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Comparability Study

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Comparability Study of GxP-Compliant Octet® R8 and the Enhanced Octet®R8e System Confirms Assay Precision and Transferability

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Abstract

This study compares the use of the GxP-compliant Octet® R8 to the enhanced Octet® R8e system for conducting binding assays, focusing on assay precision and transferability. The Octet® BLI platform is integral to biologics development, offering applications throughout the workflow, from early selection to quality control. Three rounds of experiments utilized qualified kinetic and quantitative assays to assess parameters such as K_D, on/off rates, and percentage recovery. Results demonstrated high precision and reproducibility across both systems, with coefficient of variation (CV) values consistently below 10%. The Octet® R8e system showed improved sensitivity and compatibility with 384-well plates, facilitating lower sample volumes and reduced reagent costs. The study confirms that validated assays on the Octet® R8 can be seamlessly transferred to the Octet® R8e, minimizing the need for additional assay development. This comparability ensures that both instruments can reliably support pharmaceutical and biological product regulatory applications, offering users confidence in upgrading to the Octet® R8e system. The findings underscore the Octet® BLI platform's role in enhancing assay efficiency and precision, crucial for maintaining product quality throughout the development lifecycle.

Introduction

To maintain the quality of pharmaceutical and biological products, critical processes and quality attributes should be identified early in the development phase. This allows methods for their evaluation to be implemented throughout the product development and release lifecycle. The International Conference on Harmonization (ICH) Q2(R2) offers guidance on validating analytical technologies that are used for these assessments.[1]

Depending on the type of assay being developed, different parameters should be quantified and tracked to ensure a fully validated assay can be established. For example, quantitation assays should show linearity and maintain accuracy and limits of quantitation. On the other hand, in kinetic binding assays, parameters such as kinetic rate constants, affinity and precision are generally assessed.

Biolayer Interferometry (BLI) is an analytical technique that can be used for the generation of supporting data and in lot-release protocols, as used in biologic development. This document demonstrates a comparability study between the newly released Octet® R8e and legacy Octet® R8 instruments and shows that key outputs from validated assays are consistent between the two systems. This minimizes the time and resources needed for assay development and qualification upon transferring an assay from one instrument to the other.

Benefits of the Octet® BLI Platform in GxP Applications

The Octet® BLI platform enables a variety of applications to be performed at various stages of biologics development—from early selection and validation through to manufacturing and quality control. For example, active analyte concentration determination, ligand binding, or contaminant testing for lot release and in-process testing. Benefits of the Octet® BLI platform include:

1. Parallel processing of samples to reduce assay development time.
2. Quick and easy data analysis across many samples allows for faster decision-making during assay development and qualification.
3. Simple assay set up, with minimal sample preparation, and walk-away data acquisition, which reduces hands-on time and decreases the risk of human errors.
4. Octet® BLI instruments require very little maintenance, making them ideal for a QC environment.
5. Off the shelf biosensors such as Protein A (Pro A), Anti-Human IgG Capture (AHC2) and Anti-Human Fab-CH1 (FAB2G) are available for direct capture of ligands. Streptavidin (SAX and SAX2) biosensors deliver enhanced precision for GMP applications.
6. Crude sample compatibility minimizes sample preparation and purification requirements.
7. Octet® BLI is non-destructive, therefore samples remain intact after analysis and can be reused.

Exampless of Drug Approvals that Utilized Octet® Instruments

Octet® BLI *in vitro* binding studies continue to be used in successful drug licensing applications including the assessment of functional activity of Epruvy (ranibizumab Midas), an anti-VEGF humanized monoclonal antibody fragment biosimilar of Lucentis (EMEA approved in 2024). Octet® binding studies were used to confirm that the binding of Epruvy to a panel of VEGF-A isoforms is comparable to Lucentis.[2]

Octet® BLI peptide binding studies were also used to assess binding of the bispecific antibody Elrexfio (elranatamab), which consists of a BCMA and CD3 binding domain (EMEA and FDA approved in 2023).[3]

In addition to these approved drugs, Octet® BLI instruments were used to generate supporting data for the assessment of functional activity of anti-PD-1 antibody pembrolizumab (Keytruda; EMEA approved in 2015) and the anti-PD-L1 monoclonal antibody Tecentriq (atezolizumab, EMEA approved in 2017 and FDA approved in 2018).[4,5]

Octet® BLI assays have also been used to provide supporting data for marketing authorization applications. One example of this is Oyavas (EMEA application, made in 2020 and approved in 2021), a biosimilar to Avastin (an anti-VEGF) antibody.[6] The Octet® confirmed its specificity through functional binding assessment with irrelevant antigens. These are just a few of many examples of drugs developed using the Octet® BLI systems.

Octet® R8 vs. Octet® R8e

Octet® systems use a standard microplate format that enables high-throughput, design flexibility and automated binding analysis. The Octet® R8 and Octet® R8e are both 8-channel instruments capable of analyzing up to 8 samples in tandem. Both instruments are compatible with 96-well micro-titer sample plates, whilst the Octet® R8e is also compatible with 384-well plates, facilitating the use of lower sample volumes and conservation of precious reagents.

Although evaporation covers are available for the Octet® R8 that enable long duration assays, a new Octet® AE Microplate Evaporation Cover for 96-well microplates has been designed for the Octet® R8e. The AE Microplate minimizes evaporation across all wells, to maintain consistent sample concentrations during extended (up to 16 hours) or sensitive BLI assays. This preserves sample integrity throughout the experiment and ensures greater data accuracy and reproducibility.

The length of time an assay can run depends on multiple factors, including plate type and sample volume. The improved run time of the R8e has been explored in more detail in this application note: 'Unveiling Hidden Insights: Enhanced Analytics with New Heights of Sensitivity (Sartorius, 2025)'.[7] This makes both instruments an ideal choice for probing slow effects in kinetic assays, or, for allowing more gradual loading to reduce the concentration of ligand needed and lower the risk of avidity effects.

When running an experiment on the Octet® R8, there is the option to choose an acquisition rate based on your sensitivity and signal-to-noise requirements. On the Octet® R8e system, the choice of acquisition rates has been expanded, leading to the potential for greater sensitivity detection of molecules and the capture of very fast kinetic on-rates. This improvement in sensitivity is further supported by improvements to the spectrometers in the Octet® R8e instrument compared to the R8. Both instruments come with GxP compatible products.

Octet® R8 and Octet® R8e GxP Compliance

GxP users are often looking for analytical instruments that offer simplicity, innovation and speed. Other key considerations include whether the software is user-friendly, the size of the lab footprint and automation potential. The instrument also needs to be very robust and experience minimal downtime. The Octet® R8 and Octet® R8e are designed to couple ease of use with high productivity, facilitating high capacity quality control testing.

Sartorius's Octet® GxP package comes with all of the requirements for GMP compliance including IQOQ protocols and kits, user guides, performance qualification (PQ) protocols and kits, 21 CFR Part 11 software with audit trails, software validation package and biosensor validation support.

Compound	Octet® R8	Octet® R8e
Number of spectrometers	8	8
Maximum simultaneous reads	8	8
Temperature control	15-40 °C	15-40 °C
Evaporation control	Yes - Standard	Yes - Advanced (16 hours)
Acquisition rate	Standard	Enhanced
Spectrometer type	Standard	Enhanced
96 and 384-well plate compatibility	No	Yes
GxP package availability	Yes	Yes

Figure 1: Key Features of the Octet® R8 and Octet® R8e Systems

Comparability Studies: Octet® R8 vs Octet® R8e

The Octet® R8e BLI system uses the same catalog of biosensors as the Octet® R8 and, therefore, transfer of methods developed on other Octet® BLI systems to the Octet® R8e is possible. To demonstrate comparability, three rounds of experiments were conducted, using pre-developed and qualified assays. The assays were run on both the Octet® R8 and Octet® R8e systems and included profiling of IgG samples across two kinetic and one quantitative assay. Parameters including K_D and association/dissociation rates were assessed for the kinetic assays, whilst percentage recovery was determined in the quantitative assay. Assay precision, calculated as the CV (%), was the key metric used for comparison across the systems.

The initial kinetics assay assessed the binding of human IgG (hIgG) to biotinylated CD64. The assay was conducted using the pre-diluted reagents and sensors included with the Octet® R8 PQ Kit (Kinetics), which is a validated assay used for instrument qualification. The second kinetics assay measured the binding of an anti-Fab hIgG to a Fab fragment loaded onto FAB2G biosensors. The final quantitation assay assessed the binding of human IgG (hIgG) to Protein A (ProA) biosensors.

Materials and Methods

Materials

All assays

- Instruments: Octet® R8 and Octet® R8e instrument with Octet® BLI Discovery and Analysis Studio Software
- Plates: 96-well, black, flat bottom microplate (Cat No. 655209, Greiner Bio-One)

Kinetics of hIgG binding to CD64 (PQ-K kit assay)

- Biosensors, reagents and buffers: Octet® R8 PQ Kit (Item No. 18-1177, Sartorius). This kit includes Octet® PQ Biosensors, Octet® Assay Buffer-PQ, Octet® Biotin-hCD64-PQ-K and Octet® Humanized IgG1k mAb.

Kinetics of hIgG binding to Fab fragment

- Biosensors: Octet® Anti-Human Fab-CH1 2nd Generation (FAB2G) Biosensors (Item No. 18-5125, Sartorius)
- Reagents: AffiniPure Fab Goat Anti-Human IgG (H+L) Secondary Antibody (Cat No. 109-007-003, Jackson ImmunoResearch) and ChromPure Human IgG, Fab Fragment (Cat No. 009-000-00, Jackson ImmunoResearch)
- Buffers: Octet® Kinetics Buffer 1X (Item No. 18-1105, Sartorius)

Protein A quantitation of IgG

- Biosensors: Octet® Protein A (ProA) Biosensors (Item No. 18-5010, Sartorius)
- Reagents: Octet® ProA Calibrator Set (8 IgG samples from 1-700 µg/mL, Item No. 18-1118, Sartorius)
- Buffers: Octet® Sample Diluent (Item No. 18-1104, Sartorius), Octet® 10 mM Glycine pH 1.7 (Regeneration buffer, Item No. 18-1180, Sartorius)
- Consumables: Octet® 384TW Microplates (Tilted-well microplates, Sartorius, Item No. 18-5166), 384-Well Flat-Bottom Polypropylene Microplates (Greiner, Cat No. 781209)

Methods

Kinetics of hIgG binding to CD64 (PQ-K kit assay)

PQ assay sensors were hydrated in the PQ assay buffer for 10 minutes prior to starting the assay. The pre-diluted CD64 and hIgG samples (at 1, 2 and 4 nM) were plated directly into the 96-well plate (200 µL/well) as the ligand load and analyte sample wells, respectively. The PQ assay buffer was used for baseline and dissociation steps, as well as a reference control for wells containing no analyte. The protocol was uploaded directly from the USB provided with the kit and this provided all the information on assay steps, timings and well types as required. Separate assay plates were run simultaneously on both the Octet® R8 and Octet® R8e systems. Analysis was performed using the PQ kit software module in the Octet® Analysis Studio 13.1.

Kinetics of hIgG binding to Fab fragment

A 10-minute pre-assay hydration of FAB2G biosensors was performed in 1X Octet® Kinetics Buffer (1X KB). The Fab fragment was diluted to 10 µg/mL in 1X KB and 200 µL/well was loaded onto sensors for 600 seconds. Following a 90 second baseline, association of the Fab to a range of concentrations of an anti-human Fab antibody (6.25 to 200 nM) diluted in 1X KB was assessed for 900 seconds. 1X KB was used as a zero-analyte reference control. A final dissociation step in 1X KB was monitored for a further 900 seconds. Three columns of sensors were run one after the other, with separate wells of reagents, to generate 3 replicates. A shake speed of 1000 RPM was used throughout. Assay plates were run simultaneously on the Octet® R8 and Octet® R8e systems. Analysis was performed using the Octet® Analysis Studio 13.1.

Protein A quantitation of IgG

ProA biosensors (hydrated prior to the assay in Octet® Sample Diluent) were used for quantitation (120 seconds, 400 RPM) of a range of concentrations of IgG (1-700 µg/mL) from the Octet® ProA Calibrator Set. Eight columns, each containing one well of each IgG concentration, were run sequentially, to generate 8 replicates. In between each column of IgG that was measured (and prior to assessment of the first replicate) sensors were regenerated through three cycles of 5 seconds dipped into regeneration buffer (Octet® 10 mM Glycine pH 1.7) followed by 5 seconds in neutralization buffer (Octet® Sample Diluent). Therefore, a single column of sensors was used to read all eight replicates. Identical 96-well flat bottom plates (containing 200 µL/well of samples) were run on both the Octet® R8 and Octet® R8e systems. Additional 384-well plates (flat-bottom plates containing 80 µL/well and tilted-well plates containing 40 µL/well) were quantified on the R8e system. Analysis was performed using the Octet® Analysis Studio 13.1.

Results

Loading data from the PQ assay was highly comparable between the instruments, with average loading response of 0.56 nm and 0.54 nm for the Octet® R8 and Octet® R8e instruments, respectively (Figure 2A and Table 1). Intra-assay reproducibility was also very high, with CVs for the loading response across the 16 biosensors of 1.4% on the Octet®R8 and 1.7% on the Octet® R8e.

The Octet® binding traces and affinity constant readouts for the PQ kinetics assay (Figure 2B and Table 2) also showed high similarity between the systems, with K_D values of 1.41E-10 M and 1.40E-10 M for the Octet® R8 and Octet® R8e, respectively. Low CV values (< 5%) across the 4 replicates for K_D, k_{on} and k_{off} rates indicate the high precision of this assay, which is maintained across both instruments.

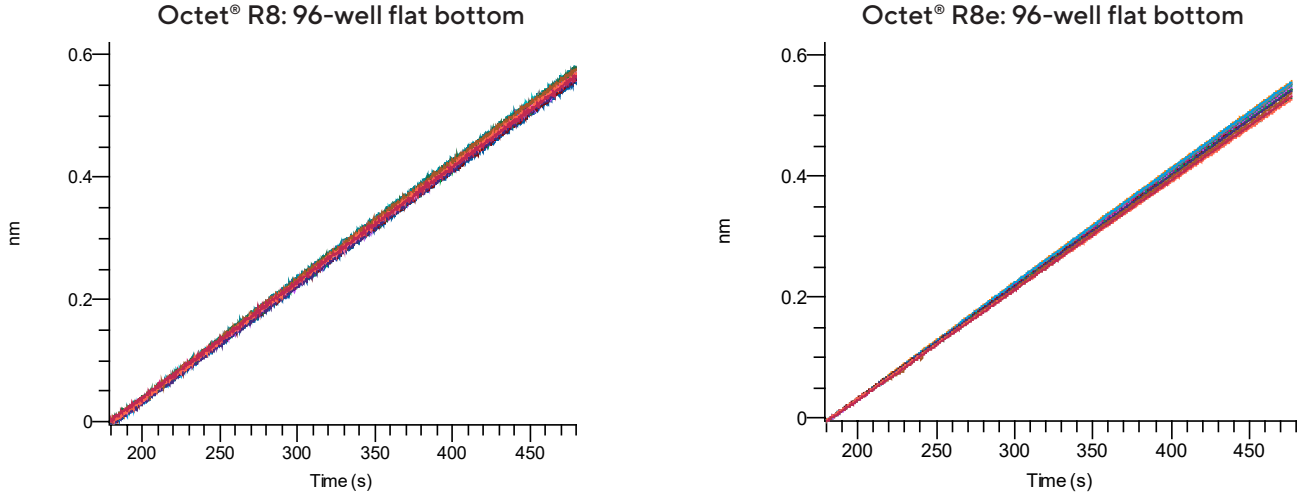

Figure 2A: CD64 loading to PQ sensors on Octet® R8 and R8e systems

Note: Traces show loading of CD64 to PQ assay sensors as measured using the Octet® R8 and Octet® R8e systems. Sixteen replicate sensors were loaded per instrument.

Instrument	Plate type	Average loading signal (nm)	Loading signal CV (%)
Octet® R8	96-well flat bottom	0.56	1.4
Octet® R8e	96-well flat bottom	0.54	1.7

Table 1. Comparison of loading parameters and CVs for CD64 loading to PQ assay sensors (n=16)

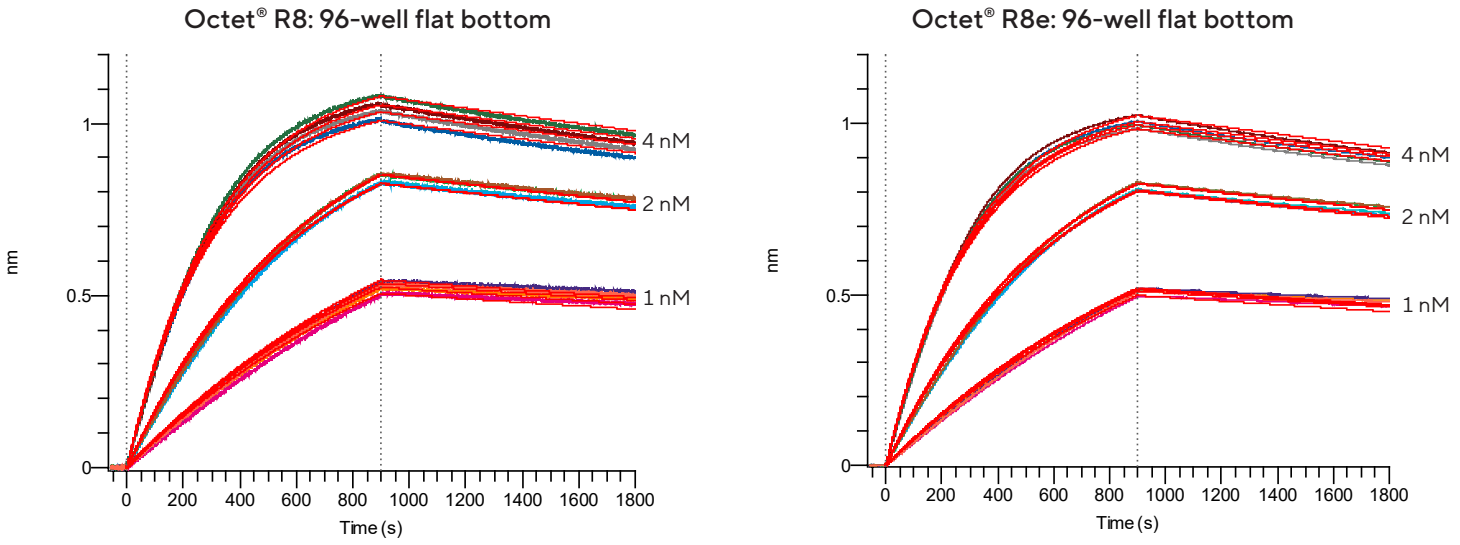

Figure 2B: PQ kinetic assay (hIgG binding to hCD64) on Octet® R8 and Octet® R8e systems

Note: PQ assay sensors were loaded with hCD64 (Figure 2A). Traces show subsequent binding of hIgG (1, 2 and 4 nM) to the loaded CD64 for 900 seconds followed by a 900 second dissociation step as measured using the Octet® R8 and Octet® R8e systems. Four replicates were measured per concentration.

Instrument	Plate type	K_D (M)	K_D CV (%)	k_a ($M^{-1}s^{-1}$)	k_a CV (%)	k_d (S^{-1})	k_d CV (%)
Octet® R8	96-well flat bottom	1.41E-10	1.4	7.63E05	1.3	1.08E-04	0.8
Octet® R8e	96-well flat bottom	1.40E-10	2.3	7.82E05	0.9	1.09E-04	1.8

Table 2. Comparison of binding kinetic parameters and CVs for hIgG binding to CD64 (n=4).

The Fab binding kinetic assay yielded similar results to the PQ assay, in that it demonstrated highly comparable readouts from both the Octet® R8 and Octet® R8e systems. The traces (Figure 3) exhibit clear separation and clustering of IgG concentrations as measured by both instruments.

Table 2 shows very closely comparable kinetic parameters as determined by each instrument, with a K_D value of 2.19E-08 M for the Octet® R8 and 2.17E-08 M for the Octet® R8e. CVs were calculated for kinetic parameters across the 3 replicates and illustrated precise quantification (< 10%) across both platforms.

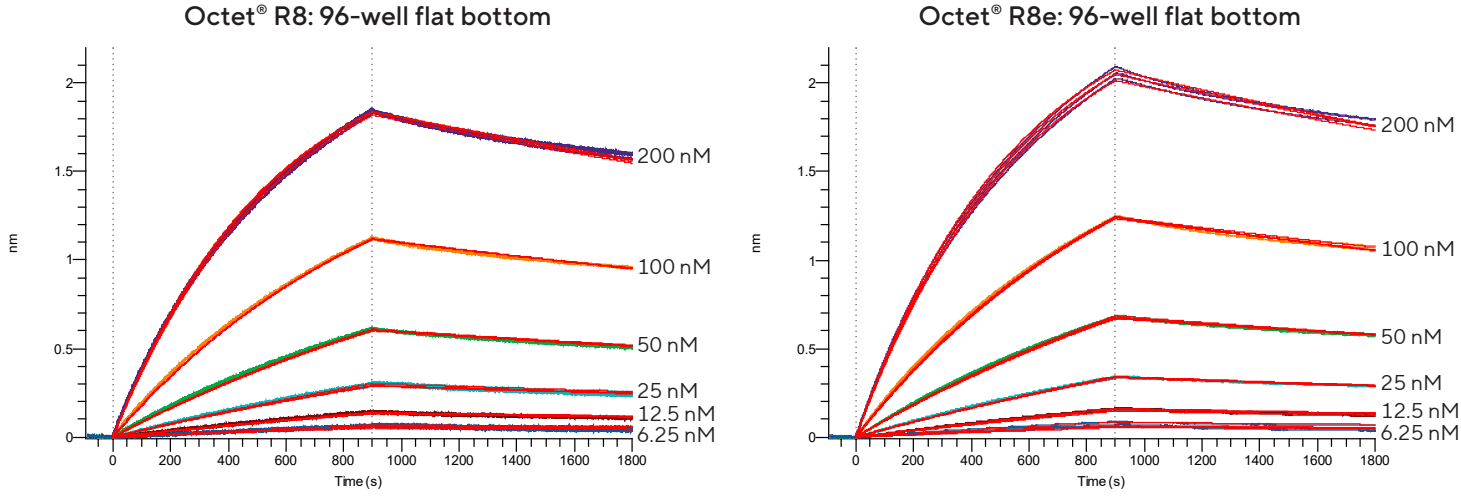

Figure 3: IgG binding to a human Fab fragment as measured by the Octet® R8 and Octet® R8e

Note: A human Fab fragment was loaded onto FAB2G biosensors. Traces show the binding to a range of concentrations of an anti-Fab IgG (6.25-200 nM) that followed (900 seconds) and its dissociation (900 seconds) as measured using the Octet® R8 and the Octet® R8e. Three replicates were measured per concentration.

Instrument	Plate type	K_D (M)	K_D CV (%)	k_a ($M^{-1}s^{-1}$)	k_a CV (%)	k_d (S^{-1})	k_d CV (%)
Octet® R8	96-well flat bottom	2.23E-08	5.4	8.04E+03	4.2	1.77E-04	1.9
Octet® R8e	96-well flat bottom	2.17E-08	2.4	8.03E+03	2.7	1.74E-04	5.1

Table 3. Binding kinetic parameters and CVs for anti-FAB binding to FAB fragment compared between Octet® R8 and Octet® R8e platforms (n=3)

Traces for the Protein A-based quantitation of IgG again showed clear, tight grouping of concentrations (Figure 4) across both instruments and all three plate types. This was reflected in the CVs that were generated across the eight replicates, which were consistently less than 9%. On top of this high precision, the percentage recovery metric for each concentration highlights that the IgG quantitation values generated using both instruments is comparable. In addition to 96-well microwell plates, 384-microwell plates were also assessed during quantitation. The precision of the 384-well data shows that similar data can be generated using much lower sample volumes (80 µL for flat-bottom plates and 40 µL for tilted-well plates) compared to the 200 µL/well used for 96-well plates. This shows that the 384-well compatibility of the Octet® R8e instrument can facilitate conservation of samples and reduced reagent costs.

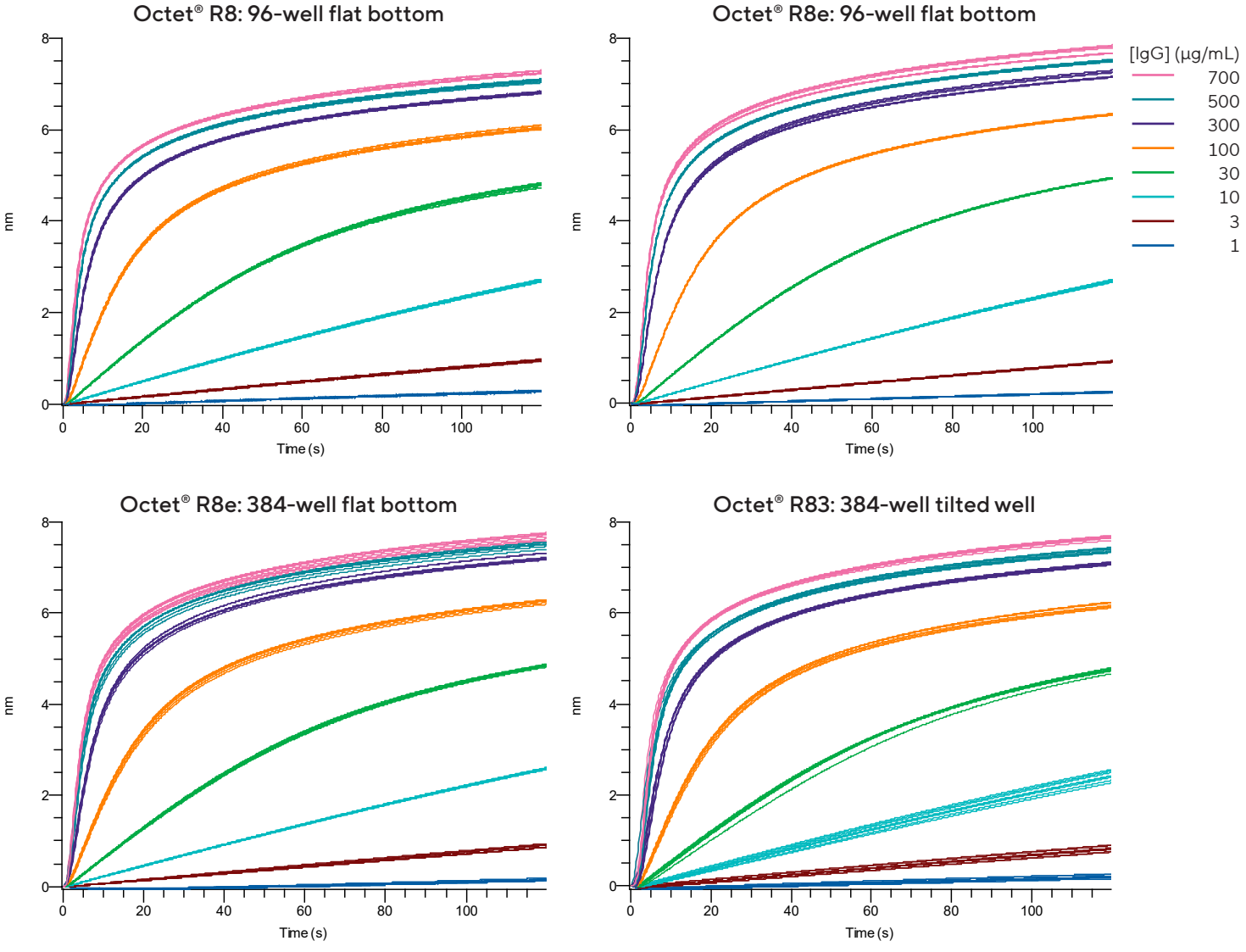

Figure 4A: Binding traces showing IgG binding to Protein A biosensors on the Octet® R8 and Octet® R8e systems

Note: Protein A biosensors were dipped into eight concentrations (1-700 µg/mL) of purified IgG for 120 seconds. Eight replicates were measured per concentration, with the sensors regenerated in between each measurement. Identical 96-well flat bottom plates were run on the Octet® R8 and the Octet® R8e systems. 384-well flat-bottom and tilted-well plates were also run on the Octet® R8e.

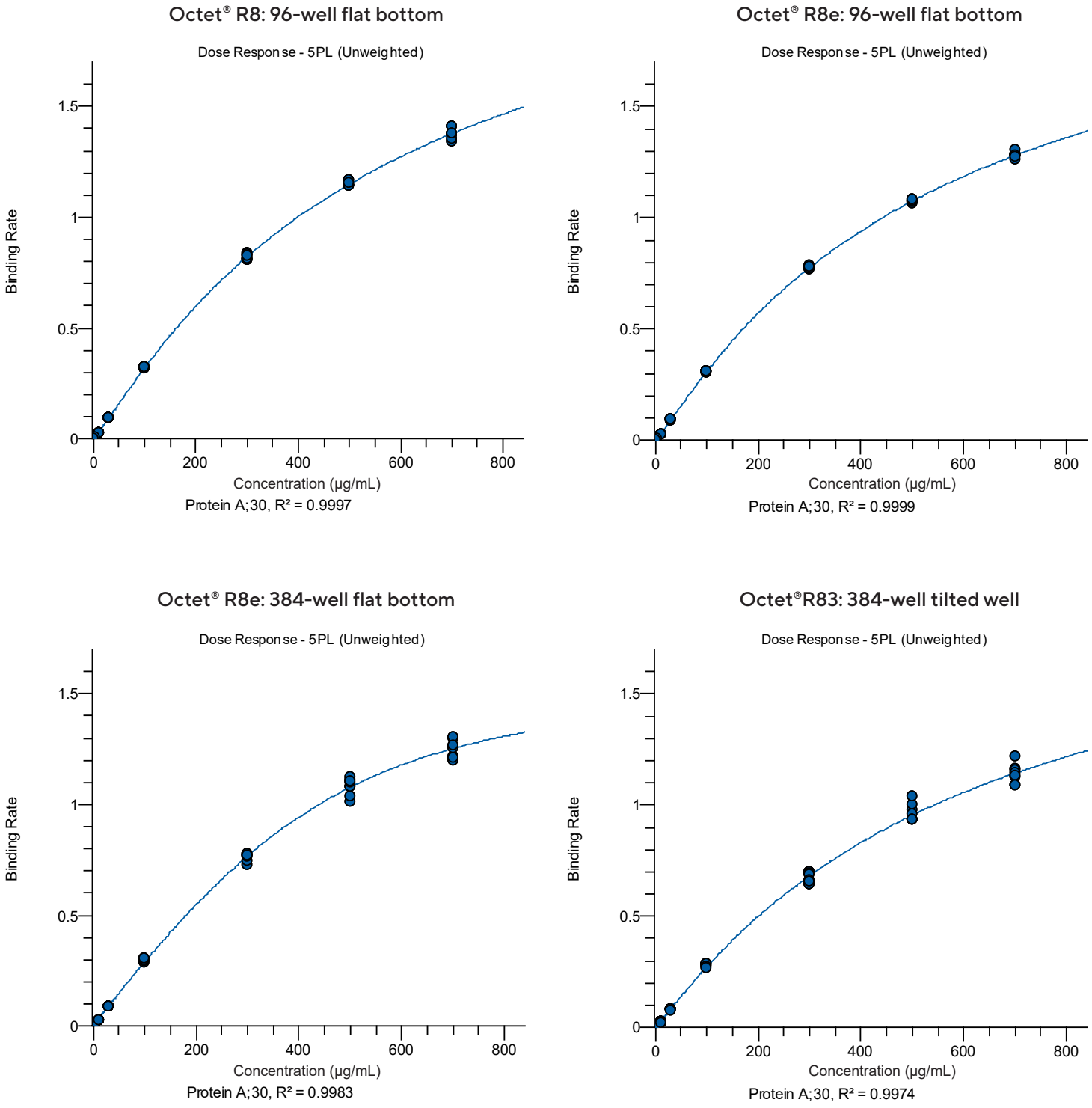

Figure 4B: Standard curves for quantitation of IgG using Protein A biosensors on the Octet® R8 and R8e systems

Note: Standard curves generated from the traces in Figure 4A by using the initial slope algorithm in the Octet® Analysis Studio 13.1 to generate binding rate. The 5PL (Unweighted) standard curve equation was used for all four combinations of instrument and plate type.

Expected concentration (µg/mL	Instrument	Plate type	1	3	10	30	100	300	500	700
Recovery (%)	Octet® R8	96-well flat bottom	100.0	106.1	95.2	101.0	99.9	100.0	100.0	100.1
	Octet® R8e	96-well flat bottom	99.8	108.2	96.9	100.8	99.9	100.1	99.9	100.1
	Octet® R8e	384-well flat bottom	100.0	92.1	80.6	94.6	102.6	99.2	101.0	100.4
	Octet® R8e	384-well tilted well	100.0	94.4	84.4	96.9	102.8	98.2	101.9	99.7
CV (%)	Octet® R8	96-well flat bottom	4.2	2.5	1.2	0.5	1.1	1.9	1.6	3.4
	Octet® R8e	96-well flat bottom	2.8	1.5	0.6	0.8	0.7	1.1	0.9	2.2
	Octet® R8e	384-well flat bottom	3.9	4.7	1.0	1.3	2.4	2.8	6.6	8.8
	Octet® R8e	384-well tilted well	7.0	6.1	5.7	3.5	2.3	5.0	7.4	7.9

Table 4. Recovery and CV for IgG binding to ProA biosensors as measured by Octet® R8 and Octet® R8e (n=8)

Conclusion

These Octet® instrument comparability studies show that while the Octet® R8e is designed with improved sensitivity over the Octet® R8 (an attribute that enables better characterization of low molecular weight or low abundance analytes)[7], larger molecular weight analytes, such as antibodies, yield comparable data on both instruments. This is reinforced by the high precision quantified for all three assays (CVs <10%) and across both instruments. These data should inform Octet® R8 users who want to upgrade to the Octet® R8e system that this can be done with the confidence that validated assays can be transferred onto the Octet® R8e, without the need for further, lengthy assay development. Both instruments generate highly reproducible data that can be used to support pharmaceutical or biological product regulatory applications.

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7. Unveiling Hidden Insights: Enhanced Analytics with New Heights of Sensitivity (Sartorius, 2025)

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