**NEW** Protein G Biosensor Available for the Octet Platform!

— Sriram Kumaraswamy, Product Manager, skumaraswamy@fortebio.com

Immunoglobulin-binding proteins such as Protein A, Protein G and Protein L are commonly used to purify, immobilize or detect immunoglobulins. They have different binding profiles with respect to species, antibody type, and the portion of the antibody that is bound. Protein A biosensors are currently used for human and other IgG quantitation on the Octet platform in a variety of applications including cell line development, clone selection, process optimization and production monitoring.

The new Protein G biosensor is useful for quantifying numerous species of IgG including murine, goat and bovine in the presence of other types of immunoglobulins or protein contaminants. It complements the use of Protein A biosensors due to its high binding affinity for rodent and many other mammalian species’ IgGs which cannot be detected with Protein A.

While native Protein G will bind to albumin in addition to immunoglobulins, the recombinant form of Protein G used in the biosensor has had the albumin- and cell-surface protein binding domains removed, to eliminate albumin binding interference. IgG can be quantified on the Protein G biosensor from buffer solutions, from cell culture media that has been appropriately diluted, or in

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**FIGURE 1.** Difference in binding for mouse IgG1 is shown between Protein G (left graph) and Protein A (right graph) biosensors on the Octet platform. Each assay was run at 1000 rpm shaking speed and 5 minute read time. The concentration of each IgG is shown in the chart legend in µg/mL.
Building an Enduring Life Science Tools Company

— Joe Keegan Ph.D., President and CEO

It is with great pleasure that I communicate some of our achievements during the first half of 2010. Driven by our initial product introduction of the Octet QK five years ago, followed by the higher-performing Octet RED in 2008 and the successful launch of the automation-friendly Octet 384 platform one year ago, ForteBio has achieved solid growth across all geographies and life science segments. We now have a global direct selling and product support team. Our extensive customer base, supported by 13 biosensor chemistries and assay kits means that we have an impact on drug discovery, development and bioprocess applications across the life sciences. We have expanded our initial key application in protein-protein kinetic characterization to a broad range of direct quantitation and real-time screening solutions including small molecule and fragment screening assays.

We have now added to our consumable offering the Dip and Read™ Protein G biosensor, enabling direct quantitation of IgG from mouse, rodent or rabbit cell line preps, strengthening our bioprocess and cell line development tool set. Earlier this year, we launched an enhanced version of our popular Octet QK instrument, the more sensitive and wider dynamic range Octet QK®. Octet Software Version 6.3 was launched simultaneously, enabling all five Octet instruments in our product line with additional fitting modes, analysis capabilities and automation tools. At ForteBio, we are committed to a product leadership strategy, aggressively investing in product development in order to deliver innovative, high quality new products — instruments, consumables, and software — that contribute to increasing performance and productivity in the laboratory.

Complementing the products we offer, the beginning of 2010 saw ForteBio launch a Custom Biosensor business. Supporting our customers’ desire to develop proprietary biosensor assays, ForteBio will develop and ship custom Dip and Read biosensors for off-the-shelf use in applications such as Host Cell Protein contamination detection or in custom proprietary assays.

The success of our new products along with high quality and responsive customer service has resulted in us being ranked among the leaders in label-free technology. ForteBio quality is evidenced by our ISO certification. Now 90% of our biosensor shipments will be ISO certified. In five years, we have become the label-free market leader as claimed by SDi, a leading Life Science market analysis firm. Many of our customers have been the catalysts for our growth through their published research and industry presentations. I want to thank them for their belief in us.

As CEO, it is my responsibility to build a company that can be counted on to serve our customers and challenge our employees. Five years ago, we set out to establish ourselves with life science customers and today we are thriving as a vibrant, independent business achieving double digit top line growth, positive earnings and cash flow. Our balance sheet is healthy. We have no debt.

Leading ForteBio the past three years has been exciting and I look forward to the future when we expect to achieve our goal to become the label-free market leader.

ForteBio Joins Forces with Tecan to Automate Label-free Assays for Bioprocess and Drug Discovery Applications

— Kevin Moore, Director of Market and Application Management for BioPharma, Tecan, kevin.moore@tecan.com

ForteBio has chosen Tecan as its preferred automation partner for real-time, label-free assays to support bioprocess and drug discovery workflows. The two companies have worked together to integrate the new ForteBio Octet® 384 platform, consisting of the Octet RED384 and the Octet QK384, on Tecan’s Freedom EVO® liquid handling workstations. The result is a robust and fully integrated off-the-shelf solution that enables Octet customers to automate sample handling and process batches of assay plates for Dip and Read™ biosensor assays, offering increased walkaway time and the option of automated liquid handling for assay plate setup.

This collaboration forms part of Tecan’s ongoing commitment to the bioprocessing market, working with leading technology providers to offer customers advanced automation solutions, and bringing the advantages of the Freedom EVO platforms to the fast growing area of label-free, real-time detection.

Label-free techniques avoid the generation of artifacts through label interference, and the Octet instruments’ proven track record of performance and reliability fitted well with Tecan’s goals, enabling high throughput measurements for protein-protein, protein-peptide, protein-small molecule and fragment screening assays. By creating an extension plate to mount the Octet 384 securely onto the side of any Freedom EVO platform, customers will be able to take advantage of ForteBio’s proprietary biolayer interferometry (BLI) technology in a format that best meets their exact needs, offering an unrivalled level of flexibility for walkaway, real-time analysis.

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Use of Octet QK to Enhance Service in a Core Hybridoma Laboratory
— Linda G. Green, Scientific Research Manager, Hybridoma Laboratory, University of Florida Interdisciplinary Center for Biotechnology Research (ICBR), lggreen@ufl.edu

The Hybridoma Laboratory is a part of the University of Florida Interdisciplinary Center for Biotechnology Research. The main service provided by this laboratory is development of new mouse monoclonal antibodies for investigators at the University of Florida. The laboratory performs ELISA and western blot screening of antibodies. In vitro production and purification services are also offered. The purchase of an Octet QK in December of 2008 has enabled several new services to be added such as antibody quantitation, affinity ranking and determination of antigen:antibody binding constants.

Antibody Quantitation Applications
Customers often request hybridoma supernatants for various applications such as ELISA, western blotting and immunohistochemistry. Prior to the acquisition of the Octet, there wasn’t an efficient way to determine the concentration of mouse antibody in a hybridoma supernatant containing serum. Currently, a two minute Octet quantitation experiment using anti-murine IgG Fv sensors is all that’s needed to generate this important information. Typically an isotype matched saved standard curve is used to analyze the data.

Quantitating the amount of mouse IgG present in supernatants from single colony wells from hybridoma cloning projects allows for selection of the highest secreting cell lines for final cell banking. When testing culture reagents such as serum supplements or other types of growth factors, quickly analyzing the antibody concentration is an important criterion. Prior to obtaining the Octet it was necessary to perform small scale affinity purifications of each sample to determine the concentration of antibody.

Most antibody production and purification performed in this core laboratory is ‘research’ scale. Customers typically request 5–20 milligrams of purified antibody. High density Cel-Line flasks are used for most production jobs. The antibody production media is supplemented with low IgG fetal bovine serum. Harvests are obtained from the cell / antibody chamber approximately once per week. The concentration of mouse antibody is determined periodically using the Octet throughout the run to determine how many harvests are needed to obtain the desired amount of purified antibody. The Cel-Line harvests are pooled prior to purification.

Protein G is the most frequently used affinity matrix in this laboratory. Octet quantitation of the pre- and post-purification samples provides critical information regarding the efficiency of the purification process. If a substantial amount of antibody remains in the post-purification sample, it may be run over the protein G column a second time in order to optimize the yield. Prior to obtaining the Octet system, a second protein G column run was performed automatically, but often the yield from the second run was negligible.

For the near future, since some of the hybridomas developed in this lab are of the IgM subclass, planning is underway to develop a mouse IgM quantitative assay on the Octet.

Kinetics Applications
Often a fusion experiment yields a panel of a dozen or more hybridoma cell lines that are secreting antibodies that score positive in various assays. Investigators may

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Why Does Academia Prefer Octet For Label-Free Protein Characterization?

With the affordable, easy-to-use Octet System, protein-protein interactions can be measured right on the benchtop in my own lab at anytime, obviating the need for a large instrumentation grant and core lab, or arranging and scheduling access.

Dr. Jay Groppe, Assoc. Professor
Department of Biomedical Sciences
Texas A&M Health Science Center

Out of the core lab, onto your benchtop
Measure concentration, kinetic and affinity constants and generate SPR-quality data right on your benchtop

Label-free kinetic characterization in a few minutes!
Get experiments completed in minutes rather than days using a Dip and Read™ assay— setup requires only pipetting reagents into a 96- or 384-well microplate

Direct quantitation assays for antibodies and other proteins
Replace HPLC and ELISA with Octet to measure antibody and other protein concentrations in crude samples accurately without secondary reagents

Visit www.fortebio.com or call 888.OCTET-QK today to learn more about the Octet platform.

Use of Octet QK to Enhance Service in a Core Hybridoma Laboratory

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only need one or two antibodies for their particular application. It may be difficult to decide which lines to clone based on antibody screening results that may be very similar for all the cell lines. Using the Octet to rank affinities by comparing off rates is a very valuable tool. Biotinylated antigen may be loaded onto a streptavidin biosensor or unlabeled antigen may be loaded onto an amine reactive sensor. The sensors can then be dipped into crude hybridoma culture supernatant. Comparing the k_dis from the panel of crude supernatants allows investigators to make a more informed choice about which antibody to pursue.

Figure 1 shows results from an affinity ranking experiment. The k_assoc is not determined since the analyte concentration is unknown. By comparing the k_dis, it is clear that SC11 and 1E3 have the lowest k_dis and therefore are likely higher affinity antibodies. These same hybridoma supernatants were tested on an indirect ELISA. SC11 gave the strongest signal. The other supernatants also gave strong readings. ELISA readings may be influenced by the antibody concentration in the supernatant: a higher antibody concentration may result in a stronger signal, which may or may not reflect the antibody affinity.

The Octet system is also used for the determination of KD (binding constants). Once a monoclonal antibody has been developed and purified antibody is available, a complete kinetics experiment can be performed on the Octet to determine the antibody: antigen affinity. The antigen can be labeled with biotin and loaded on streptavidin sensors. The sensors can then be dipped into several different concentrations of purified antibody. The reverse experiment is also possible: loading biotin labeled antibody on the SA sensor and dipping into several concentrations of purified antigen.

Determining the antibody/antigen affinity allows more complete characterization of antibodies. Figure 2 is an example of a kinetics experiment using biotinylated antigen loaded SA biosensors dipped in purified antibody. Global fitting of binding data for three concentrations of the antibody measured in duplicate resulted in a measured association rate constant of 2.1E5, dissociation rate constant of 7.65E-5 and affinity constant of 3.64E-10.

Octet Service Delivery

There are several modes that customers can choose from for Octet service in this core laboratory:

- **Self service:** customer pays an instrument fee based on elapsed time the instrument is used (this will help pay for the service contract) plus customer pays for the cost of expendables (sensors, sensor plate, sample plate, buffers, etc.).
- **Staff assisted:** customer pays for self service charges plus an additional fee for some staff assistance.
- **Complete service:** customer does not participate in the experiment. The complete service charge includes instrument time, expendables and staff time.

So far, several self service users have been trained to use the Octet. Most users are able to run their first kinetics experiment as a part of the two-hour training. Typically users are able to run repeat experiments on their own without further assistance. Binding constants are routinely obtained within one day, including the initial experiments which may be required to optimize conditions.

This facility also has a Biacore 3000 which requires more extensive training. The time required to develop optimum conditions is typically longer than with the Octet. Since the Octet biosensors are so inexpensive, most users choose not to regenerate, which saves a lot of time. Scouting for regeneration conditions on the Biacore is very time consuming.
Synergism Between Two High Throughput Platforms Efficiently Predicts Productivity

— Rachel Legmann, Principal Scientist, SimCell Applications, Seahorse Bioscience, rlegmann@seahorsebio.com

Small differences in manufacturing processes can affect the efficacy and safety of a therapeutic product. In efforts to rapidly bring high quality drugs to market, statistically designed experiments are a powerful strategy for effective cell culture clone screening and process development. Only through the use of miniaturized high throughput technologies can one realize the full benefits of multi-factorial experimentation for process development.

The SimCell is one such system that fulfills this need by providing a high throughput controlled culture environment equivalent to the bench scale reactors used today. It performs fed-batch protocols with pH, dissolved oxygen (DO), and glucose control across hundreds of micro-bioreactors. Cell density, metabolites and titer profiles permit rapid and more accurate analysis using multiple metrics. Such content results in more predictive experiments earlier in the development process. Very high throughput allows wider design spaces to be covered with greater statistical depth, providing better optimization and process robustness.

Unfortunately, many analytical techniques are not designed to meet the throughput requirements of the SimCell output as they are challenged by small harvest samples volumes and lack of a rapid, reliable and easy to use analysis. These challenges were addressed in this case study with the use of the ForteBio Octet QK platform to quantify monoclonal antibody during the production phase and at the end of the experiment. The results demonstrate that early selection of productivity levels is key in developing a robust process.

A mixed level, full factorial design was implemented in this DOE study to optimize and characterize production of a monoclonal antibody by a CHO cell line. DO and pH were varied at two levels, while feed rate and feed schedule strategy were varied across three levels. The impacts of these variations on cell growth and product yield were examined and compared. Each of the 36 unique experimental conditions were run with six replicates to yield a total of 216 micro-bioreactors. The temperature was set to 36.5°C for the duration of a 13-day experiment. DO and pH were set according to the DOE. Glucose was controlled to 2 g/L by the addition of glucose feed, if necessary, based on the off-line measurements starting on day 3 of culture. Feed rate and time of addition were performed according to the DOE. Samples were used to measure total cell density, viability and product titer on day 7, day 10 and at harvest on day 13.

After the completion of the factorial DOE in the micro-bioreactors (SimCell), a subset of 2 conditions were duplicated in conventional bench-scale bioreactors using 3L glass vessels (Applikon) operated at a 1L working volume. Two replicates for each condition were performed. MAb concentrations in crude samples from bench top bioreactors and micro-bioreactors were quantified using a plate-based, high throughput assay on an Octet QK instrument (ForteBio). Twenty microliters of a 1:10 diluted culture was transferred to a 96-well plate and further diluted, 1:10, with CD-CHO media. The plate was then transferred to the Octet instrument where MAb concentrations for each sample were measured. The total analysis time for a single 96-well plate was approximately 30 minutes.

The overall objective of this study was to examine the potential synergies between two high throughput platforms, SimCell and Octet QK, to predict titer outcome and compare the data to standard predictions made by bench-scale bioreactors.

In this study a DOE with 216 micro-bioreactor experiments was conducted in the span of less than a month. The response of an IgG...
Octet in the News at Recent Conferences
— Sriram Kumaraswamy, Product Manager, skumaraswamy@fortebio.com

With a rapidly growing customer base, Octet systems and related products are finding ever greater use in academic and pharmaceutical research. The growth in Octet use is reflected in the flurry of recent publications, patent applications and conference presentations around the world. ForteBio is also actively engaged in meeting researchers involved in drug discovery and protein research at various conference venues and promoting discussion of the utility of the Octet platform in their daily work.

At Screening Europe, held at Barcelona in February, Genmab’s Arnout Gerritsen presented a poster discussing the success they have achieved in increasing the throughput of label-free assays and in accommodating more projects for antibody discovery by integrating the Octet RED384 system with the Tecan Freedom EVO robotic workstation and IDBS’s ActivityBase data management suite. On a related note, read Kevin Moore’s description of the partnership between ForteBio and Tecan on page 2 of this issue. More information on automation of the Octet platform is available at www.fortebio.com/automation.html.

At the SBS conference, held in Phoenix in April, ForteBio conducted a workshop on the use of the Octet RED384 system for small molecule and fragment screening. The workshop was presented by John Wang, an expert researcher in small molecule and protein drug discovery and until recently, an Octet user at Novartis. He talked about the higher throughput, greater ease-of-use and faster time-to-results achieved on the Octet RED384 for screening small molecule and fragment compound libraries to identify lead candidates. Roche Discovery Technologies presented a poster at the conference reporting their use of the Octet RED384 system, integrated with a Hudson PlateCrane workstation and a Multidrop liquid dispensing station, in a fragment screening campaign with a kinase that contains a known allosteric binding site. Please download a copy of the poster from our website. I presented a poster on the screening of a 500 compound Maybridge library against Carboxic Anhydrase II (work performed by Charles Watchow et al. of ForteBio).

ForteBio conducted a successful workshop at AAPS National Biotechnology conference, held in San Francisco in May. The workshop was moderated by Laura Lerner, Director, Protein Science at Macrogenics and the seminars consisted of a presentation on detection of low affinity anti-drug antibodies and improved drug tolerance in immunogenicity testing by Jian Li of Centocor, ligand binding assays on the Octet platform for bioprocess contaminants by Sae Choo of ForteBio, study of inhibitors of Wnt/LRP6 signaling complex by Eric Bourhis of Genentech and biopharmaceutical production using Pfenex expression technology and high throughput assays on the Octet system by Jeff Allen of Pfenex.

At the Protein Engineering Summit, held in San Diego in May, Yasmina Abdiche discussed the use of the Octet QK384 system at Rinat/Pfizer for affinity screening of crude antibodies, epitope binning and cross-blocking studies. Robin Barbour of Neotope compared the Octet system to other label-free methods.

Readers can download many of the above presentations and much more from Octet customers who presented at various other conferences on ForteBio’s website at www.fortebio.com/literature.html.

ForteBio Joins Forces with Tecan
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A robotic manipulator arm automates handling and transport of plates and sensor trays, and an 8-channel liquid handling arm with either fixed or disposable tips is used to prepare assay plates. A MultiChannel Arm™ (MCA) with a 384- or 96-channel pipetting head can also be specified to further increase throughput and efficiency. The Octet platform also complements a range of other technologies within Tecan's portfolio of bioprocessing solutions — covering everything from construct generation to protein purification — providing customers with a reliable method for product quantitation and quality assurance at almost every step.

This close collaborative partnership will ensure that customers receive the best solution available, combining the advanced liquid handling and scheduling capabilities of the Freedom EVO workstation with the innovative Dip and Read biosensors of the Octet 384 platform. This integrated set-up offers a straightforward route to reliable and reproducible performance of label-free assays, with the option to automate as much of the workflow as the customer desires.

To learn more about automation on the Octet platform, visit www.fortebio.com/automation; learn more about Tecan’s bioprocessing solutions at www.tecan.com/drugdiscovery.
producing CHO fed-batch process to 4 factors with respect to large scale production was extensively characterized. Statistical analysis of the results identified interactions and effects of four factors on product yield as determined by ForteBio for this defined process space. Figure 2 shows the overall performance of the 36 unique experimental conditions in the factorial design and two conditions in bench top bioreactors using titer as the metric. Manipulation of process parameters is shown to have very significant impact on titer. It is clear that the titer results between the two systems have strong quantitative correlation between the predictions of the micro-bioreactor and the results of the bench-scale bioreactor models. A previous study showed good correlation ($R^2$ value of 0.97 with a slope of 1.02) between SimCell micro-bioreactors and bench-scale bioreactors for terminal product titer as measured by the Octet (Legmann et al, 2009).

The results demonstrate that combination of these two high throughput platforms acts synergistically as an effective and efficient tool to predict productivity. This combined platform permits a single experimenter to increase the scope of experimentation by a factor of twenty or more. These larger experiments allow the potential design space to be penetrated at greater statistical depth in less time.


the presence of media containing biotin (for example, RPMI).

**Quantitation Assays**

The Protein G biosensor is specifically designed for use in quantitation assays on the Octet platform. Immunoglobulin binding to the biosensor is monitored in real time using an Octet system. The real-time binding information is used to quantify the amount of IgG by comparing the binding rate of the unknown sample to a standard curve of the binding rates of known concentrations of the same species and subtype of immunoglobulin. Protein G’s binding kinetics vary greatly depending on both the species and the subtype of IgG, therefore it is critical that the IgG used as a calibration standard be the same species and subtype as the antibodies that are being quantitated.

**Assay Range**

IgG can be detected on the Protein G biosensor from 0.05 µg/mL to 2000 µg/mL in Sample Diluent. Typically, assays run at 200 rpm shaking speed and a 2-minute read time provide a dynamic range of 0.5 µg/mL to 2000 µg/mL. By extending the read time to 5 minutes and increasing the shaking speed to 1000 rpm, the detection range is lowered to 0.05 µg/mL to 300 µg/mL. As an added benefit, a low-pH buffer such as 10 mM Glycine, pH 1.7 to 2.0, can be used to regenerate the Protein G biosensor.

For more information on the Protein G biosensor, visit www.fortebio.com.
**Introduction**

Quantitation assays on the Octet platform have many similarities to enzyme-linked immunosorbent assays (ELISA). Both are performed on a solid support on which the capture molecule is immobilized and the analyte is bound from solution. Signal reported in the assay is either directly or inversely proportional to the amount of bound analyte. In fact, Octet quantitation assays can be considered automated forms of ELISA. The conversion to an Octet assay often involves simply re-optimizing and/or validating the conditions and configurations of the already existing ELISA assay. However, in some cases where the minimum essential requirements are stringent, more development work is required.

It is often beneficial to convert ELISA assays to the Octet platform as it allows the scientist to:

1. Choose from a number of assay formats (label-free direct binding, sandwich, sandwich followed by signal amplification etc.) to suit detection limit requirements
2. Detect low-affinity analytes often missed by ELISA
3. Minimize handling via automated and wash-free steps
4. Fully recover and re-use samples and reagents
5. Regenerate the assay surface and re-use for some binding pairs (e.g., Protein A/ human IgG).

This article provides guidance on converting an existing ELISA-based assay to an Octet assay. The process can be broken down into five steps:

1. Defining assay requirements
2. Selecting biosensor type
3. Selecting assay format
4. Minimize non-specific binding (NSB)
5. Optimizing assay buffer

Details on each of these steps are presented in the following sections.

**Defining Assay Requirements**

Conversion activity can start with defining the minimum assay requirements. Sensitivity and throughput requirements often become critical factors in determining the Octet instrument and sensor type and assay format, while the sample matrix has the biggest impact on reagent formulation and the blocking protocol. Sample volume requirement also influences the choice of instrument (Octet QK, QK² and RED require 80–200 µL sample volume while Octet QK384 and RED384 require 40–80 µL). Sample volume also indirectly influences the sensitivity of detection when dilution is required to compensate for limited availability of sample. Physical characteristics (pI, size, polymeric status, hydrophobicity, stability etc.) of the analyte should be considered when selecting the surface and the immobilization protocol.

**Selecting Biosensor Type**

There are four types of biosensors to be considered in building quantitation assays on the Octet platform. They are Streptavidin (SA), Aminopropylsilane (APS), Amine-reactive (AR) biosensors and analyte-specific biosensors. SA, APS, and AR biosensors can be used to build custom quantitation assays for analytes that are process-specific.

SA biosensors are most often preferred for its flexibility to accommodate a variety of capture molecules (ligand) through Streptavidin/Biotin interaction and its superior surface capacity derived from the use of specially designed cross-linked streptavidin conjugates. There are several advantages of immobilizing the ligand that is labeled with a long-chain biotin at a low molar coupling ratio (biotin: ligand):

1. Loss of binding capacity due to cross-linking and steric hindrance are reduced.

2. Biosensors can be prepared in batch mode and stored for later use.
3. The biotin-ligand solution may be used to prepare multiple batches of biosensors.
4. The strong, nearly irreversible binding between the biotinylated ligand and the streptavidin biosensor allows easy regeneration of the ligand-loaded biosensor.

APS biosensors are ideal for immobilizing ligands that are not suitable for covalent linking chemistry. The mode of immobilization is a combination of hydrophobic and/or electrostatic interactions. The APS biosensor surface is somewhat more hydrophobic in comparison to the surface of ELISA microtiter plates, so the stability of the ligand on the biosensor surface should be tested prior to loading to avoid denaturation.

AR biosensors can be used to covalently immobilize amine group containing ligands onto carboxylate groups on the biosensors. Batch mode preparation and regeneration of the biosensor are possible.

Analyte-specific biosensors are pre-immobilized with specific capture proteins such as anti-human IgG Fc, anti-murine IgG (Fab′)2, Protein A, Protein G, and anti-Penta HIS. These ready-to-use biosensors are suited for quantitative analysis of human IgG, mouse IgG and rat IgG, all proteins that bind Protein A, those that bind Protein G, and penta-HIS-tagged proteins, respectively.

**Selecting Assay Format**

The choice of an assay format is dependent on the concentration range of analyte to be quantified. Octet systems offer the advantage of a direct binding assay (also called 1-step), that generally affords a dynamic range of detection from low ng/mL up to low mg/mL, depending on the analyte. The direct binding assay is fast, easy and eliminates the need for secondary reagents and steps. It also allows regeneration of the biosensor in some cases.
Multi-step assays provide enhanced sensitivity down to low pg/mL, depending on the analyte. Octet systems measure signal as a function of the thickness and density of the analyte binding layer, so increasing analyte binding translates to bigger signals. When analyte is present in low concentrations and the binding signal is low even after a long incubation, building additional layers of secondary reagents over the analyte binding layer enhances signal.

Minimize Non-specific Binding (NSB)
Managing NSB and matrix effects are critical parts of the assay development process that ensure acceptable specificity and sensitivity. The major sources of NSB are hydrophobic, electrostatic, and cross-reactive interactions between the molecules on the biosensor surface and in the solution. In addition to NSB, unrelated proteins and other components of the sample matrix often cause assay interference.

The most effective way to minimize such effects is passivation of the biosensor surface by including blocking step(s) prior to sample incubation. Often, blocking buffer formulation needs to be matched to the sample matrix (e.g. block with the serum for immunogenicity sample in serum).

Optimizing Assay Buffer
Assay buffer formulation has a significant effect on NSB, sample matrix interferences and signal over background. The goal of optimizing reagent formulation is to maximize specific signal and minimize NSB. A direct adaptation of previously developed ELISA formulation may be sufficient in most cases, unless the formulation contains components or conditions that are not compatible with Octet biosensors. Examples of such incompatible reagents include those with low pH (< 4), high pH (>10), and certain types of organic solvents. The characteristics of the analyte (pI, hydrophobicity and stability) and sample matrix components are important to keep in mind when developing an assay buffer.

On a related note, the rinsing step employed in Octet assays is often performed in physiological buffers such as TBS and PBS in the presence of a detergent.

The following is a list of common assay buffer components that are useful to keep in mind during optimization.

1. **Salt:** high salt concentrations slow down the reaction, reduce stickiness of antigen with very high/low pI values, reduce charge-induced NSB and denature proteins in solution when needed (e.g., Protein A contamination assay sample pre-treatment buffer).
Recent References To ForteBio’s Products In Published Literature

Hundreds of researchers throughout the world are using Octet systems in their work every day. The type of work done on the Octet systems spans the entire drug discovery and development pipeline from early discovery to late stage production and QC, as well as in a variety of academic research areas. Some of this work is published by our customers in journals; a substantial portion of work is collected in patent applications and not available for peer review. The following is a collection of peer-reviewed literature published in recent months. We hope that reading these articles inspires you in your work!

Structural Correlates of Antibodies Associated with Acute Reversal of Amyloid Beta-Related Behavioral Deficits in a Mouse Model of Alzheimer Disease.
Basi GS and others.

Neutralizing and Non-neutralizing Monoclonal Antibodies against Dengue Virus E Protein Derived from a Naturally Infected Patient.
Schieffelin JS, Costin JM, Nicholson CO, Orgeron NM, Fontaine KA, Isern S, Michael SF, Robinson JE.
Virology Journal, 2010, 7, 28–38

Reconstitution of a Frizzled8-Wnt3a-LRP6 Signaling Complex Reveals Multiple Wnt and Dkk1 Binding Sites on LRP6.
Bourhis E, Tam C, Franke Y, Bazan JF, Ernst J, Hwang J, Costa M, Cochran AG, Hannoush RN.

Molecular Mechanism of the Syanptotagmin-SNARE Interaction in Ca²⁺-Triggered Vesicle Fusion.
Vrljic M, Strop P, Ernst JA, Sutton RB, Chu S, Brunger AT.

Recombinant Respiratory Syncytial Virus F Protein Expression is Hindered by Inefficient Nuclear Export and mRNA Processing.
Huang K, Lawlor H, Tang R, MacGill RS, Ulbrandt ND, Wu H.
Virus Genes, 2010, 40 (2), 212–221.

Human Framework Adaptation of a Mouse Anti-Human IL-13 Antibody.
Fransson J, and others.

Grading the Commercial Optical Biosensor Literature — Class of 2008: The Mighty Binders.
Rich RL, Myszka DG.

Binding Rate Screen — A High-Throughput Assay in Soluble Lysate for Prioritizing Protein Expression Constructs.

Conclusions

ELISA-based assays and Octet quantitation assays share many similarities. Therefore, conversion of a pre-configured ELISA assay to the Octet platform may only require transfer of assay conditions. When assay conditions need re-optimization on the Octet platform, considerations are often similar to those employed in ELISA.

The Octet platform’s direct binding assay method is simple, fast and accurate; the multi-step methods offer high sensitivity and expanded dynamic range. The automated assay formats enhance time-to-results and walk-away time and reduce operating expenses.

buffer capacity: higher buffer capacity minimizes pH changes and stabilize sample solutions.

non-specific antibodies: reduce NSB from multi-species cross-reactions.

detergents: reduce sample aggregation, reduce hydrophobic NSB and reduce sample coating out of very low concentration samples.

bulk proteins (BSA, casein, antibodies): serve as blocking agents, reduce sample coating out of solution, and stabilize analyte proteins in solution in other ways.

sugars (trehalose, dextran, sucrose): stabilize some proteins, enhance signal by increasing effective concentration in solution.

PEG: helps reduce non-specific binding and can enhance signal in some cases by increasing the effective concentration of proteins in solution.

pH: low pH may often reduce the affinity of competing interactions and improves the proportion of ligand-analyte binding to the biosensor.

dilution: to minimize matrix effects.
Octet Technology Platform

Systems and Features

Analyte MW: > 150 D
LOD: 25 ng/mL for hlgG on Protein A biosensors in 5 min assay

Analyte MW: > 10 kD on Octet QK

Analyte MW: > 5 kD on Octet QK®, QK384
LOD: 100 ng/mL for hlgG on Protein A biosensors in 5 min assay

Octet RED384

Octet QK®

Octet QK

• 16-channel
• 96-, 384-well
• ≥ 40 µL sample volume
• 2 plate positions
• Biosensor re-racking
• Automation-friendly

• 8-channel
• 96-well
• 1 plate position
• Re-racking on Octet QK®

Accessories

SIDEKICK OFFLINE IMMOBILIZATION STATION

TILTED-BOTTOM 384-WELL MICROPLATE (384TW)

Biosensors

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<th>Biosensor Type</th>
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<tbody>
<tr>
<td>Anti-hlgG-fc</td>
<td>None</td>
<td>hlgG, human fc-fused proteins</td>
<td>Q</td>
</tr>
<tr>
<td>Anti-mlgG-fv</td>
<td>None</td>
<td>mlG, rat IgG, mouse-fab, rat fab</td>
<td>Q</td>
</tr>
<tr>
<td>Protein A</td>
<td>None</td>
<td>Many human and other IgG types</td>
<td>Q</td>
</tr>
<tr>
<td><strong>NEW!</strong> Protein G</td>
<td>None</td>
<td>Many murine and other IgG types</td>
<td>Q</td>
</tr>
<tr>
<td>Anti-Penta HIS</td>
<td>None</td>
<td>His-tagged proteins, peptides</td>
<td>Q</td>
</tr>
<tr>
<td>Streptavidin</td>
<td>Biotin-tagged peptides, proteins</td>
<td>Native proteins, peptides</td>
<td>Q, S, K</td>
</tr>
<tr>
<td>Super Streptavidin</td>
<td>Biotin-tagged peptides, proteins</td>
<td>Native proteins, peptides</td>
<td>S, K</td>
</tr>
<tr>
<td>Amine Reactive</td>
<td>Native proteins, peptides</td>
<td>Native proteins (MW &gt; 30 kD)</td>
<td>S, K</td>
</tr>
<tr>
<td>Anti-hlgG-fc Capture</td>
<td>hlgG, human-fc fusion protein</td>
<td>Native proteins, peptides</td>
<td>S, K</td>
</tr>
<tr>
<td>Aminopropylsilane</td>
<td>Native proteins, peptides</td>
<td>Native proteins, peptides</td>
<td>S, K</td>
</tr>
</tbody>
</table>

Q - Quantitation, S - Screening for Yes/No, rank ordering, K - Kinetics

Assay Kits

<table>
<thead>
<tr>
<th>Assay</th>
<th>Item</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunogenicity</td>
<td>Kit</td>
<td>Bridging and Direct assay formats to detect high- and low-affinity anti-drug antibodies</td>
</tr>
<tr>
<td>Residual Protein A Detection</td>
<td>Kit</td>
<td>Protein A and biosimilars such as MabSelect SuRe</td>
</tr>
<tr>
<td>Host Cell Protein</td>
<td>Custom</td>
<td>Generic and process-specific assays</td>
</tr>
</tbody>
</table>
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