FortéBio Bio-layer Interferometry Quantitation Tutorial

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Outline

- Introduction to Biolayer Interferometry (s. 3-9)
- Quantitation Basics (s. 10-17)
- Basic Quantitation Acquisition (s. 18-29)
- Advanced Quantitation (s. 30-50)
- Data Analysis HT (s. 51-66)
- Conclusions (s. 67-68)



• ForteBio – A Leader in Biomolecular Analysis



Full life-cycle offering of instruments

- Label-free assays based on Bio-Layer Interferometry (BLI) and
 - Surface Plasmon Resonance (SPR) platforms









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Dip and Read Biosensors

The Octet Dip and Read[™] Biosensor consists of a fiber optic embedded into a polypropylene hub with a sensor-specific chemistry at the tip



- Two-dimensional binding surface
- Biocompatible Matrix (minimizes non-specific binding)
- Uniform
- Non-denaturing





Bio-Layer Interferometry

- In BLI, light is directed down an optical fiber (the sensor) toward two interfaces separated by a thin layer at the end of the fiber
- The two reflected beams interfere constructively or destructively at the spectrometer CCD detector array



Reflections R & T are in phase Constructive interference <u>Strong</u> signal at the spectrometer



Reflections R & T are out of phase Destructive interference <u>Weak</u> signal at the spectrometer





Monitoring nm-shift Against Time



Time (sec)



Octet Versatility in Interaction Analysis



Current Octet assays within blue area













	Octet RED96e	Octet K2	Octet QKe	Octet RED384	Octet HTX				
Molecular Weight Range	> 150 Da	> 150 Da	> 5000 Da	> 150 Da	> 150 Da				
# Spectrometers	8	2	1	16	16				
# Channels per Read	8	2	8	16	1 - 96				
Microplate Positions	1	1	1	2	2				
Biosensor Reracking	Yes	Yes	Yes	Yes	Yes				
Robot Compatible	No	No	No	Yes	Yes				
Sample Vessel Formats	96	96 96, 96 HA		96 / 96HA 384 / 384TW	96 / 96HA 384 / 384TW				
Minimum Sample Volume	180 μL per well	180 μL per well	180 μL per well	40 μL per well	40 μL per well				
Affinity range	1 mM to	1 mM to	0.1 mM to	1 mM to	1 mM to				
(approximate)	10 pM	10 pM	10 pM	10 pM	10 pM				
Sample Usage		Non-dest	ructive and reco	verable					
Temperature Control	15 – 40 ° C		4°C above an	nbient to 40°C					
Analysis time per sample	Up to 12 hrs with evaporation cover	Up to 4 hrs							
21 CFR Part 11 Compliance	Available as option for all systems								





Quantitation Basics



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Quantitation Biosensors

Biosensor	Application
Antibody-Specific Capture	
Anti-Human IgG Fc Capture (AHC)	Human IgG Fc region, kinetic analysis
Anti-Human IgG Fc Capture (AHQ)	Human IgG Fc region, quantitation
Anti-Mouse Fc Capture (AMC)	Mouse IgG1, 2a & 2b Fc regions, kinetic analysis
 Anti-Mouse Fc Capture (AMQ) 	Mouse IgG1, 2a & 2b Fc regions, quantitation
 Anti-Human Fab-CHI (FAB2) 	Fab-CH1 domains of human IgG
 Protein A (ProA) 	Quantitation of various species IgG
 Protein G (ProG) 	Quantitation of various species IgG
Protein L (ProL)	Quantitation of IgG via kappa light chain
Affinity Tag Capture	
Streptavidin (SA)	Biotinylated ligands
High Precision Streptavidin (SAX)	Biotinylated ligands (4% CV loaded SA)
 Super Streptavidin (SSA) 	Biotinylated ligands (high-density surface)
• Anti-GST (GST)	GST-tagged recombinant proteins
 Anti-Penta HIS (HIS1K) 	HIS-tagged recombinant proteins
 Anti-Penta HIS 2nd Gen (HIS2) 	HIS-tagged recombinant proteins
• Ni-NTA (NTA)	HIS-tagged recombinant proteins
Immehilization	
• Amine Reactive 2nd Gen (AR2G)	Covalent coupling to reactive amine groups
	Adsorption to hydrophobic mojetios
	Ausorption to Hydrophobic moleties

• Quantitation biosensors listed in black





Differences Between Kinetic vs Quant Biosensors

Kinetics Biosensors

- Minimal Baseline Drift
- Higher Coefficient of Variation (CV)

Quant Biosensors

- Precise CV must be within a certain range
- Not checked for baseline drift Short assays, high signal

Different Manufacturing and QC Criteria









A new standard curve is created for each target molecule and may be utilized to analyze the same target over time under identical conditions





When do I need a new standard curve?

- For each new condition, i.e. temp, buffers, pH, shake speed, etc.
- For each new target molecule
- With a new lot of biosensors



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Octet Workflow for Quantitation



- Quantitation in as little as 2 mins ٠
- 1 step, no washing ٠

Concentration



Octet Workflow for Quant with Regeneration



- Binding rates of test samples are measured and interpolated from the standard curve to determine concentration
- Ability to reuse standard curve when sample is run under comparable conditions





Considerations for Biosensor Regeneration





Basic Quantitation Workflow with Data Acquisition V11



Double click the Data Acquisition 11.0 software icon to start the program





Select Assay Type





Acquisition Steps for Basic Quantitation

Work from left to right, from Tab 1 to Tab 4.



- 1. Plate Definition In this tab, all the information about the sample plate and its wells will be entered
- 2. Sensor Assignment In this tab, sensors are assigned to samples
- 3. Review Experiment In this tab, you can review the steps that make up the experiment
- 4. Run Experiment In this tab, you can select where you would like your data saved and name the data file. Run settings may also be changed



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Tab 1. Plate Definition – Assay Settings





Tab 1. Plate Definition (96-well Plate)

- 1. Click on column number or specific wells to highlight wells of interest
- 2. Right click on highlighted wells or select from options below plate to select well type





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Tab 1. Plate Definition (384-well Plate)

- 1. Hold down the Shift key on the keyboard and click to the upper left most well of choice to highlight the number of "associated" wells
- 2. Right click on highlighted wells or select from options below plate to select well type





Tab 1. Plate Definition (96 well Plate)

3. Make sure that units are correct for the assay

V

Plate 1 Table (96 wells)

2. Add concentration

1. Label Sample ID →

4. By using a unique identifier, replicate groups can be assigned fo each sample where mean, standard deviation, and % CV (coefficient of variation) data is required

	Concent	ration units:	µg/ml	\sim	Export	Import		Print	K		
	Well	Sample ID	7	Replicat	e Group	Туре	Conc	: (µg/ml)	Dilution	Factor	Information
•	🔵 A1	hlgG in Sample	Diluent	0		Reference	n/a		n/a		
	🔵 B1	hlgG in Sample	Diluent	3		Standard	3		n/a		
	O C1	hlgG in Sample	Diluent	10		Standard	10		n/a		
	O D1	nlgG in Sample	Diluent	30		Standard	30		n/a		
	E 1	hlgG in Sample	Diluent	100		Standard	100		n/a		
/	🔵 F1	hlgG in Sample	Diluent	300		Standard	300		n/a		
	🔵 G1	hlgG in Sample	Diluent	500		Standard	500		n/a		
	O H1	hlgG in Sample	Diluent	700		Standard	700		n/a		
	A2	hlgG in Sample	Diluent	700		Standard	700		n/a		
	O B2	hlgG in Sample	Diluent	500		Standard	500		n/a		
	O C2	hlgG in Sample	Diluent	300		Standard	300		n/a		
	O D2	hlgG in Sample	Diluent	100		Standard	100		n/a		
•	O E2	hlgG in Sample	Diluent	30		Standard	30		n/a		
	O F2	hlgG in Sample	Diluent	10		Standard	10		n/a		
	🔵 G2	hlgG in Sample	Diluent	3		Standard	3		n/a		
	H2	hlgG in Sample	Diluent	0		Reference	n/a		n/a		
	A3	hlgG in Sample	Diluent	3a		Unknown	n/a				3
	B 3	hlgG in Sample	Diluent	3a		Unknown	n/a				3
	O C3	hlgG in Sample	Diluent	3a		Unknown	n/a				3
	O D3	hlgG in Sample	Diluent	3a		Unknown	n/a				3
	🔵 E3	hlgG in Sample	Diluent	10a		Unknown	n/a				10
	F 3	hlgG in Sample	Diluent	10a		Unknown	n/a				10
	🔵 G3	hlgG in Sample	Diluent	10a		Unknown	n/a				10
	O H3	hlgG in Sample	Diluent	10a		Unknown	n/a				10
	O A4	hlgG in Sample	Diluent	100a		Unknown	n/a				100
	O B4	hlgG in Sample	Diluent	100a		Unknown	n/a				100
	O C4	hlgG in Sample	Diluent	100a		Unknown	n/a				100
	O D4	hlgG in Sample	Diluent	100a		Unknown	n/a				100
	🔵 E4	hlgG in Sample	Diluent	500a		Unknown	n/a				500
	F 4	hlgG in Sample	Diluent	500a		Unknown	n/a				500
	🔵 G4	hlgG in Sample	Diluent	500a		Unknown	n/a				500
	O H4	hlgG in Sample	Diluent	500a		Unknown	n/a				500

5. Notes in this column will be carried over to data analysis



Tab 1. Plate Definition

1. To set up a dilution series, highlight the concentrations of interest, right click, and select Set Well Data^{*}

	Set Well Data		×
Impose Prote Type Cone: (ug/w) Dilation Factor Information Reference n/a N/a Sandard 10 n/a Standard 10 n/a Sandard 10 n/a Standard 10 n/a Sandard 100 n/a Standard 00 n/a Sandard Sandard 100 n/a Standard 300 n/a Sandard Sandard	Well Information Sample ID: hIgG in Sample Diluent Replicate Group: Well Information:	Dilution Series Starting value (µg/ml): Series operator: Series operand: Dilution orientation Series operand: Dilution orientation	2. Check the box by dilution series
Standard 300 nra Standard 100 ••••••• Standard 300 •••••• Standard 100 ••••• Standard 100 ••••• Standard 100 ••••• Standard 100 •••••• Standard 100 ••••••• Standard 100 •••••••••• Standard 100 ••••••••••••••••••••••••••••••••••••	Concentration (µg/ml): Standards only	OK	Cancel
Urknown ry'e Der wiel Data Stream urknown ry'e Clear Date		3. Select a sta	rting value.

3. Select a starting value, series operator, series operand, and the dilution orientation, and click OK

*The concentration range displayed here was not set up using a dilution series

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Tab 2. Sensor Assignment

Protein A

Protein A

Protein A

Protein A

Protein A

Protein /

Protein A

180716

807161

807161

180716

180716

1807161

1807161

1807161

1807161

180716

1807161

1807161

1807161

1807161

1807161

180716

180716

1807161

1. Check box to have biosensors replaced back in the tray instead of being ejected to the waste container

2. Initially, one column of biosensors is assigned to each column on the plate. This is color coded, as displayed in the figure

If you have a partial sensor tray it can be accomodated by selecting the missing sensors and clicking 'Remove Only the first sensor tray can be a partial plate Sensor Tray Replace sensors in tray after use Well Sensor Type Lot Number Information A1 B1 В C1 С D1 D E1 F1 H1 G A2 н B2 C2 \otimes Missing sensors Legend D2 Print. E2 Fill Plate Remove F2 Plate 1 (96 wells G2 10 11 12 H2 A 000000 A3 **B**3 EOOOOOO F000000 GOOOOOO HOOOOOO Unassigned samples Leaend: 4. Select the sensor type and enter the lot number for your biosensor. The lot number will auto populate G6

🕦 Plate Definition 🛛 🥝 Sensor Assignment 👩 Review Experiment 🔞 Run Experiment

In this step, sensors are assigned to samples.

1807161 Protein A D3 Protein A 180716 F3 180716 Protein A F3 Protein A 180716 G3 Protein A 180716 H3 1807161 Protein A A4 180716 Protein A B4 180716 Protein A C4 Protein A 1807161 1807161 D4 Protein A F4 Protein A 180716 F4 Protein A 180716 Protein A 1807161 H4 Protein A 1807161 A5 Protein A 180716 **B5** 180716 Protein A C5 Protein A 1807161 D5 Protein A 1807161 1807161 E5 Protein A F5 Protein A 180716 G5 Protein A 180716 H5 Protein A 1807161 A6 1807161 Protein A **B6** Protein A 1807161 C6 Protein A 1807161 D6 1807161 Protein A F6 180716 Protein A F6 180716 Protein A

Protein A

Protein A

H6

180716

1807161

re ir	eus eus nore) se ea	rege bio ase	ene ser reg	rate nso ene bit	e/ rs, eration
 In this step, sensors are assigned to samples. Four have a partial sensor tray is can be a social plate. Sensor Tray can be a partial plate. Sensor Tray is the sensor tray can be a partial plate. 	ated by selecti	ng the	missing sensors a	nd clicking 'Re	move'.	Regenerations Times sensors will be reused Reply Reply
✓ Replace sensors in tray after use	11 12	Wall	Consor Tuno	Lot Number	Information	
		A1	Protein A +	1807161	moniation	
		B1	Protein A	1807161		
		C1	Protein A	1807161		
		D1	Protein A	1807161		
		E1	Protein A	1807161		
		F1	Protein A	1807161		
F		G1	Protein A	1807161		
		H1	Protein A	1807161		
		A2	Protein A	1807161		
H		B2	Protein A	1807161		
	_	C2	Protein A	1807161		
Legend: Unassigned sensors 🔯 Missing sens	ors	D2	Protein A	1807161		
Remove Fill Fill Plate	Print	E2	Protein A	1807161		
Plate 1 (96 wells)		F2	Protein A	1807161		
rideo r (oo mello)		G2	Protein A	1807161		
	11 12	H2	Protein A	1807161		

Recenerations

Times sensors will be reused

*ForteBio's recommendation is to utilize fresh biosensors during initial assay development; only optimize regeneration conditions after you have successfully run your assay multiple times



Tab 3. Review Experiment





Tab 4. Run Experiment

1. Saving an experiment is a two-step process. A. First, click on the box by the three dots and select the folder where you would like to save the experiment. B. Name the experiment in the Experiment run name (sub directory).



2. Change settings of interest, including plate temperature. The Red96e has a temperature range between 15 to 40 °C. Other instruments have heating up to 40 °C but do not have cooling. For these instruments, the recommendation is to set the temperature from 2 °C above ambient temperature up to 40 °C. This will allow the instrument to consistently heat to the recommended temperature.

Delayed experiment start	Open runtime charts automatically
Start after (s): 600 🚔	Automatically save runtime chart
Shake sample plate while waiting	Set plate temperature (°C):



Tab 4. Run Experiment

late Definition	n 🌝 Sensor Assignment									
Data File Loca	ation and Names									
Assay type:		Basic Quantitation with Regeneration Standard Assay								
Quantitation o	data repository:	C:\Users\jennifer.codding-bui\Desktop\Quant Presenta								
Experiment ru	in name (sub directory):	t Experiment for Quant Presentation, 2018 →								
Plate name/b	arcode (file prefix):	181214								
Auto-increme	nt file ID start:) start: 1								
Data files will	be stored as follows:									
Desktop \Qua Desktop \Qua Desktop \Qua	ant Presentation\Quant Expe ant Presentation\Quant Expe ant Presentation\Quant Expe	eriment for Quant Presentation, 2018/181214_002.frd eriment for Quant Presentation, 2018/181214_002.frd								
Desktop \Qua Desktop \Qua Desktop \Qua	ant Presentation (Quant Expe ant Presentation (Quant Expe	riment for Quant Presentation, 2018/181214_00.7fd eriment for Quant Presentation, 2018/181214_00.7fd eriment for Quant Presentation, 2018/181214_00.3frd								
Desktop \Qua Desktop \Qua Desktop \Qua Run Settings	ant Presentation (Quant Expe	riment for Quant Presentation, 2018/181214_002.frd eriment for Quant Presentation, 2018/181214_002.frd								
µesktop∖Qua Desktop∖Qua Desktop∖Qua Run Settings ☑ Delayed e	xperiment start	Open runtime charts automatically								
µesktop\Qua Desktop\Qua Desktop\Qua Nesktop\Qua Run Settings ☑ Delayed e	experiment start Start after (s): 600 mple plate while waiting	Open runtime charts automatically Open runtime charts automatically Automatically save runtime chart Set plate temperature (°C):								
µesktop∖Quz Desktop∖Quz Desktop∖Quz Run Settings ☑ Delayed e ☑ Shake sar General Infom	IX Presentation (Vaunt Expendent Presentation (Vaunt Expendent Presentation (Vaunt Expendent Expendent Start after (s): 600 ()	Open runtime charts automatically Open runtime charts automatically Automatically save runtime chart Set plate temperature (°C):								
Desktop\Qua Desktop\Qua Desktop\Qua Run Settings ☑ Delayed e ☑ Shake sar General Infom User name:	ant Presentation (Quant Expendent Presentation) (Quant Expendent Presentation) (Quant Expendent Start Start after (s): 600 () () () () () () () () () () () () ()	Machine name: AMFREL-PC0N7Z1Q								
Desktop \Qua Desktop \Qua Desktop \Qua Run Settings ☑ Delayed e ☑ Shake sar General Inform User name: Description:	ant Presentation (Quant Expendent Expendent Presentation) (Quant Expendent E	Machine name: AMFREL-PCON7Z1Q								
Uesktop\Qua Desktop\Qua Desktop\Qua Run Settings ☑ Delayed e ☑ Shake sar General Inform User name: Description:	xperiment start start after (s): 600 € mple plate while waiting nation	Automatical Value Presentation, 2018/181214_002.frd eriment for Quant Presentation, 2018/181214_002.frd eriment for Quant Presentation, 2018/181214_003.frd								

rior to pressing "Go" confirm the Assay.

Total experiment time 0:19:50

1. After confirming the assay setup, place the biosensors in assay buffer in the pre-hyrdation plate, pipette the sample plate as defined in the plate definition, and hit "GO"

2. Upon hitting Go, a box will appear as a reminder to pre-hydrate your biosensors. You may hit OK

3. If the Delayed experiment start box is checked, a timer will appear. If your biosensors have already been incubating in assay buffer for 10 mins, you are welcome to override the timer and begin the assay*. If using the timer, the assay will begin automatically after the timer is up

*Even if the biosensors have already been properly hydrated at the start of the experiment, ForteBio recommends keeping this box checked and running the timer for 1-2 mins to allow your sample plate to obtain the desired temperature. Shaking the sample plate for a short period of time may also remove air bubbles from solution.





Advanced Quantitation Workflow with Data Acquisition V11

- In basic quantitation, binding of the analyte of interest to the ligand on the biosensor generate robust signal.
- In advanced quantitation, the initial binding of the analyte of interest to the ligand on the biosensor generate very small signal. In order to amplify the signal, additional "detection" step(s) are needed.
- We will show two examples of the advanced quantitation.
 - 2-Steps Sandwich-style Quantitation Assay
 - Multi-Steps Enzyme-linked Quantitation Assay





Select Assay Type





Acquisition Steps for Advanced Quantitation 384 Model

Work from left to right, from Tab 1 to Tab 4.



- 1. Plate Definition In this tab, all the information about the two sample plates and their wells will be entered.
- 2. Sensor Assignment In this tab, sensors are assigned to samples.
- 3. Review Experiment In this tab, you can review the steps that make up the experiment.
- 4. Run Experiment In this tab, you can select where you would like your data saved and name the data file. Run settings may also be changed.



Tab 1. Plate Definition





Tab 1. Plate Definition

Octet Data Acquisition 11.0.0.64 - [Advanced Quantitation Experim Image: State of the state of t	nent]	Alternative
Plate Definition Sensor Assignment Review Experiment In this step, all the information about the sample plate and its well First, check the assay settings. Then highlight one or more wells of Read Head: 8 channels Acquisition Rate: Standard (5.0 Hz, averaging by 20) Assay Settings Assay: Advanced Quantitation Modify	Run Experiment s will be entered. on the sample plate, and right-click to enter/modify well data. Plate 1 Table (96 wells) Concentration units: µg/ml v Export Well Sample ID Replicate Group Type Conc (µg/ml) Dilution Factor Information	
Standard Assay Single analyte Time (s): Shake speed (rpm): Sample 120 1000 Detection 120 1000	Assay Parameters Assay Parameters X Available Assays: Single analyte Basic Quantitation Single analyte Arti-FLAG Quantitation Replicates per sensor type: Arti-GST -High sensitivity Single analyte Arti-HIS (HIS2) Quantitation Sample Arti-Perta-HIS -High sensitivity Image: Single analyte Arti-Perta-HIS -High sensitivity Image: Single analyte Arti-Perta-HIS -High sensitivity Image: Single analyte Arti-Perta-HIS -Standard range Sample Image: High sensitivity Image: Single analyte Arti-Perta-HIS -Standard range Capture Antibody Sample Use odropodowup Mumore Information Sample Image: Signal analyte Sample Image: Signal analyte Capture Antibody Sample Use odropodowup	
Plate 1 (96 wells) Modify 1 2 3 4 5 6 7 8 9 10 11 12 A Image: Control standard Image: Control stand	 Immunology Culturation Protein L -Standard range Standard Assay Basic Quantitation with Regeneration Arti-Human Fab-CH1 (FAB) with regeneration Protein L -Standard range Standard Assay Advanced Quantitation Immunogencity - Enzyme Linked Standard Assay Burfer 2nd Antibody Between assay steps: Pre-condition sensors Post-condition sensors Post-condition sensors Blue indicates a built-in assay. 	g ssay. Ike



Т	ab 1	. Pl	ate	e D)efir	nitic	n		
Codeb Data Association 11.00.64. [Advanced Occupitation Function									- v
Cite View Superiore Letternet Window Unit	mentj							-	
								: • °●	- <u>-</u> ×
🏝 🖆 🚰 😣 🖆 🔇								• • • •	PALL FortéBio
🜖 Plate Definition 🥝 Sensor Assignment 🔞 Review Experiment 🄇	O Run Experiment								
In this step, all the information about the sample plate and its we First, check the assay settings. Then highlight one or more wells	Ils will be entered. on the sample plate, and r	ight-click to enter/mo	odify well data.						3
Read Head: 8 channels ~	Plate 1 Table (96 wells))							
Acquisition Pate: Standard (5.0 Hz. successing by 20)	Concentration units:	µg/ml ∨	Export	Import	Print				
Assav Settings	Well Sample ID	Replicate Group	Type C	onc (µg/ml)	Dilution Factor	Information		^	
Assay: Advanced Quantitation Modify	O A1		Standard		n/a				
Standard Assay Single analyte		ight click	r on		n/a				
Time (s): Shake speed (rpm):					n/a				
Detection 120 1000		المما ما ما	مالمين		n/a				
		niightea	weiis		n/a				
	🔵 G1				n/a				
	🔵 н1 to d	lefine the	e well		n/a				
	○ A2	-			n/a				
	e type	e. Selec	t well		n/a				
	O C2				n/a				
	tv	pe from	the		n/a				
Plate 1 (96 wells) V Modify	F2	p o o			n/a				
		ndown d	noleil		n/a				
			lalog		n/a				
	Standard	hov							
	Unknow	DUX.							
	Control			-					
	Reference		Unknown n/	a					
	Detection		Unknown n/	a					
	Detection		Unknown n/	a 5					
	Set Well Data		Unknown n/	a					
	Clear Data		Unknown n/	a					
Standard O Control Unassigned	Copy to Clipboard		Unknown n/	a					
Unknown Reference Reserved	Extended Sample Typ	es	Unknown n/	a					
	O D4		Unknown n/	a					
	🔵 E4		Unknown n/	a					
	🔵 F4		Unknown n/	a				*	



Tab 2. Sensor Assignment

1. Check box to have biosensors replaced back in the tray instead of being ejected to the waste container

2. Initially, one column of biosensors is assigned to each column of samples on ____ the plate. They are color coded, as displayed in the figure. For 384 model, the 2 columns of biosensor pickers are tied together and moved in unison. In order to pick 1 column of 8 biosensors at a time, an empty column is needed in between each column of the biosensors. Follow the figure to prepare the biosensor tray.



3 Right click to bring out the dialog box to select the sensor type.

4. Enter the lot number for your biosensor once.The software will populate the rest.







Tab 3. Review Experiment & Tab 4. Run Experiment

are the same as example shown for basic

quantitation.



3 CONFIDENTIA

Multi-Step, Enzyme-linked Quantitation Assay



- Detect and quantify the presence of anti-drug Ab
- Additional sensitivity over direct binding format
- Better performance than other bridging assays such as ELISA
 - Higher drug tolerance
 - Fewer assay steps and faster time to result
- Allows use of up to 50% serum



Select Assay Type





Acquisition Steps for Advanced Quantitation HTX Model

Work from left to right, from Tab 1 to Tab 5.



- 1. Sensor Loading In this unique tab for the HTX model, custom biosensors can be prepared. The number of read heads can be defined separately from the next tab. You may skip this tab if no custom biosensor is needed.
- 2. Plate Definition In this tab, all the information about the sample plates and their wells will be entered.
- 3. Sensor Assignment In this tab, sensors are assigned to samples.
- 4. Review Experiment In this tab, you can review the steps that make up the experiment.
- 5. Run Experiment In this tab, you can select where you would like your data saved and name the data file. Run settings may also be changed.





Creating Custom Biosensors



 Steps to Prepare Custom Biosensors

1. Dip bare biosensor into wells containing buffer

2. Dip into wells containing biotinylated ligands

 The number of biosensors can be defined as needed. In the upcoming example, 2 rounds of 32 streptavidin biosensors were loaded with the desirable biotinylated-ligands



Tab 1. Sensor Loading

Octet Data Acquisition 11.0.0.64 - [Advanced Quantitation Experiment] \times 🐘 File View Experiment Instrument Window Help _ 8 × 🔌 🔀 🖪 🔞 (PALL) FortéBio 🕣 Sensor Loading 🛛 Plate Definition 🔞 Sensor Assignment 🗿 Review Experiment 🗔 Run Experiment Select Number of Read Heads In this step, all the information about the sensor loading reagent positions will be entered. well data First, check the assay settings. Then highlight one or more wells on the sample plate, and right-click to te 1 Table (96 wells) (8, 16, 32, 48, or 96 for HTX) Read Head: 32 channels (high throughput) Concentration units: ug/ml Export.. Acquisition Rate: Standard (0.6 Hz, averaging by 5) Well Sample ID Replicate Group Type Conc (µg/ml) Dilution Factor Information Sensor Loading Settings Advanced Quantitation Assav Modify Assay Parameters \times Standard Assav Single analyte Sensor Loading ssay Parameters ailable Assays: Time (s) Shake speed (rpm): 400 basic Quantitation Read Head: Buffer X 60 Load 120 400 32 channels Select "Modify" to Step Type Time (s) Shake (rpm) Insert (x) Buffer X 60 400 Remove bring out dialog Load 120 400 Plate 1 (96 wells) Modify (none) box and then Sample 2 3 4 5 6 10 12 Load Use dropdown manual to Activation specify the "Sensor **Click on Modify to** Buffer X complete programing individual Loading" tab to Buffer Y select 96 or 384 wells Ouench steps. Adjust time & shake modify assay for Plate 1 & 2. speed of each step accordingly. parameters for HOOOO custom biosensor Standard Control Unassigned \bigcirc Reference Unknown Reserved creation OK Cancel



Tab 1. Sensor Loading

😳 Octet Data Acqui	sition 11.0.0.64 - [Advanced Quar	titation Experiment]								- 0	×	
🔝 File View Exp	eriment Instrument Window	Help									Б×	
🗟 🖄 🛱	è 😣 🖪 🕢									For	rtéBio	
🜖 Sensor Loading	2 Plate Definition Sensor As	signment 🧿 Revie	w Experiment 🛛 🚭	Run Experiment								
In this step First, chec	o, all the information about the sensor k the assay settings. Then highlight o	loading reagent positio ne or more wells on the	ns will be entered. sample plate, and	l right-click to enter/r	nodify well	data.)	
Read Head:	32 channels (high throughput)	→ Pla	te 1 Table (96 wel	s)	Export	Import	Print					
Acquisition Rate:	Standard (0.6 Hz, averaging by 5)	~		Poplicate Group	Type	Cope (ug/ml)	Dilution E-					
- Sensor Loading Sett	ings	A		nepilcale croup	Duffor V	conc (µg/mi)						
Assay: Ac	dvanced Quantitation	Modify	1	n/a	Duffer V	n/a	n/a					
Si	ngle analyte		1	n/a	Duffer V	n/a	n/a	Hold de	own the Shift kev on	the		
П	me (s) Shake speed (rpm):		1	n/a	Duffer X	n/a	n/a		- - - - - - - - -			
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		E .	1	n/a	Buffer X	n/a	n/a	reybuai	in and click the uppe			
		-	1	n/a	Buffer X	n/a	n/a					
		u 1	1	1/2	Buffer X	n/a	n/a	most wel	ll of choice to highlig	nt the		
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Plate 1 (96 wells)		V Medify P	2	n/a	Duffer X	n/a	n/a	numbe	number of "associated" wells			
1 2 3	4 5 6 7 8 9 1	0 11 12 0	2	n/a	Buffer X	n/a	n/a	numbe	115.			
			2	n/a	Buffer X	n/a	n/a					
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		П	2	n/a	Duffor V	n/a	n/a		, , , , , , , , , , , , , , , , , , ,	,		
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$F \times \times$		0.	2	n/a		n/a	n/a	CIICKING	on the myringined w	EIIS		
GXXX) (x) (^(X) Buffer X	0	2	n/a		n/a	n/a					
	Set Well Data	0	3	n/a		n/a	n/a	and bri	nging out the dropdo	wn		
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Standard			2	n/a	Buffer X	n/a	n/a		dialog box			
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Unknown	Extended Sample	Types H	3	n/a	Buffer X	n/a	n/a					
		A	+	n/a	Buffer X	n/a	n/a					
		В	4	n/a	Butter X	n/a	n/a					
		C	+	n/a	Butter X	n/a	n/a					
		D	4	n/a	Butter X	n/a	n/a					
		E	4	n/a	Butter X	n/a	n/a					
		F	•	n/a	Buffer X	n/a	n/a			~		
		G	4	n/a	Ruffer X	n/a	n/a					



Multi-Steps Enzyme-linked Quantitation Assay



- 8 Custom biosensors were used in each cycle
 - 1. Dip into wells containing standards or unknown samples
 - 2. Dip into wells containing HRP enzyme-conjugated detecting Ab
 - 3. Dip into wells containing HRP substrate metal DAB



Octet Automated Workflow for Multi-Steps Enzyme-linked Quantitation Assay



- Low detection reagent consumption
- No wash format allows • fewer steps and faster time to result





Tab 2. Plate Definition

Octet Data Acquisition 11.0.0.64 - [Advanced Quantitation Experiment]





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Tab 2. Plate Definition

Octet Data Acquisition 11.0.0.64 - [Advanced Quantitation Experiment] \times 🜇 File View Experiment Instrument Window Help _ 8 × PALL FortéBic E) 🔀 🖪 📿 🚹 Sensor Loading 🕗 Plate Definition 🔞 Sensor Assignment 🚯 Review Experiment 🟮 Run Experiment In this step, all the information about the sample plate and its wells will be entered. First, check the assay settings. Then highlight one or more wells on the sample plate, and right-click to enter/modify well data. Plate 2 Table (96 wells) Read Head: 8 channels (high sensitivity) ~ Export. Print. Concentration units: µg/ml Import. Acquisition Rate: Standard (5.0 Hz, averaging by 20) ~ Well Sample ID Replicate Group Type Conc (µg/ml) Dilution Factor Information ~ Assay Settings A1 Standard n/a Advanced Quantitation Assay Modify Standard Assay 🔵 B1 Standard n/a Single analyte 🔵 C1 Standard n/a Time (s): Shake speed (rpm); 🔵 D1 Standard 120 1000 n/a Sample Enzyme 120 400 O E1 Standard n/a Detection 120 400 🔵 F1 Standard n/a 🔵 G1 Standard n/a 🔵 H1 Standard n/a Click to highlight the number of A2 Standard O B2 Standard n/a "associated" wells O C2 n/a O D2 Standard n/a E2 Standard n/a ~ Modify Plate 2 (96 wells) Standard n/a 10 11 12 G2 Standard n/a А Standard Standard n/a В Unknown Unknown n/a С Unknown n/a Control Define the well types by right Unknown n/a D Reference Unknow E Load Unknown n/a clicking on the highlighted wells D Detection F Unknown n/a Enzyme E Unknown n/a G X Buffer X and bringing out the dropdown Unknown n/a н Unknown n/a Set Well Data Unknown n/a Clear Data dialog box. Control Unassigned Standard Unknown n/a \bigcirc Unknown Reference Reserved Copy to Clipboard Unknown n/a **Extended Sample Types** Unknown n/a F4 Unknown n/a G/ G/ Unknown n/a



Tab 3. Sensor Assignment

1. Check box to have biosensors replaced back in the tray instead of being ejected to the waste container

2. Based on the assay design, 64 biosensors are needed for this experiment. Unlike in the 384 models, individual columns of biosensor pickers in HTX can moved independently. No empty column is needed. Follow the figure to prepare the biosensor tray.



3 Right click to bring out the dialog box to select the sensor type.

4. Enter the lot number for your biosensor once and the software will populate the rest.







Tab 4. Review Experiment & Tab 5. Run Experiment

are the same as example shown for basic

quantitation.



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Advanced Quantitation Workflow with Data Analysis HT V11



Double click the Data Analysis HT 11.0 software icon to start the program







Opening file New Document





Loading Data into the Analysis Software





			Data Analy	ysis HT 11.0.0.50 - C:\Us	ers\m.I.Richard.Yip\D	esktop∖Prese	ntations\Quant Presentation\	Quant Experiment\Quant Exper	iment for Quant Presenta	tion with R	1. Select the "Preprocessed
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Assay	#1										stone
🛠 Refer	ence Subtractions a	and Data C	Correction Setti	ings		📈 Quar	titation Cycle and Step Graphs				sieps.
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4	4	1	1	acquisitit			hIgG in Sample Diluent	D1	30	C:\User	rs/m.l.Richard.Yip/Desktop/Presentations/Quant Presentation/Quant Experiment/Quant Exper
5	5	1	1	t1E1	Protein A	E1	hIgG in Sample Diluent	E1	100	C:\User	rs/m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Ouant Exper
6	6	1	1	t1F1	Protein A	F1	hIgG in Sample Diluent	F1	300	C:\User	rs\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Ouant Exper
7	7	1	1	t1G1	Protein A	G1	hIgG in Sample Diluent	G1	500	C:\User	rs\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper
8	8	1	1	t1H1	Protein A	H1	hIgG in Sample Diluent	H1	700	C:\User	rs \m.l.Richard.Yip \Desktop \Presentations \Quant Presentation \Quant Experiment \Quant Exper
9	9	1	1	t1A1	Protein A	A2	hIgG in Sample Diluent	A2	700	C:\User	rs\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper
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🖽 Quan	Quantitation Cycle and Step Tables												
Data Cycle	Quantitation Step												
Index	Sensor Number	Tray	Repetition Number	Sensor Name	Sensor Type	Sample Location	Sample ID	Ref Well Subtraction Formula	Concentration(µg/ml)	File location			
1	1	1	1	t1A1	Protein A	A1	hIgG in Sample Diluent	A1	1	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
2	2	1	1	t1B1	Protein A	B1	hIgG in Sample Diluent	B1	3	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
3	3	1	1	t1C1	Protein A	C1	hIgG in Sample Diluent	C1	10	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
4	4	1	1	t1D1	Protein A	D1	hIgG in Sample Diluent	D1	30	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
5	5	1	1	t1E1	Protein A	E1	hIgG in Sample Diluent	E1	100	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
6	6	1	1	t1F1	Protein A	F1	hIgG in Sample Diluent	F1	300	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
7	7	1	1	t1G1	Protein A	G1	hIgG in Sample Diluent	G1	500	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
8	8	1	1	t1H1	Protein A	H1	hIgG in Sample Diluent	H1	700	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
9	9	1	1	t1A1	Protein A	A2	hIgG in Sample Diluent	A2	700	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
10	10	1	1	t1B1	Protein A	B2	hIgG in Sample Diluent	B2	500	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
11	11	1	1	t1C1	Protein A	C2	hIgG in Sample Diluent	C2	300	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper			
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Data Cycle	ata Cycles Quantitation Step										
Index	Sensor Number	Tray	Repetition Number	Sensor Name	Sensor Type	Sample Location	Sample ID	Ref Well Subtraction Formula	Concentration(µg/ml)	File location	
1	1	1	1	t1A1	Protein A	A1	hIgG in Sample Diluent	A1	1	C: \Users \m.l.Richard.Yip \Desktop \Presentations \Quant Presentation \Quant Experiment \Quant Exper	
2	2	1	1	t1B1	Protein A	B1	hIgG in Sample Diluent	B1 - Average(A1,H2)	3	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper	
3	3	1	1	t1C1	Protein A	C1	hIgG in Sample Diluent	C1 - Average(A1,H2)	10	C: \Users \m.l.Richard.Yip \Desktop \Presentations \Quant Presentation \Quant Experiment \Quant Exper	
4	4	1	1	t1D1	Protein A	D1	hIgG in Sample Diluent	D1 - Average(A1,H2)	30	C: \Users \m.l.Richard.Yip \Desktop \Presentations \Quant Presentation \Quant Experiment \Quant Exper	
5	5	1	1	t1E1	Protein A	E1	hIgG in Sample Diluent	E1 - Average(A1,H2)	100	C: \Users \m.l.Richard.Yip \Desktop \Presentations \Quant Presentation \Quant Experiment \Quant Exper	
6	6	1	1	t1F1	Protein A	F1	hIgG in Sample Diluent	F1 - Average(A1,H2)	300	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper	
7	7	1	1	t1G1	Protein A	G1	hIgG in Sample Diluent	G1 - Average(A1,H2)	500	C: \Users \m.l.Richard.Yip \Desktop \Presentations \Quant Presentation \Quant Experiment \Quant Exper	
8	8	1	1	t1H1	Protein A	H1	hIgG in Sample Diluent	H1 - Average(A1,H2)	700	C: \Users \m.l.Richard.Yip \Desktop \Presentations \Quant Presentation \Quant Experiment \Quant Exper	
9	9	1	1	t1A1	Protein A	A2	hIgG in Sample Diluent	A2 - Average(A1,H2)	700	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper	
10	10	1	1	t1B1	Protein A	B2	hIgG in Sample Diluent	B2 - Average(A1,H2)	500	C: \Users \m.l.Richard.Yip \Desktop \Presentations \Quant Presentation \Quant Experiment \Quant Exper	
11	11	1	1	t1C1	Protein A	C2	hIgG in Sample Diluent	C2 - Average(A1,H2)	300	C:\Users\m.l.Richard.Yip\Desktop\Presentations\Quant Presentation\Quant Experiment\Quant Exper	
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Quantitation calculation completed



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Quantitation calculation completed.











Quantitation calculation completed.





Tips for Quantitation Assays

- Fully equilibrate all reagents to room temperature
- Hydrate biosensors in sample matrix for 10min
- Match specific calibration standards with samples, e.g. Ab isotype and subtype
- Match neutralization solution to sample matrix
- Reference subtract using blank medium matrix
- Optimize shake speed and step time
- Use corresponding biosensors when making comparisons to other technologies
- Use black, polypropylene, flat bottom plates [absolutely no exception]
 - Black 96 well plates: Greiner catalogue # 655209; 200μL sample volume
 - Black 384 well plates: Greiner catalogue # 781209; 100μL sample volume
 - Black 384 tilted-well plates: ForteBio 18-5080 or 18-5076; 50µL sample volume
- Keep Octet on at All Times. Turning the system on / off will shorten the lifespan of the Lamp
- Knowledge Base link: <u>www.fortebio.com/kb</u>







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