## FortéBio Bio-layer Interferometry Kinetic Analysis Tutorial

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#### Outline

- Introduction to Biolayer Interferometry (s. 3-12)
- Kinetic Analysis Basics (s. 13-18)
- Basic Kinetics Data Acquisition (s. 19-35)
- Data Analysis HT (s. 36-63)



#### ForteBio is a Market Leader in Label-Free Biomolecular Analysis



#### Full life-cycle offering for biomolecular interaction analysis

- Label-free assays based on Bio-Layer Interferometry (BLI) and Surface Plasmon Resonance (SPR) platforms
- Instruments, consumables, software, post-sale services (one-on-one training)













	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	o 4 hrs	
21 CFR Part 11 Compliance		Available	as option for all	systems	



#### **Biosensor-based Technology**



#### Use of biosensors is core to BLI technology



### **The Octet Design Features**

#### Octet 96/QKe



- Optics box moves the biosensors to samples
- One biosensor tray
- One 96-well sample/reagent plate
- Upto 8 interactions simultaneously in one experiment for Octet RED96e

#### Octet 384/HTX



- Microplate format for samples allows for a large number of interactions to be studied in one experiment.
- Compatible with 2-96-well or 384-well sample plates.
- <u>Upto 16 interactions</u> simultaneously in one experiment for Octet RED384
- <u>Upto 96 interactions</u> simultaneously in one experiment for **Octet HTX**



#### Dip and Read<sup>™</sup> Biosensors

The Octet Dip and Read<sup>™</sup> 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 out of phase Destructive interference <u>Weak</u> signal at the spectrometer





### Monitoring nm-shift Against Time



Time (sec)



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### Versatile Applications On ForteBio Label-Free Systems



#### **Kinetics**

- $k_{a}, k_{d}, K_{D}$
- Proteins, Abs
- Peptdes, oligos
- Small molecules
- Fragments







#### Quantitation

- Direct, 1-step
- Sandwich
- ELISA
- mg/mL pg/mL

#### Diversity

- Function testing
- Epitope binning
- Rank ordering
- Isotyping

#### cBLI

- Cell capture
   based assays
- Dynamic Mass Redistribution (DMR)
- Toxicity assays



### Size Range & Octet Versatility in Interaction Analysis





### The Art of Biosensor Regeneration





# Kinetic Characterization on

#### the Octet



#### The Ideal Binding Behavior



In a simple 1:1 binding model, the association and dissociation phases are described by a single exponential function



## **Biosensors for Kinetic Analysis**

Biosensor	Application
Antibody-Specific Capture	
Anti-Human IgG Fc Capture (AHC)	Human IgG Fc region, kinetic analysis
<ul> <li>Anti-Human IgG Fc Capture (AHQ)</li> </ul>	Human IgG Fc region, quantitation
<ul> <li>Anti-Mouse Fc Capture (AMC)</li> </ul>	Mouse IgG1, 2a & 2b Fc regions, kinetic analysis
<ul> <li>Anti-Mouse Fc Capture (AMQ)</li> </ul>	Mouse IgG1, 2a & 2b Fc regions, quantitation
• Anti-Human Fab-CHI (FAB)	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
<ul> <li>High Precision Streptavidin (SAX)</li> </ul>	Biotinylated ligands (4% CV loaded SA)
<ul> <li>Super Streptavidin (SSA</li> </ul>	Biotinylated ligands (high-density surface)
• Anti-GST (GST)	GST-tagged recombinant proteins
<ul> <li>Anti-Penta HIS (HIS1)</li> </ul>	HIS-tagged recombinant proteins
<ul> <li>Anti-Penta HIS 2<sup>nd</sup> Gen (HIS2)</li> </ul>	HIS-tagged recombinant proteins
• Ni-NTA (NTA)	HIS-tagged recombinant proteins
Immobilization	
<ul> <li>Amine Reactive 2nd Gen (AR2G)</li> </ul>	Covalent coupling to reactive amine groups
Aminopropylsilane (APS)	Adsorption to hydrophobic moieties

#### **Kinetic Biosensors are Highlighted**



#### Differences Between Kinetic and 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**



#### Label-free Terminology





## Automated Kinetic Work Flow on the Octet







### Kinetic analysis Workflow with Data Acquisition V11



#### **Double click the Data Acquisition 11.0 software icon**

to start the program



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### Setting up Kinetics Assays

• Use either **Experiment Templates** or the **Experiment Wizard** for assay design and optimizations such as pH scouting, regeneration scouting, etc.

Octet Data	Acquisition 10.0.1.3						
File View E	xperiment Instrument Window	Help					
	<ul> <li>New Experiment Wizard</li> <li>Edit Assay Parameters</li> </ul>	Ctrl+N					
	Edit Sensor Types Set Plate Temperature						Option 2: Templates
	Show Temperature Log						
	Templates		Epitope Binning 🔹 🕨				
	Skip Step		Kinetics +	Biomolecu	Ile kinetics - AHC biosensor		
	Stop		Quantitation +	Biomolecu	Ile kinetics - AMC biosensor		
		_		Biomolecu	ile kinetics - AR biosensor	•	
				Biomolecu	Ile kinetics - SA biosensor	•	Kinetic Characterization_8CH_96W.fmf
				Small Mol	ecule and Fragment Kinetics - SSA biosensor	•	RegenerationConditionScouting_8CH_96W.fmf
Option	n 1: Wizard	Choose	Meent Wizard	nt Regeneration	Templates will be available after instrument is initia	alized	
			Basic Kinetics     Epitope Binning     Recent Methods	]			



### Setting up Kinetics Assays





## **Acquisition Steps for Basic Kinetics**

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



- 1. Plate Definition In this tab, all the information about the sample plate and its wells will be entered
- 2. Assay Definition In this tab, specific experimental steps are established
- 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





### **Setting up Kinetics Methods**

#### Tab 1: Plate Definition





#### Tab 1: Plate Definition

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

Wel	Sample ID	Replicate Group	Туре	Conc (µg/ml)	MW (kD)	Molar Conc (nM)
🖲 C1	1 x KB		Buffer			
🖲 D1	1 x KB		Buffer			
🖲 E1	1 x KB		Buffer			
🖲 F1	1 x KB		Buffer			
🖲 G1	1 x KB		Buffer			
🖲 H1	1 x KB		Buffer			
() A2	BPA		Load	6.25		
C 82	BPA		Load	6.25		
() C2	BPA		Load	6.25		
C D2	BPA		Load	6.25		
() E2	BPA		Load	6.25		
🕻 F2	BPA		Load	6.25		
0 62	BPA		Load	6.25		
<u>с)</u> н2	BPA		Load	6.25		
O A3	HIgG		Sample	3	150	20
<b>B</b> 3	HIgG		Sample	1.5	150	10
0 🛛	HIgG		Sample	0.75	150	5
<b>D</b> 3	HIgG		Sample	0.375	150	25
<b>E</b> 3	HIgG		Sample	0.1875	150	1.25
<b>F</b> 3	HIgG		Sample	0.09375	150	0.625
63	HIgG		Sample	0.04688	150	0.3125
HB	HIgG		Referen	0	0	0
-						

et Well Data				×	
Well Information		Dilution Series			
Sample ID:		Apply to:	<ul> <li>Concentration</li> </ul>	R	
HlgG			<ul> <li>Molar Concentration</li> </ul>	n N	
Replicate Group:		Starting value (nM):	20	2	Check the box
W-ll l-f		Series operator:	/ ~		
		Series operand:	2	by	dilution series
		Dilution orientation			
		Right	888 O Left		
Well Data - Sample only		👸 💿 Down	u 🞇 🔿 Up		
Molecular Weight (kD):	150	00	••		
Molar Concentration (nM):					
Concentration (µg/ml):			OK Can	cel	



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

## **Setting up Kinetics Methods**

#### Tab 2: Assay Definition



- Move black arrow (→) to the desired step
- Use mouse cursor to click on the corresponding column (number)
  - Set Shake speed to 1000 rpm.
  - Stacking the Baseline, Association, and Dissociation steps consecutively is important!



## **Setting up Kinetics Methods**

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#### Tab 3: Sensor Assignment



- Always input biosensor lot # for troubleshoot if error happened
   Ensure the "replace biosensor after use" is checked if biosensors are being used for next experiment
  - Click on the "Fill Plate" button to start from the A1 position from the Sensor Tray
  - If need to move biosensor location, highlight the corresponding columns follow by clicking on "Remove"







Slider



#### Tab 5: Run Experiment

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

Plate Definition O Sensor Assignment	Review Experiment Q Run Experiment	
Data File Location and Names		
Assay type:	Basic Quantitation with Regeneration Standard Assay	
Quantitation data repository:	C:\Users\jennifer.codding-bui\Desktop\Quant Presenta	← 8
Experiment run name (sub directory):	t Experiment for Quant Presentation, 2018	← k

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 not cooling. For these instruments, the recommendation is to set the temperature from 2 °C above ambient up to 40 °C, to allow the instrument to consistently heat to the recommended temperature.

nun seungs		
Delayed experiment start	Open runtime charts automatic	cally
Start after (s): 600 🚔	Automatically save runtin	ne chart
Shake sample plate while waiting	Set plate temperature (°C):	25





Assay type:		Basic Quantitation with Regeneration Standard Assay
Quantitation d	ata repository:	C:\Users\jennifer.codding-bui\Desktop\Quant Presenta
Experiment rur	n name (sub directory):	t Experiment for Quant Presentation, 2018 →
Plate name/ba	arcode (file prefix):	181214
Auto-incremen	nt file ID start:	1
Data files will I	be stored as follows:	
Desktop\Qua Desktop\Qua Desktop\Qua 	nt Presentation\Quant Ex int Presentation\Quant Ex int Presentation\Quant Ex	periment for Quant Presentation, 2018\181214_001.frd periment for Quant Presentation, 2018\181214_002.frd periment for Quant Presentation, 2018\181214_003.frd
Desktop\Qua Desktop\Qua Desktop\Qua  Run Settings -	nt Presentation\Quant Ex Int Presentation\Quant Ex Int Presentation\Quant Ex	periment for Quant Presentation, 2018\181214_001.frd periment for Quant Presentation, 2018\181214_002.frd periment for Quant Presentation, 2018\181214_003.frd
Desktop∖Qua Desktop∖Qua Desktop∖Qua  Run Settings – Delayed ex	nt Presentation \Quant Ex Int Presentation \Quant Ex Int Presentation \Quant Ex Kperiment start	periment for Quant Presentation, 2018\181214_001.frd periment for Quant Presentation, 2018\181214_002.frd periment for Quant Presentation, 2018\181214_003.frd
Desktop∖Qua Desktop∖Qua Desktop∖Qua  Run Settings ☑ Delayed ex	nt Presentation \Quant Ex nt Presentation \Quant Ex nt Presentation \Quant Ex kperiment start Start after (s): 600	periment for Quant Presentation, 2018\181214_001 frd periment for Quant Presentation, 2018\181214_002 frd periment for Quant Presentation, 2018\181214_003.frd
Desktop∖Qua Desktop∖Qua Desktop∖Qua  Run Settings ☑ Delayed es ☑ Shake san	nt Presentation\Quant Ex nt Presentation\Quant Ex nt Presentation\Quant Ex xperiment start Start after (s): 600 [ nple plate while waiting	periment for Quant Presentation, 2018\181214_001 frd periment for Quant Presentation, 2018\181214_002 frd periment for Quant Presentation, 2018\181214_003 frd  Open runtime charts automatically  Quant Presentation Charts automatically  Set plate temperature (°C): 25
Desktop \Qua Desktop \Qua Desktop \Qua Sektop \Qua  Delayed ex Delayed ex Shake san	nt Presentation\Quant Ex nt Presentation\Quant Ex nt Presentation\Quant Ex xperiment start Start after (s): 600 nple plate while waiting lation	periment for Quant Presentation, 2018\181214_001.frd periment for Quant Presentation, 2018\181214_002.frd periment for Quant Presentation, 2018\181214_003.frd  Open runtime charts automatically  Open runtime charts automatically  Set plate temperature (°C): 25
Desktop∖Qua Desktop∖Qua Desktop∖Qua  Run Settings ☑ Delayed ex ☑ Shake san àeneral Inform User name:	Int Presentation \Quant Ex Int Presentation \Quant Ex Int Presentation \Quant Ex Start after (s): 600 [ Inple plate while waiting Intion	periment for Quant Presentation, 2018\181214_001 frd periment for Quant Presentation, 2018\181214_002 frd periment for Quant Presentation, 2018\181214_003 frd  Open runtime charts automatically  Open runtime charts automatically  Automatically save runtime chart  Set plate temperature ("C): 25

#### Prior to pressing "Go" confirm the Assay.

Total experiment time: 0:19:50

 After confirming the assay setup, placing the
 biosensors in assay buffer in the pre-hyrdation plate, and pipetting the sample plate as defined in the plate definition, you are ready to hit "GO"

**2.** Upon hitting Go, a box will appear as a reminder to prehydrate 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<sup>\*</sup>. 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

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## Setting up Kinetics Assays

open the door and th	e stage will come out.	
	Esperiment Waard	
Select Basic Kinetics	Choose an option to stat  Choose an option to stat  Mew Guanttation Expensent  Insu: Quanttation with Regeneration  Advanced Quantgation  Advanced Quantgation  Reset Relation  Basic Relation  Experiment  Experi	Select Blank Experimer
		Click to Start
	Recert Methods.	



### Setting up Kinetics Assays on the High throughput Octet Instruments





## 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





# • 16-Channel Movement on Octet 384 Systems



96 Well Microplate



384 Well Microplate



## 8 Channel Assay "WITH" Re-racking

#### Important:

 If the Replace Sensors In Tray After use is Checked, you will have to put biosensors in every other column

Follow the diagram to load your biosensors

Biosensor Location #1 will be used in this mode with re-racking





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## 8 Channel Assay "WITHOUT" Re-racking

#### 🖄 🖆 🖄 😫 🕢 **Important:** Sensor Assignment Plate Definition Assay Definition If you are running 4 different assays in In this step, sensors are assigned to samples. 8 channels mode, then make sure you If you have a partial sensor tray it can be accomodated by sel Only the first sensor tray can be a partial tray. Right dick to have the "Replace Sensors in Tray Sensor Tray after use" is unchecked (biosensors Replace sensors in tray after use will be discarded after use) 10 11 12 2 8 9 6 Then, you can place biosensors in Α adjacent columns B D F **Biosensor Location #2 will be used** F in this mode without re-racking G Unassigned sensors Legend: XX Missing sensors Fill Plate Fill Remove Print...



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## Kinetics Analysis Workflow with Data Analysis HT V11.0



#### **Double click the Data Analysis HT 11.0 software icon**

#### to start the program





#### Locating Data File



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#### HT Kinetics Analysis Preprocessing Kinetics Data Sets

- Idea is same as before (subtracting references and making other data corrections), but with much more flexibility
- Now you can see the baseline after subtraction

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• Experiments with multiple assays will show up as separate assays as before, but will combine in final data table/fitting graph.



In preprocess data tab there is a new K to Q option. Using this new feature you were able to quantify any of the kinetic steps by selecting the appropriate step list. For example if you load unknown amount of ligand by using this option you should be able to quantify the amount of loading concentration.

However, now if you would be able to quantitate multiple step, you can select quantitate step type means that will analyte all the loading steps in different experiment.



Exclude	Color	Sensor Number	Location	Tray	Sensor	Sensor Type	Sensor Info	Sensor Subtraction Formula	Assay	
		1	t1A7	1	Ligand Sensor	SA (Streptavidin)			1	
		2	t1B7	1	Ligand Sensor	SA (Streptavidin)			1	
		3	t1C7	1	Ligand Sensor	SA (Streptavidin)			1	
<										



## HT Kinetics Analysis Reference Tabs



- Reference Sensors, Ref Wells/Samples and Data Corrections options have been separated into sub-tabs
- Edit sample or sensor information, Exclude samples or sensors
- This is a new look to the interface



Data Anal

## Subtracting Reference Sample



### **Subtracting Reference Sample**





#### **Data Correction**





## **Fitting Parameters**



# Double Reference on

#### **Kinetic Analysis**



#### Refence Sensor vs. Reference Well

- Reference WELLS are wells in the sample plate containing assay buffer only (no analyte)
- The sensor dipped into them DOES have ligand loaded on it
- Their purpose is to subtract any drift in the BLI signal
  - --- Drift in the signal is usually caused by certain buffer components, like Tween or DMSO
- Usually there is one reference well per column of samples
- Reference wells should ALWAYS be included in kinetics assays

#### **Sample Plate**





#### Refence Sensor vs. Reference Well

- Reference SENSORS are a second complete set of sensors that are NOT loaded with ligand (or are loaded with a control ligand that does not bind to the analyte)
- The reference sensors are dipped into all the same samples as the sensors with the ligand loaded on them
  - --- Note that when using reference sensors, you MUST replicate your assay steps exactly with the reference sensor
  - --- The only allowable difference is that reference sensors can be "loaded" in buffer or a different ligand well
- Refence sensors are used to subtract NSB, and therefore are not always used

#### **Sensor Tray**



Assay	No.	Sample	Step Name	Step Type	Sensor Type	Assay Time Commen
1	1	1	Baseline 👻	🔜 Baseline	SA (Streptavidin) 👻	
1	2	2	Loading	🖌 Loading	SA (Streptavidin)	
1	3	1	Baseline	🔜 Baseline	SA (Streptavidin)	
1	4	3	Association	🞽 Association	SA (Streptavidin)	
1	5	1	Dissociation	📐 Dissociation	SA (Streptavidin)	0:25:40
2	1	1	Baseline	🕳 Baseline	SA (Streptavidin)	
2	2	1	Loading	🖌 Loading	SA (Streptavidin)	
2	3	1	Baseline	Baseline	SA (Streptavidin)	
2	4	3	Association	🞽 Association	SA (Streptavidin)	
2	5	1	Dissociation	<b>Dissociation</b>	SA (Streptavidin)	0:25:40





#### **Double Referencing**





Customize





#### **Double Referencing**





### Loading Data into the Analysis Software



Combined Dataset Valid





#### **Reference Sensor Subtraction**





#### **Reference Sensor Subtraction**





## HT Kinetics Analysis Increased Flexibility in Reference Subtraction

#### **By Column**



#### By Row



#### **By Pattern**





### **Double Referencing**

Hide Steps: Show All Graph Assay #1	Auto Scale - Sa	etic Analysis Re w Data Prepro E	eport Cressed Data Excel	Report Points to et	ant Vipi Desktop (Presentations nt time value (sec) 100 verage: 20	Add Point Remove Al Approx Traces - Report Point	a Export Seve	HTSettings.efrd	K to Q+ Operations	Kinetic -		
Reference Subtractions :	and Data Conection Set	tings		Full Tree	ce and Cycle Graphs							
Reference Sensor Reference	e Sample Data Correcti	on		Pull Traces :	Data Cydes							
Rest Ungroup	*Reference () - Bri Signal	senso(s) into groups only s from r d referen btracted	no-ligand nce biose	ensors				2				
	ligand and th found	-loaded e formu here.	biosens la can be	ors	50 100	150 200	250 308	1111111 350 400 Time (5)	450	500 550	 603	650 700
	ligand and th	-loaded e formu here.	biosense la can be	ors	50 105	150 200	250 308	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	450	500 550	eod	650 700
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E C C C C C C C C C C C C C C C C C C C	ligand and th found	-loaded e formu here.	biosense la can be	Sensor Info Color	Sensor Subsection Parmula	Assay	250 300	Time (s)	450	500 550	115_001.8w	**************************************
E C C C C C C C C C C C C C C C C C C C	Location Tray	-loaded e formu here.	biosense la can be sensor Type	Sensor Info	Sensor Subjection Pormule 1981-1982 1981-1982 1981-1982	Assay 11 C.V.arvy 11 C.V.arvy 11 C.V.arvy	250 300	File location File location File location File location File location File location File location File location	450	500 550	115_001.fre 115_001.fre 115_001.fre 115_001.fre	**************************************
E C C C C C C C C C C C C C C C C C C C	Location Tray	-loaded e formu here.	biosense la can be sensor Type Sensor Type Sensor Type Sensor Type Sensor Type	Sensor Info	Sensor Subsection Pormula 1441-1442 1491-1422 1491-1492	Assay 1 C.(V.em/y) 1 C.(V.em/y) 1 C.(V.em/y) 1 C.(V.em/y)	1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 250 - 300 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 250 - 300 1 - 2 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 -	File location Time (s) File location Colony Varial Fitsler Colony Varial Fitsler Colony Varial Fitsler	450 Mitch Off and Com anon WT and Com attorney T and Com attorney T and Com	1 / 1 / 1 / 1 / 550 550 550	eod eod 115.301.84 119.302.84 119.302.84 119.303.84 119.304.87	**************************************
Construction     C	Locator Tray	-loaded e formu here. Sersor User Server User Server User Server User Server User Server	biosense la can be sensor Type Sensor Type	Sensor Info	Sensor Subtraction Pormula 1441-1441 1481-1482 1481-1482 1481-1482 1481-1482	Assay 1 CiV/Jennin 1 CiV/Jennin 1 CiV/Jennin 1 CiV/Jennin 1 CiV/Jennin 1 CiV/Jennin 1 CiV/Jennin 1 CiV/Jennin 1 CiV/Jennin	1 - Charl Pro Centro Print 250 300	Pile location Time (a) Pile location Debuny () = 11.5 Protect Debuny () = 11.5 Protect	450	1 Palo Soverco (181 197 No Novelco (181 197 No No No No No No No No No	e00 119:001.htt 119:002.htt 119:002.htt 119:002.htt 119:005.htt 119:005.htt	**************************************
E C C C C C C C C C C C C C C C C C C C	Location Tray	-loaded e formu here.	biosense la can be sensor Type Sensor Type SA Desenver() SA Desenver() SA Desenver() SA Desenver() SA Desenver() SA Desenver() SA Desenver()	Sensor Info	Sensor Subvector Pormula 1011 - 1022 1021 - 1022 1021 - 1022 1021 - 1022 1021 - 1022 1021 - 1022	Assay 1 C/Usersy 1 C/Usersy	250 300	Time (a) Time (a) Pile location Debug (a) dis Present Debug (a)	Hon (VIT and Com address) and Com	al Pub Street co. (18) al Pub	600 600 115_011.8v 115_021.8v 115_021.8v 115_021.8v 115_025.8v 115_026.8v 115_026.8v	**************************************
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### Subtracting Reference Well





#### Subtracting Reference Well Data Analysis HT 11.0.0.50 - C/Users/m/LRichard/Yig/Desidog/Presentations/Kinetics Presentation/WT and Control Fab Kinetics/HTSettings effot - 🗆 x Preprocessed Data Kinetic Analysis Report loime. A n ħ, 2 2 Report point time value (sec) 100 100 115 A Points to average: 20 Kinetic. Show All Auto Scale -Raw Data Preprocessed Data Excel Report Add Point Remove All Export Save Load View Graph Export Sensor Traces - Report Points Operation Assay #1 Relevence Subtractions and Data Connection Setting Full Trace and Cycle Graph Full Traces Data Cycles Reference Sensor Reference Sample Data Correction Processed Data (IIII ) Reference Sample Subtraction: ĕÇ. 1. Select and set sample well(s) as reference well(s) 2. Group available sample well(s) into groups to deter Reset Ungroup Chore. = Reference Well ) = Buffer 🛄 = Sample/Load ) = Empty 10 11 10 0.3 B C D E 0.2 F مقاملة الاما ومرد المراجع والمراجع ومعامل والمتعادة والمتعادة والمراجع والمراجع والمراجع والمالية G \*\*\*\*\*\* 120 140 160 180 200 220 240 260 × Set Reference ٠ Time (a) Subtract Reference . In Column 7 Full Trace and Cycle Data Tab Subtract Reference in Selected Wells In flow Full Traces Data Cycles Copy By Pairs Right rdude ubtraction Formula Double Ref Subtraction Formula After specifying the "Reference Paste By Pays Left p1A4 1A1c1 **Remove Reference Subtraction** By Pattern Hight p154 151ct Edit Sample Info p3C4 1C3c1 By Pattern Left Sample Well", right click to bring out p1D4 ID1c1 Exclude Wells from Analysis By Whole Plate p1E4 dE1c1 Include Wells for Analysis 3F1c1 SA (Streptavidin) p1F4 dropdown dialog box to select the 1 t161 SA (Streptavidn) p164 163c1 81A2 SA (Streptavidin) p1A4 appropriate reference subtraction 1A2c1 10 10 t182 SA (Streptavidin) p184 :182:1 1 11 11 t1C2 SA (Streptavidn) 0104 1C2c1 algorithm



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-		2	2	1	1	11A1 1151	SA (Streptavidiri)	p184	p1A5	p184	WT	A5 - H5 85 - H5	tiBici - tiHici	(1181 - 1182) - (1181 - 1182) (1181 - 1182) - (1181 - 1182)	
-		3	3	1	1	tiC1	SA (Streptavidin)	p1C4	p1C3	p1C4	WT	C5-H5	t1Clcl-t1H1cl	(t3C1-t1C2) - (t1H1-t1H2)	
C		4	4	1	3	t101	SA (Streptavidin)	p104	p1D5	p1D4	WT	DS - HS	tiDici-tiHici	(t1D1-t1D2) - (t1H1-t1H2)	
		5	5	1	1	t£1	SA (Streptavidin)	p1E4	p tE5	p1€4	WT	E5 - H5	tiEici - tiHici	(t1E1 - t1E2) - (t1H1 - t1H2)	
2		6	6	1	1	t2#1	SA (Streptavidin)	p1%4	p1/5	p1#4	WT	P5H5	tiPici-tiHici	(t1F1 - t1F2) - (t1H1 - t1H2)	
	-	1	T	- 4	-	1361	SA (Streptsjudin)	p364	p105	P304	WI	GS - HG	TIGICI - TIHICI	(1201 - 1102) - (11H1 - 11H2)	
1		9	9	1	1	t1A2	SA (Streptavidin)	p1A4	plAS	plAt	WT	AS	t1A2c1		
		10	50	1	1	t182	SA (Streptavidin)	p184	p185	p.84	WT	85	t182c1		
F _		11	11	1	1	tiC2	SA (Streptavidin)	p3C4	piC5	p1C4	WT	C5	11C2c1	¥	
c														3	



#### **Data Correction**





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### **Fitting Parameters**



Kinetic calculation completed



#### **Kinetic Analysis**



#### **Kinetic Analysis**



Enstic calculation completed





#### HT Quantitation/Kinetics Analysis Excel or PDF Report



Can make traditional excel report or generate a customized PDF report









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