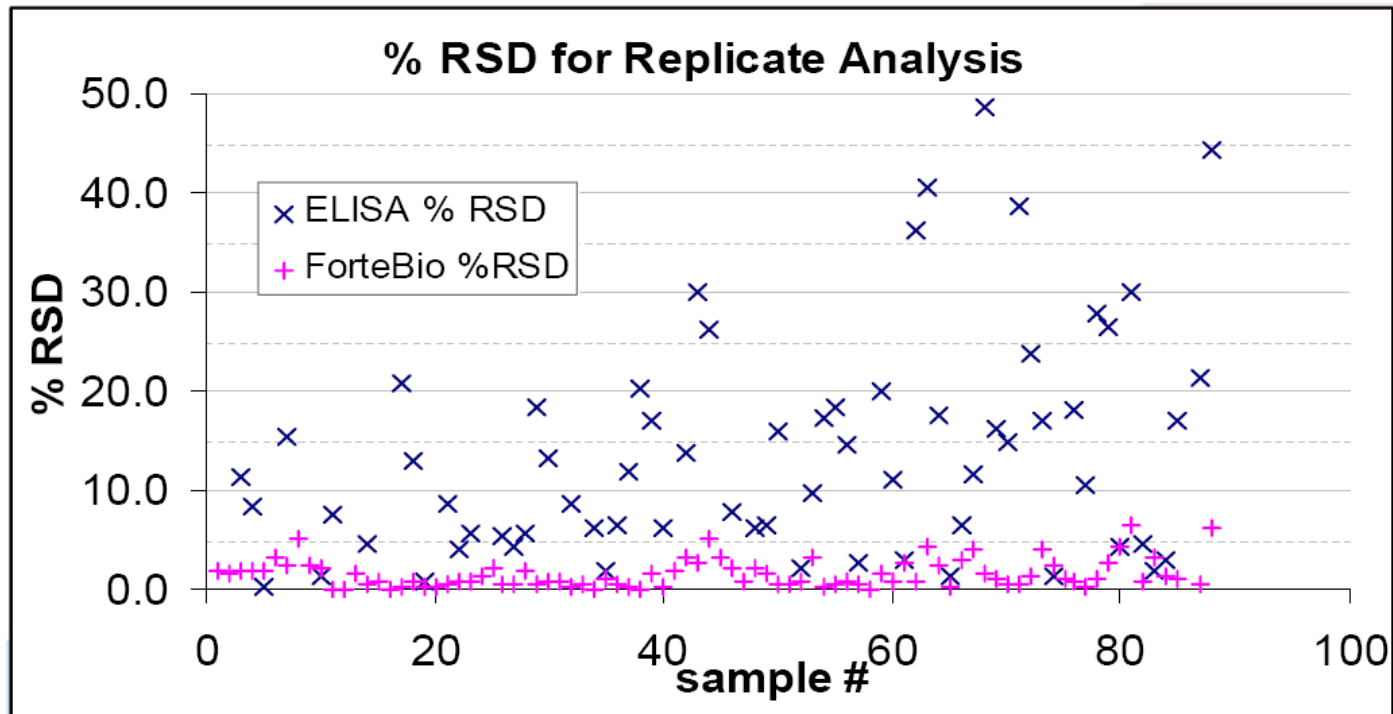
Protein A biosensors were compared to Protein A HPLC and ECL technology.



Data presented at  
Bioprocessing International  
2007 by Sarah Koob from Wyeth

# 1-Step Example: Protein-A Biosensor

## Octet Reproducibility Comparison to ELISA



Data presented at IBC Antibody Production,  
2008 by Keith Davis from Pfizer MO.

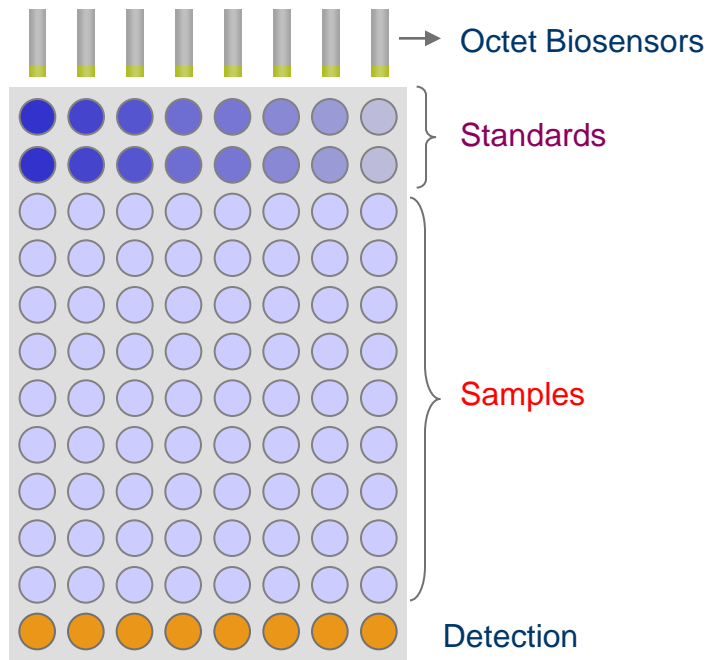
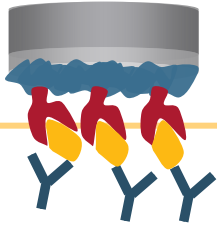
### Typical RSD Values:

ELISA	5 - 20%
Octet	< 5%

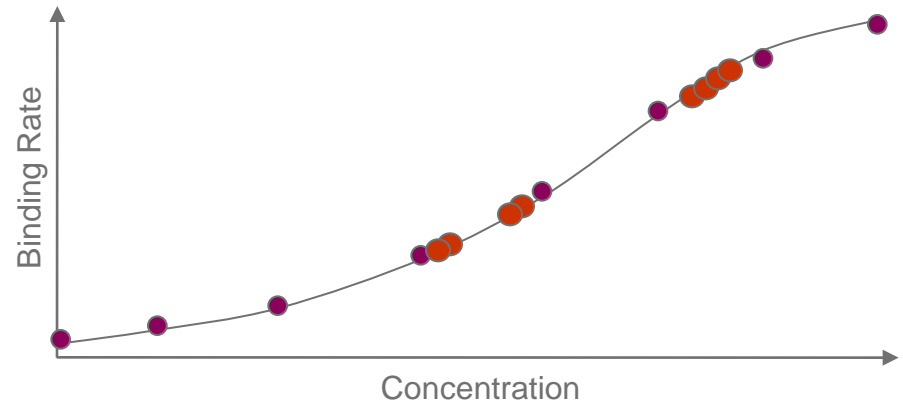
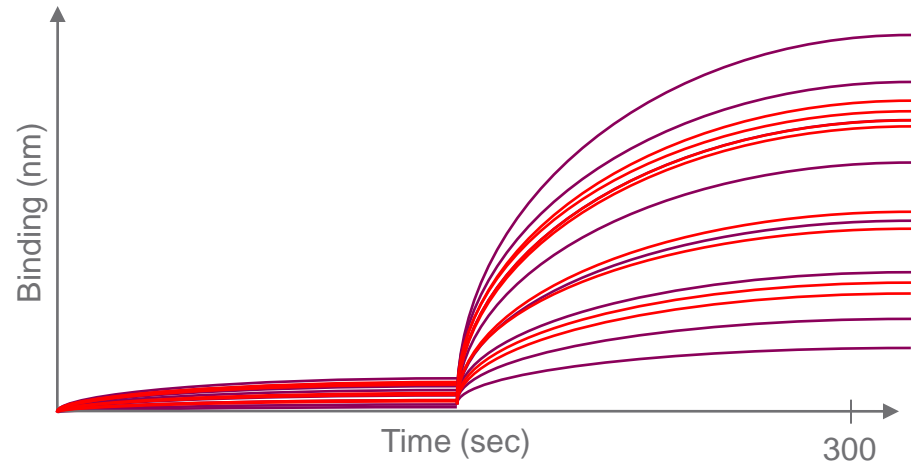
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# Octet Automated Workflow for 2-Step Sandwich-style Quantitation



- Detection reagent reused
- No washing external to instrument



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# Biosensor Products that Support 2-step Assays

Biosensor Name	Immobilization Chemistry
<b>Protein A</b>	The protein A immobilized on the biosensor binds to the Fc region of human IgGs. Will also bind to the Fc of many subtypes of mouse and rabbit IgG.
<b>Streptavidin (SA)</b>	Streptavidin coated biosensor with a high binding capacity for biotinylated proteins, peptides and nucleic acids.
<b>Residual Protein A Detection Kit</b>	Binds specifically to Protein A or MabSelect column resin molecules in IgG samples.



# 2-Step Example: Residual Protein A Detection Kit

1. **Residual ProA Biosensor** || : \*ProA : @ProA

2. Denaturing Buffer

3. Neutralization Buffer

4. Sample Diluent A

5. Sample Diluent B

6. Secondary Antibody

7. Recombinant ProA Stock Solution (calibrator)



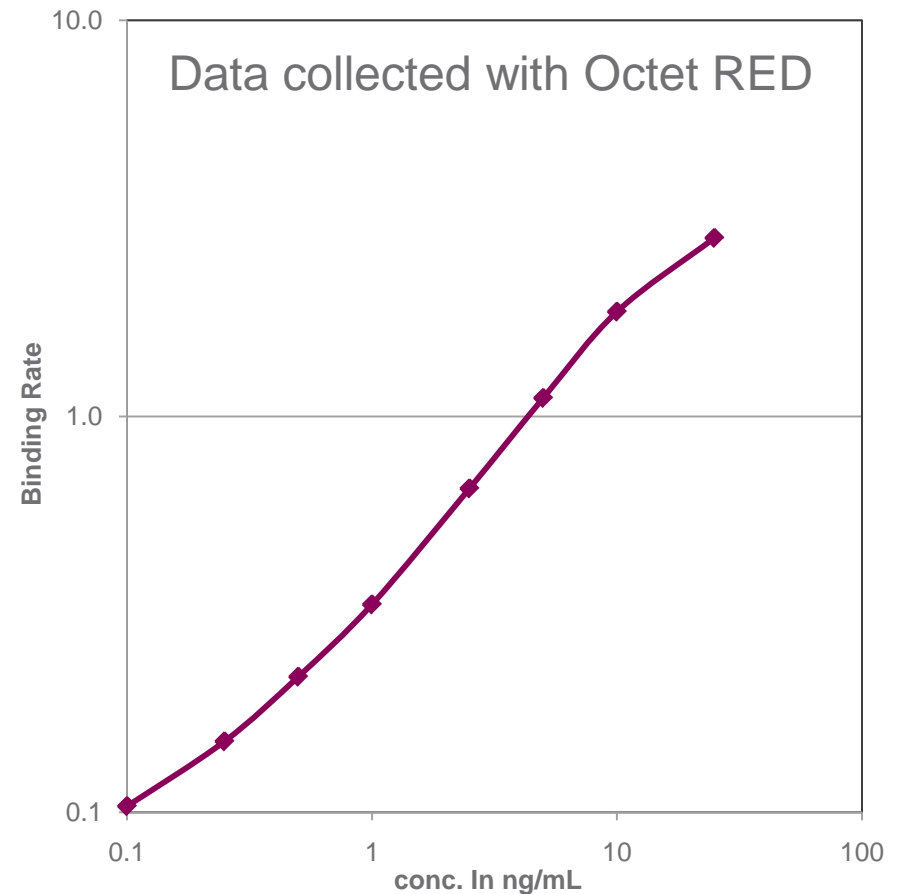
# 2-Step Example:

## Residual Pro-A Contamination Assay STD Curve

### Concentration of Protein A (N=4)

ng/ml	Binding Rate	%CV
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0.1	0.10	4.2%
0.25	0.15	6.1%
0.5	0.22	3.6%
1	0.33	3.4%
2.5	0.66	5.4%
5	1.11	3.6%
10	1.84	2.2%
25	2.82	1.5%



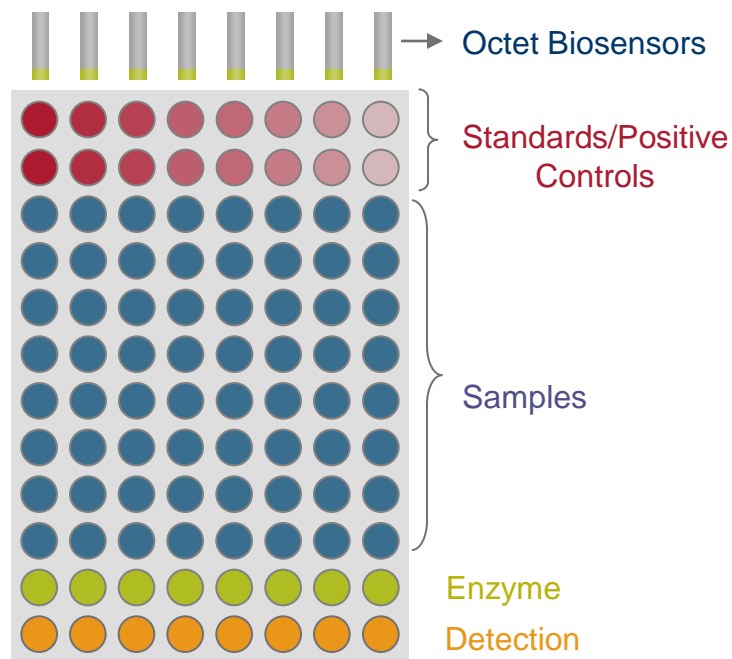
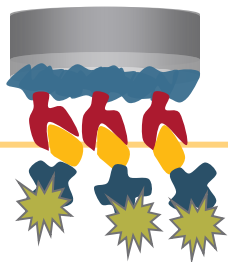
All dilutions include 0.5 mg/mL human Human IgG

## 2-Step Example:

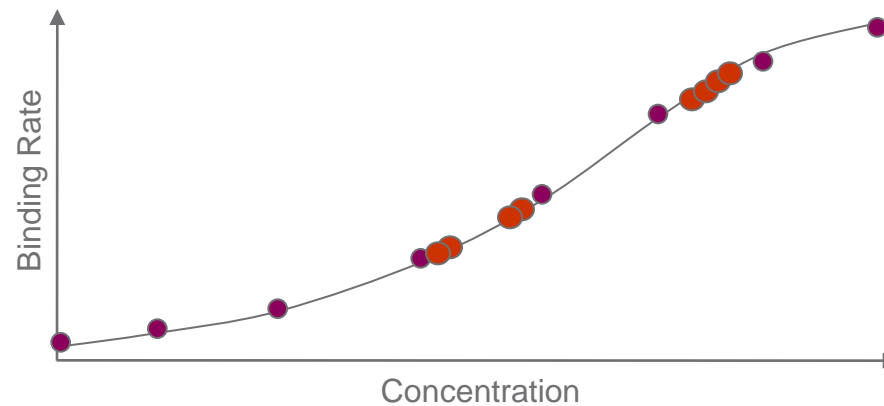
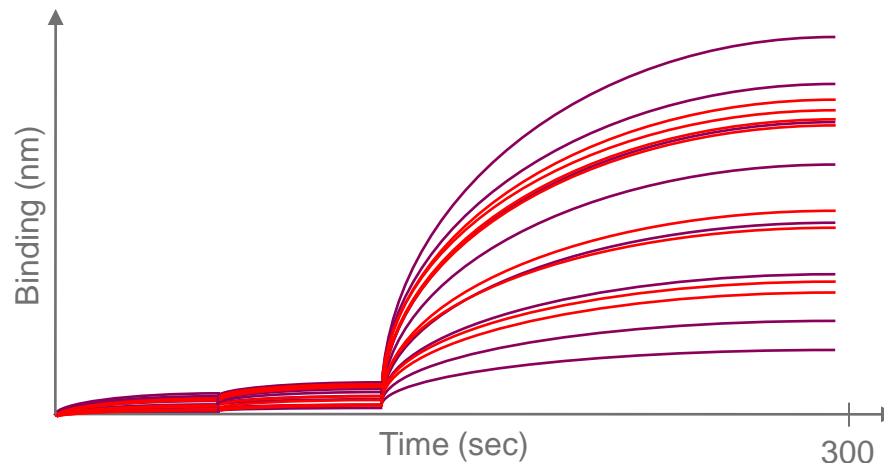
### Performance Comparison of Residual Pro-A Contamination Assay

Important Feature	RepliGen (ELISA)	Cygnus (ELISA)	ForteBio
Dynamic Range	0.1 – 1.6 ng/ml	0.1 – 10 ng/ml	0.1 – 25 ng/ml
Precision	< 10%	> 10%	< 10%
Read Time	5 minutes	5 minutes	1 hour
Prep Time	~2.5 hours	~2.5 hours	1.25 hours
Throughput	1 plate/3 hours	1 plate/3 hours	1 Plate / 2h15m

# Octet Workflow for Enzyme-linked Bridging Assay (3-Step)



- Detection reagent reused
- ➔ No washing external to instrument



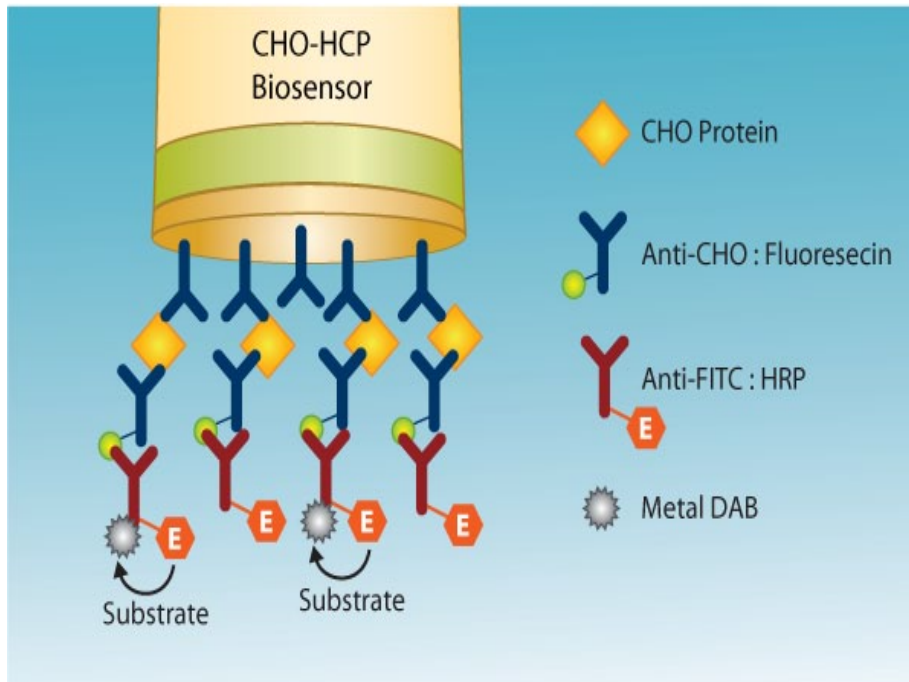
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# Biosensor Products that support 3-Step Quantitation

Biosensor Name	Immobilization Chemistry
<b>Streptavidin (SA)</b>	Streptavidin coated biosensor with a high binding capacity for biotinylated proteins, peptides and nucleic acids.
<b>Immunogenicity Kit</b>	Streptavidin coated biosensors supplied with Sample Diluent and the Immunogenicity Reagent for detection of anti-drug antibodies. Supports enzyme-linked bridging and direct formats for detection of both high and low affinity ADA's.
<b>Residual CHO HCP Detection Kit</b> (Build-to-order through ForteBio's Custom Services Program)	Binds specifically to HCP proteins.

# 3-Step Example:

## Built-to-order Residual CHO HCP Detection Kit



All kits will be custom kits made to customer specifications

### Kit Contents

1. Anti-CHO HCP biosensor
2. Fluorescein-anti-CHO antibody
3. HRP-anti-FITC antibody
4. CHO HCP standard
5. ForteBio Sample Diluent
6. Metal DAB

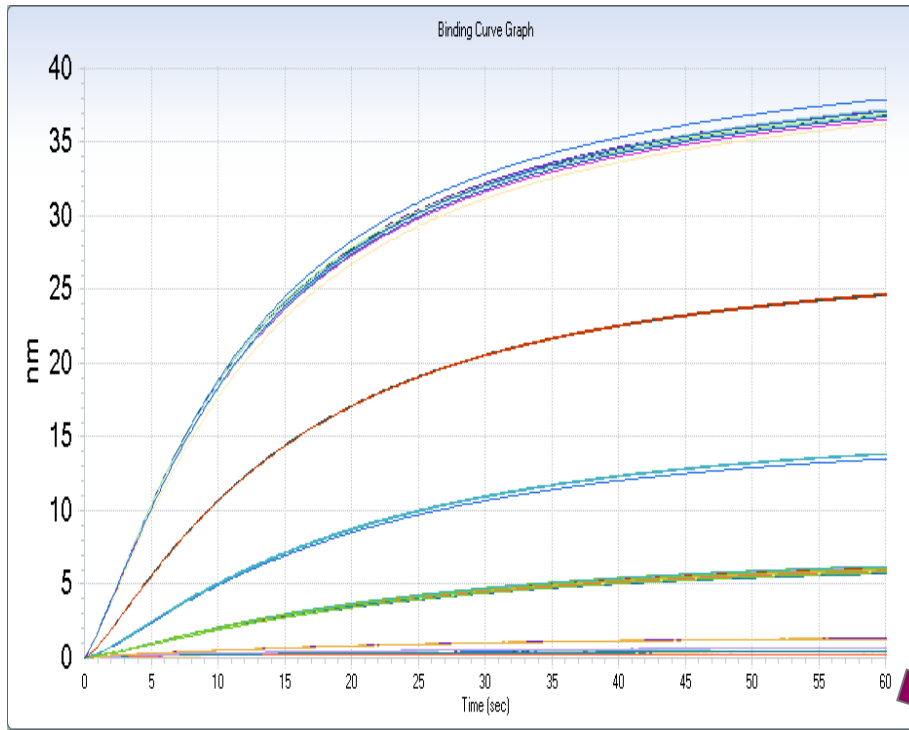
# 3-Step Example:

## Performance Comparison of Residual CHO HCP Contamination Assay

Important Feature	Cygnus ELISA Kit	ForteBio Custom Assay using Commercially available CHO Ab
Dynamic Range	1 – 75 ng/mL	0.3 – 100 ng/mL
Precision	> 10%	< 10%
Read Time	5 minutes	30 minutes (Octet 384) 60 minutes (Octet classic)
Incubation Time	3.5 hours	60 minutes
Hands on time (sample prep)	20 min	15 min
Throughput	1 plate/4 hours	1 plate/ 1.75 hours (Octet 384) 1 plate/ 2.5 hours (Octet classic)

# 3-Step Example:

## Residual CHO HCP Contamination Assay STD Curve



CHO Conc. (ng/mL)

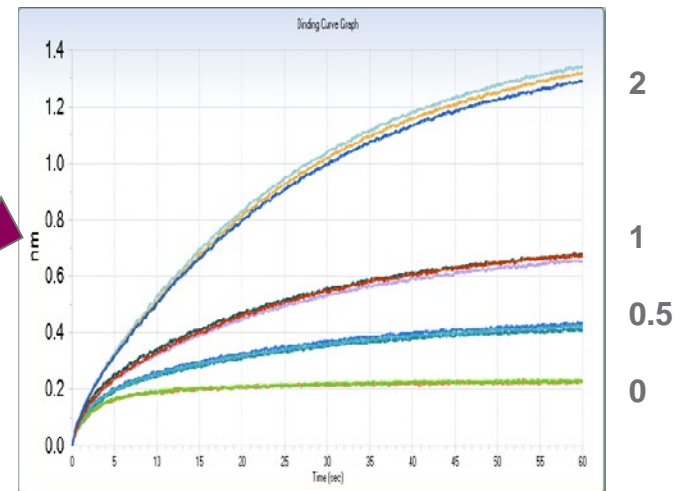
200

75

25

8

Low end zoom in



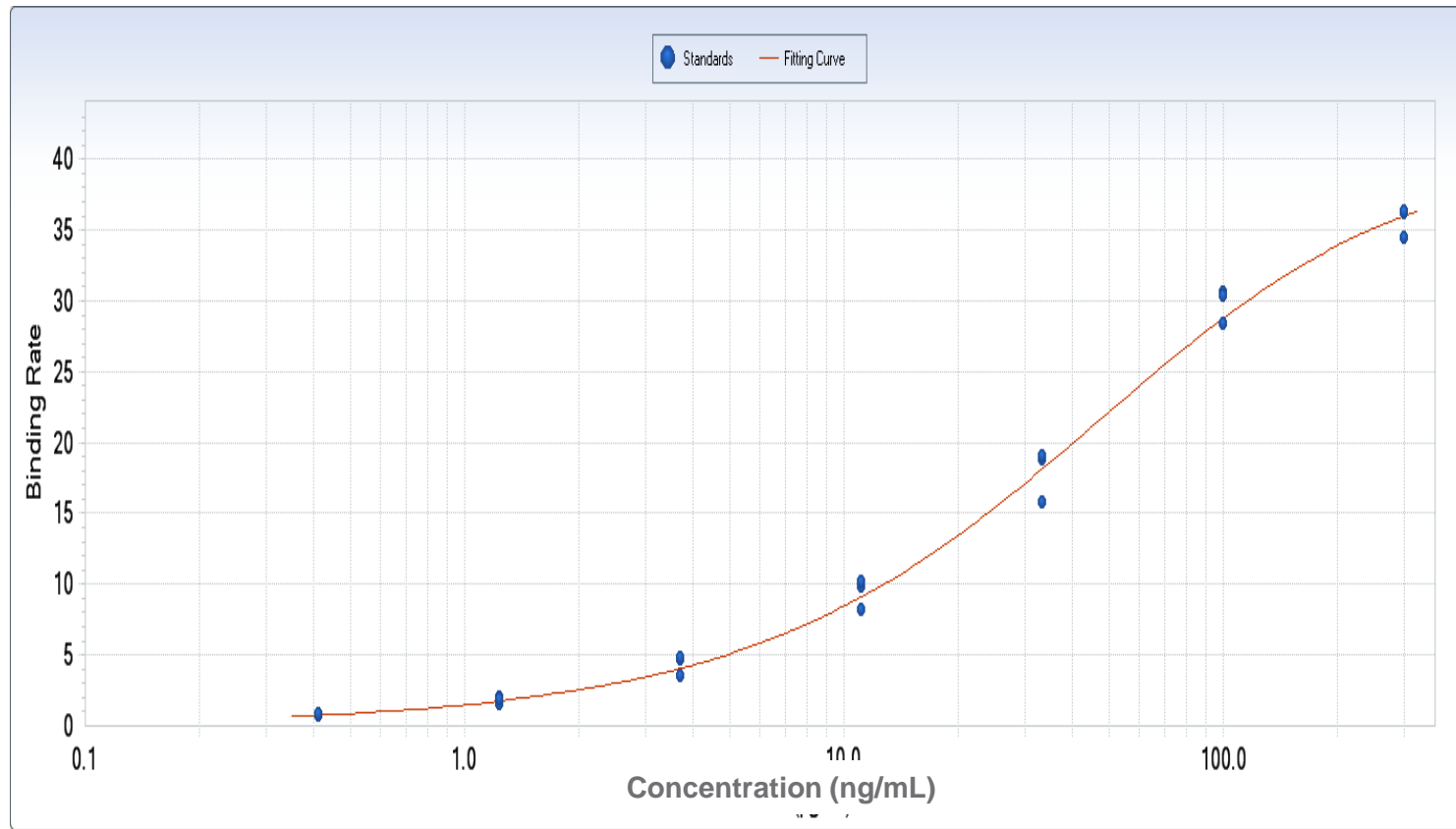
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# 3-Step Example:

## Residual CHO HCP Contamination Detection Assay STD Curve



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# Conclusion:

- 1) Flexible Octet platform enables maximizing the advantages in **time to answer, throughput, dynamic range** (100pg/mL ~ 2 mg/mL), and **sensitivity** through various assay formats, as simple as direct real time binding to highly amplifying multi-steps.
- 2) Throughput of each assay can be increased using the Sidekick™ shaker for off-line incubations and/or running on the 16 channel RED384 or QK384 instruments.
- 3) Octet enables many contamination assays on a single platform simplifying user's assay design and saving time.