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)

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ForteBio – A Leader in Biomolecular Analysis

BLI
Octet & BLItz

SPR
Pioneer

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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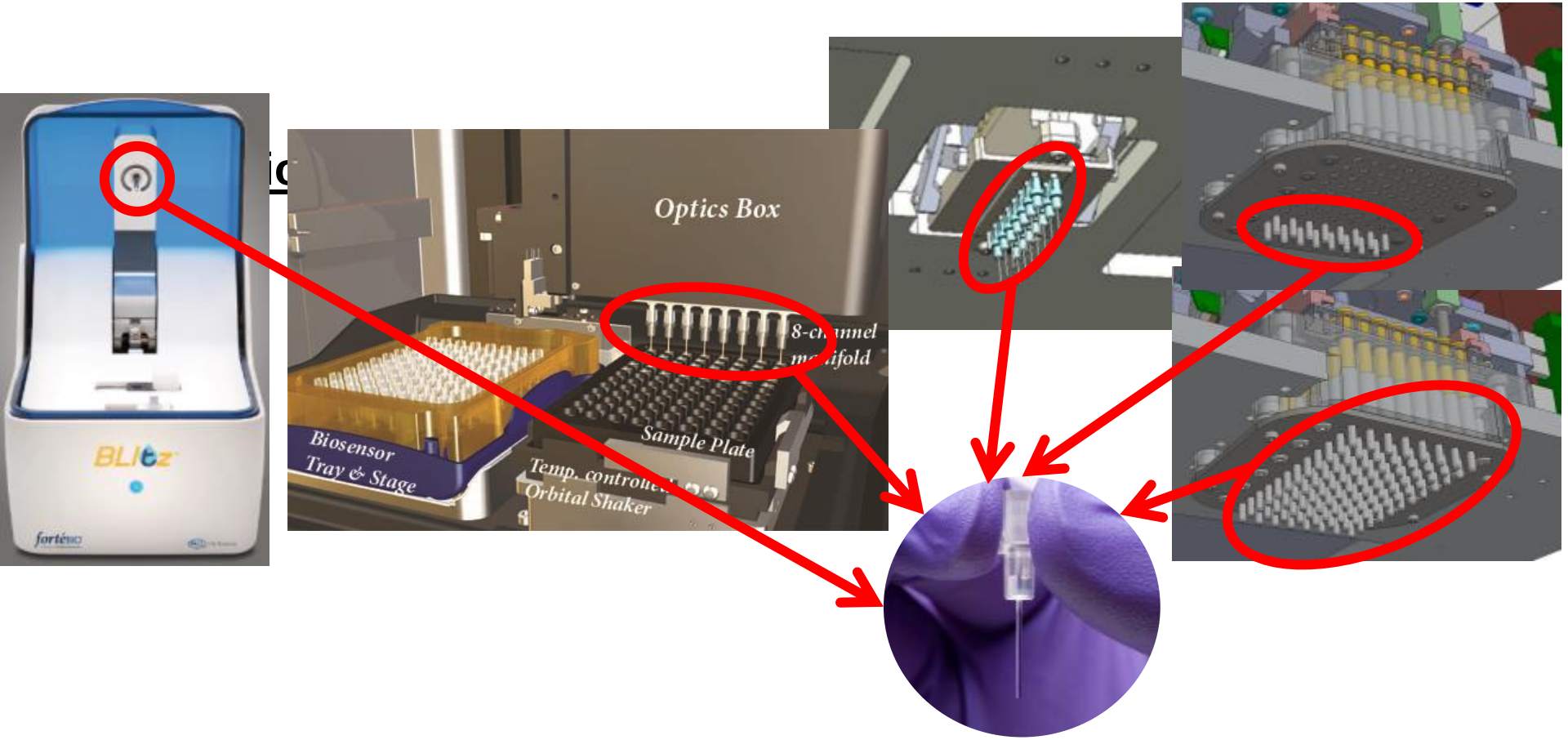
Biosensor-based Technology



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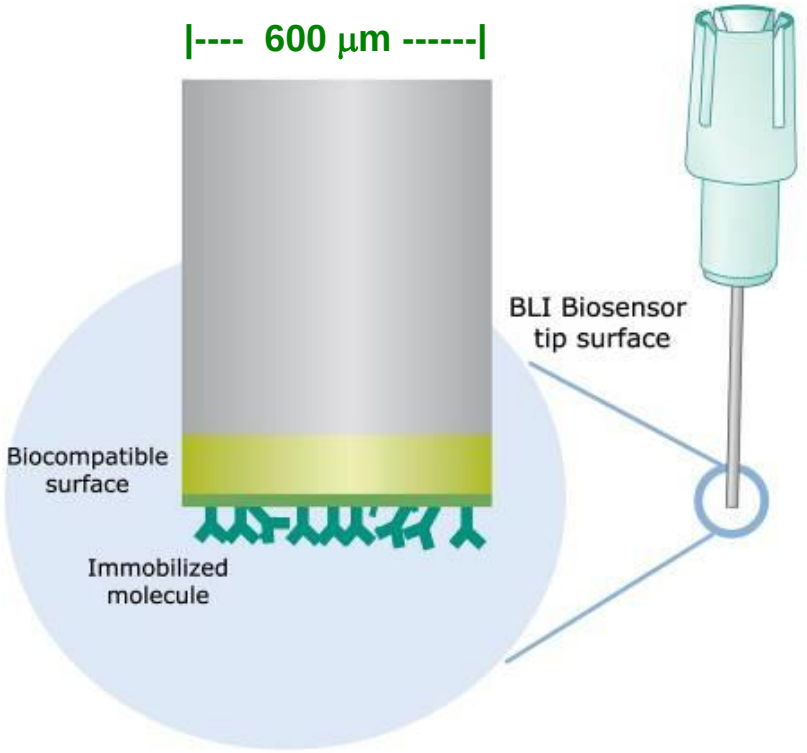
Dip and Read Biosensors

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*

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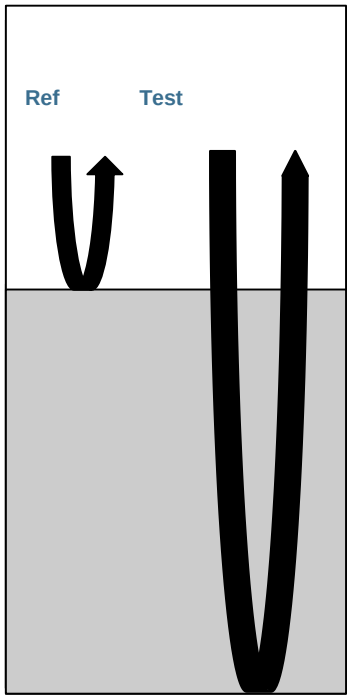
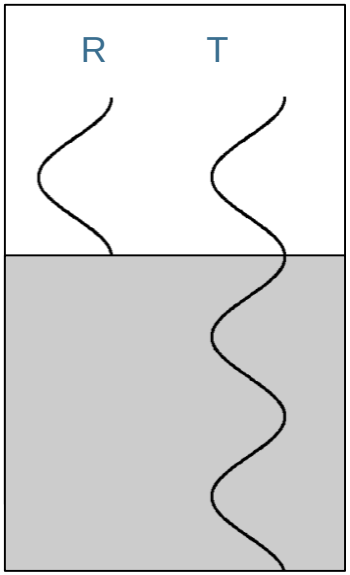
Bio-Layer Interferometry

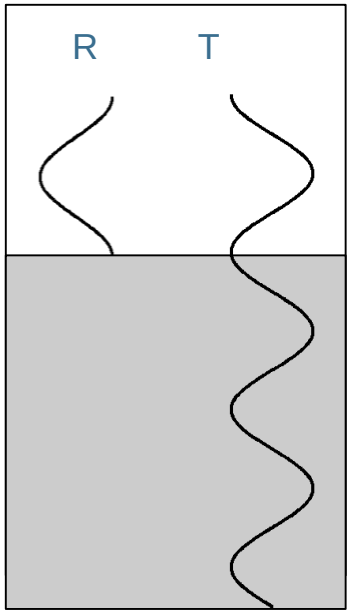
- In BLI, light is directed down an optical fiber (the sensor) toward two interfaces separated by a thin layer at the end of the fiber
- The two reflected beams interfere constructively or destructively at the spectrometer CCD detector array



Reflections R & T are in phase
Constructive interference
Strong signal at the spectrometer



Reflections R & T are out of phase
Destructive interference
Weak signal at the spectrometer



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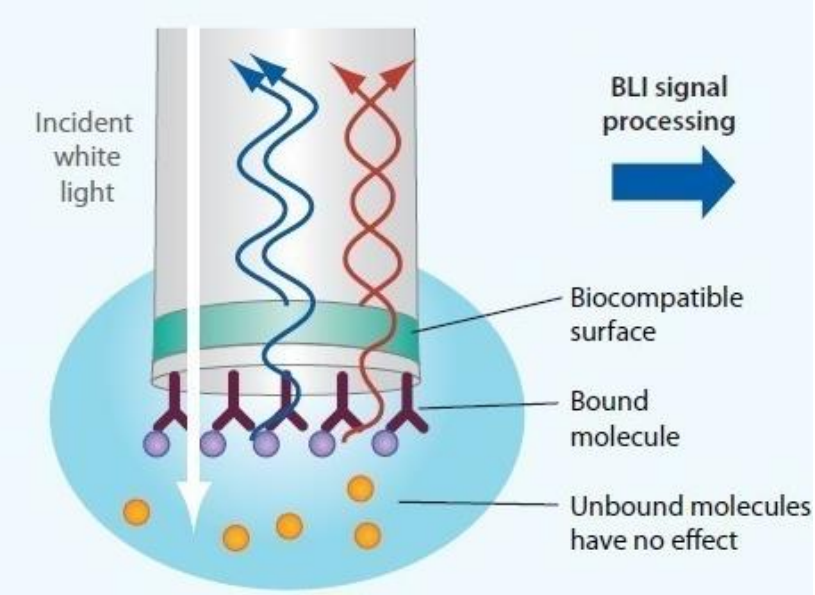
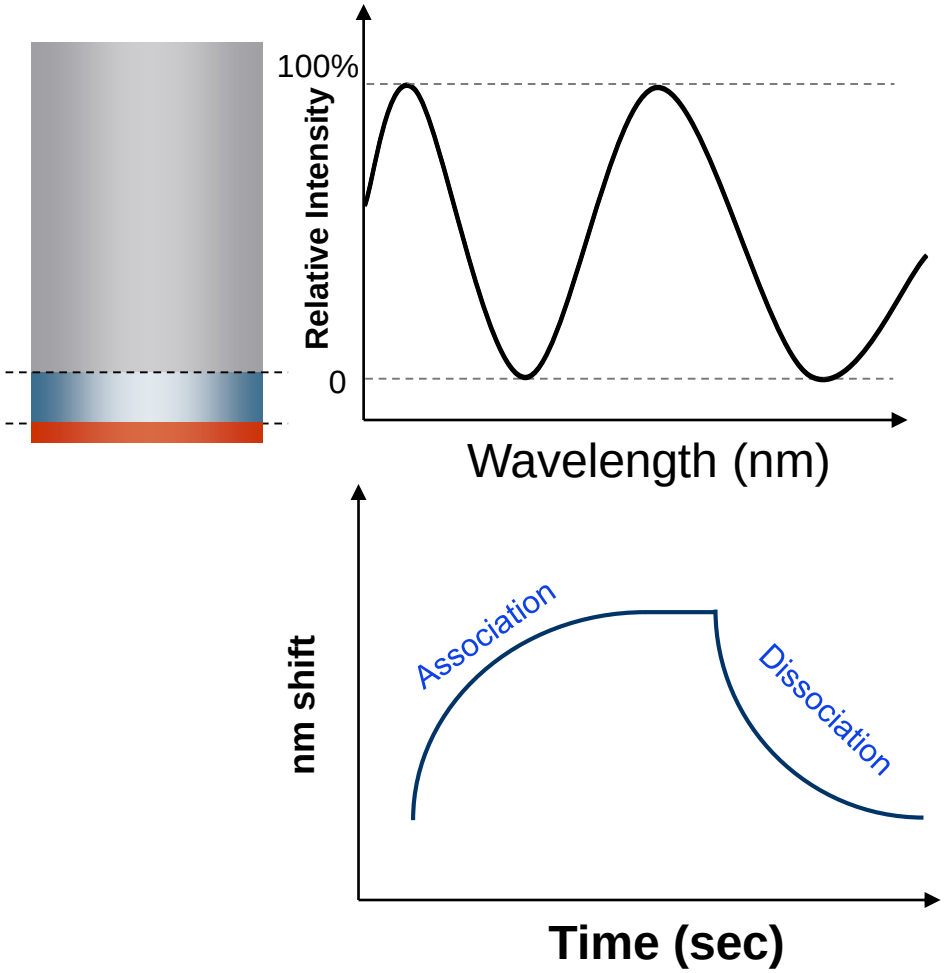
Monitoring nm-shift Against Time





- Analyte binding changes bio-layer thickness; thus, the increased distance that the light travels is detected

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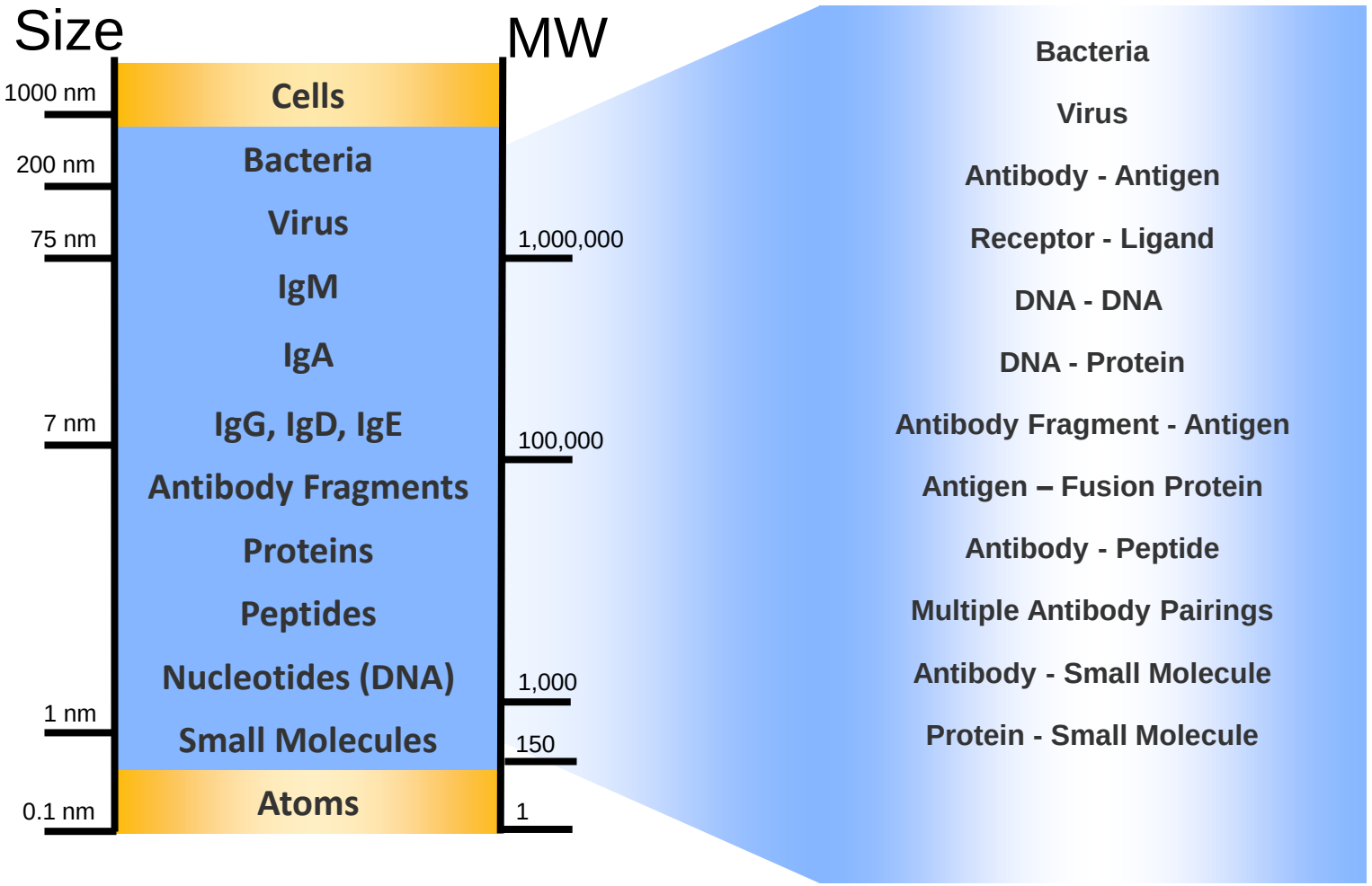
Octet Versatility in Interaction Analysis



Current Octet assays within blue area

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Octet Models





	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 (approximate)	1 mM to 10 pM	1 mM to 10 pM	0.1 mM to 10 pM	1 mM to 10 pM	1 mM to 10 pM
Sample Usage	Non-destructive and recoverable				
Temperature Control	15 – 40 ° C	4° C above ambient 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				

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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**
Immobilization	
• Amine Reactive 2nd Gen (AR2G)	Covalent coupling to reactive amine groups
• Aminopropylsilane (APS)	Adsorption to hydrophobic moieties

- Quantitation biosensors listed in black

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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

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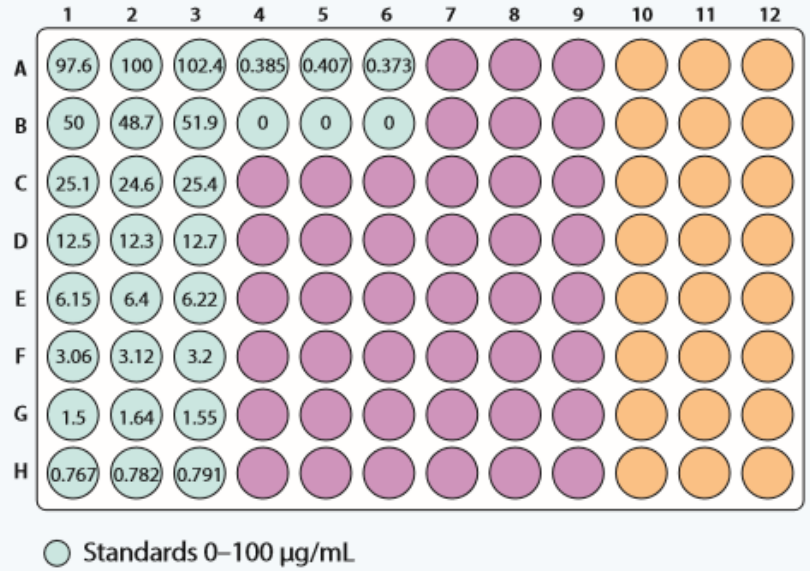
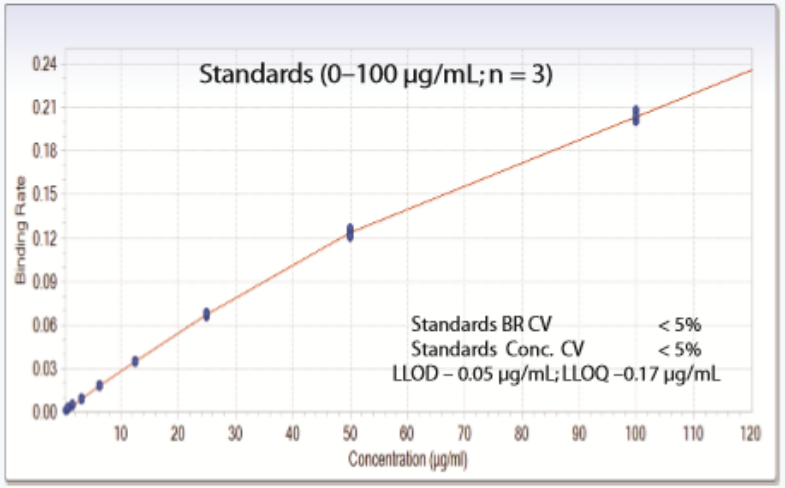
Quant Experimental Setup – Start with a Standard Curve





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

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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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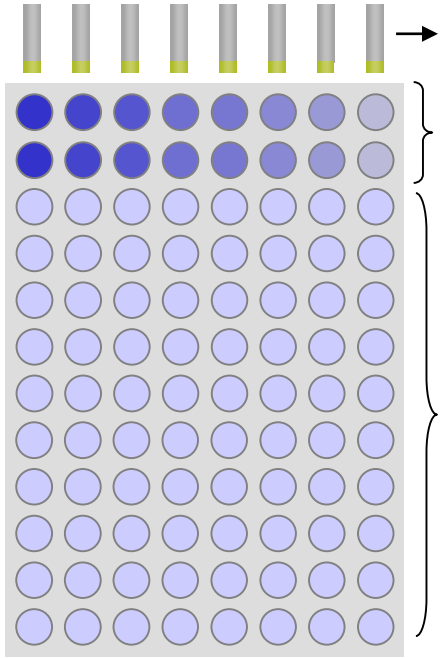
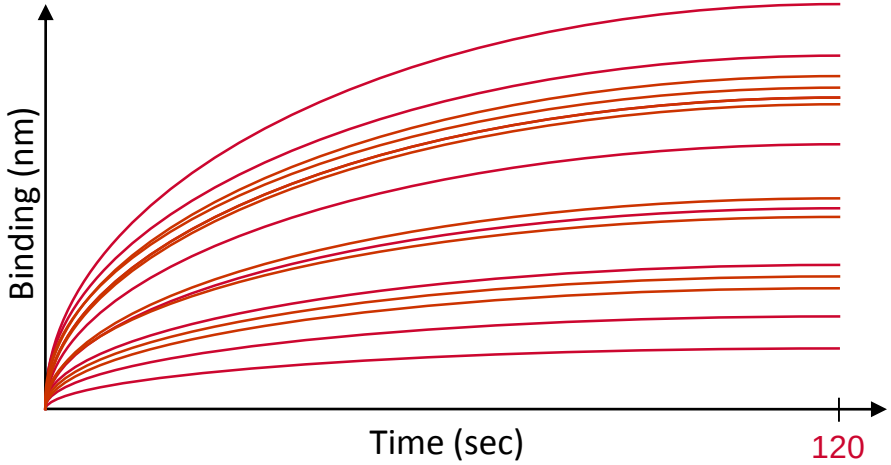
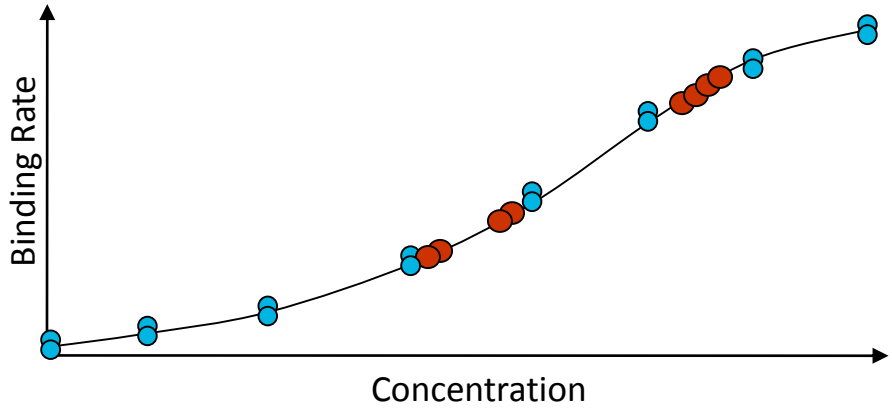
Octet Workflow for Quantitation







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

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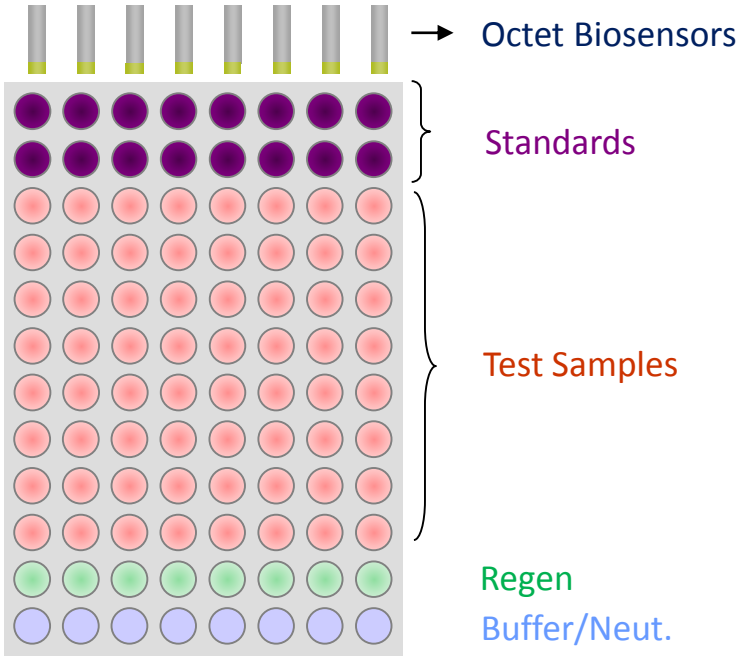
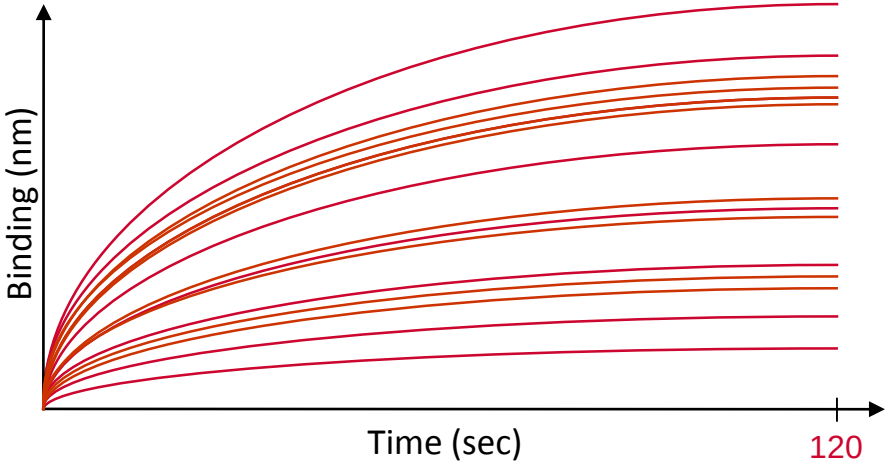
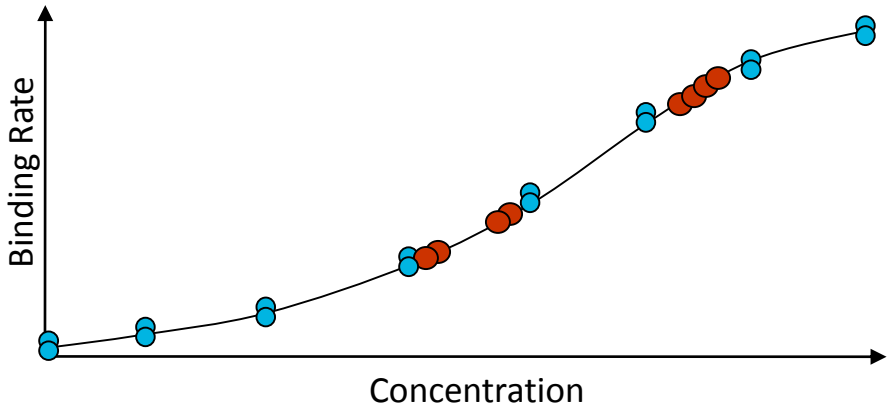
Octet Workflow for Quant with Regeneration







- 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

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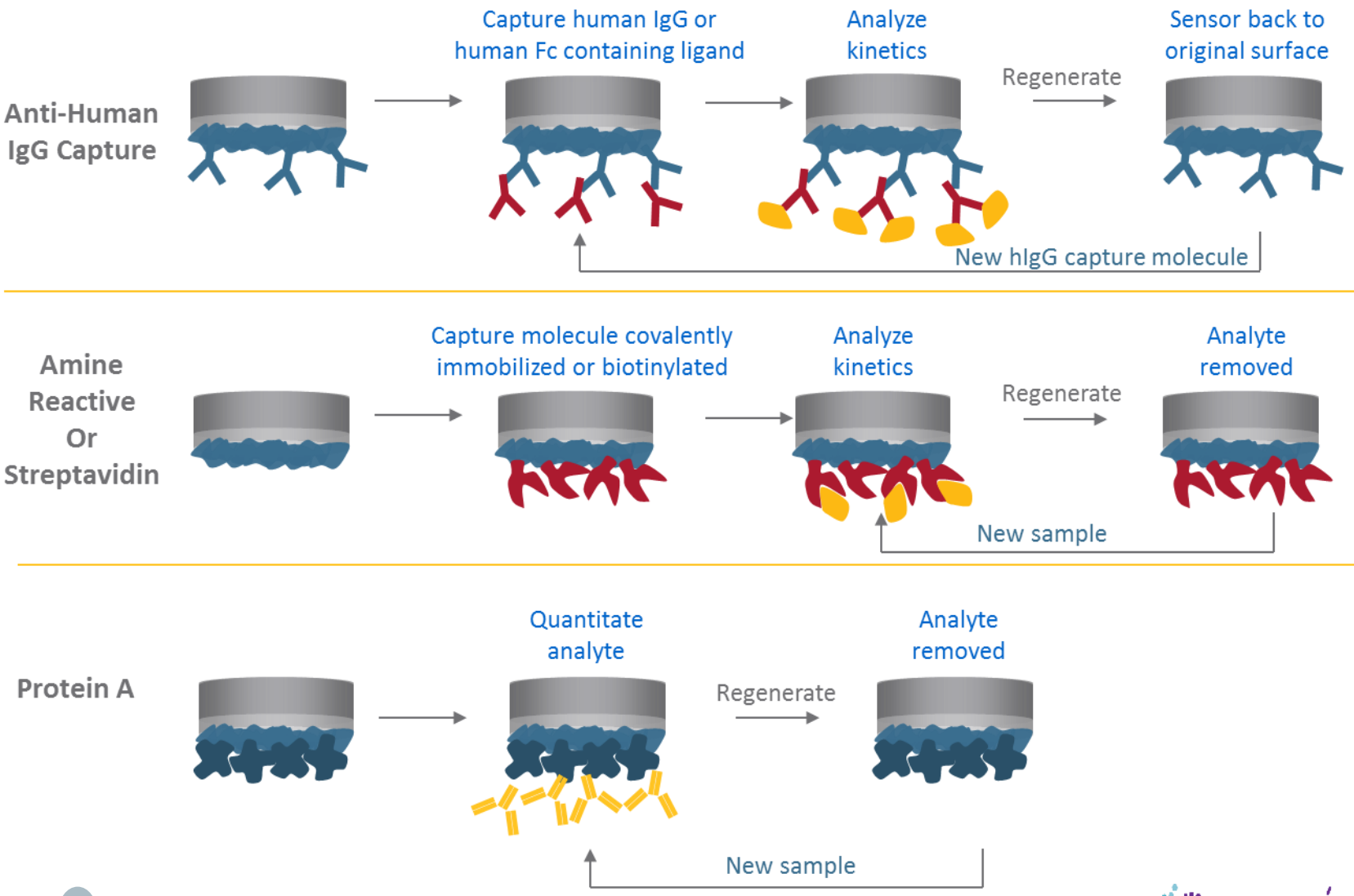
Considerations for Biosensor Regeneration



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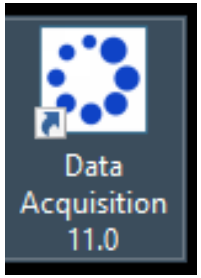
Basic Quantitation Workflow with Data Acquisition V11



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

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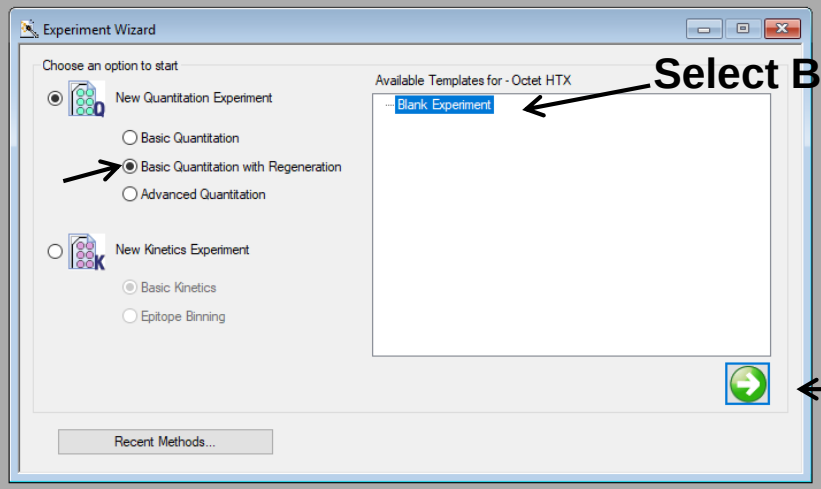
Select Assay Type

For 384 and HTX models, click on the Present Button to open the door and the stage will come out.

Select Basic Quantitation or Basic Quantitation with Regeneration



Select Blank Experiment

Click to Start

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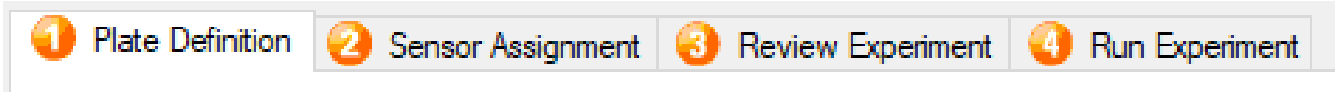
Acquisition Steps for Basic Quantitation

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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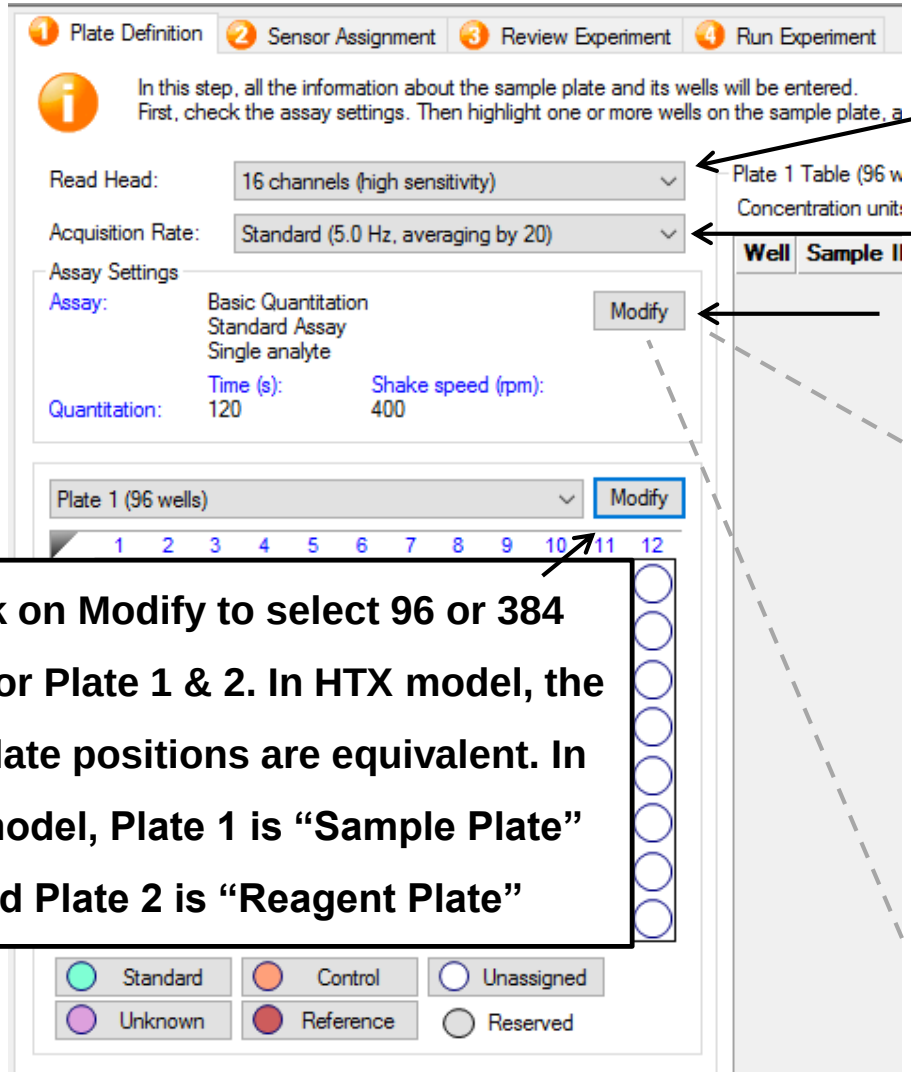
Tab 1. Plate Definition – Assay Settings

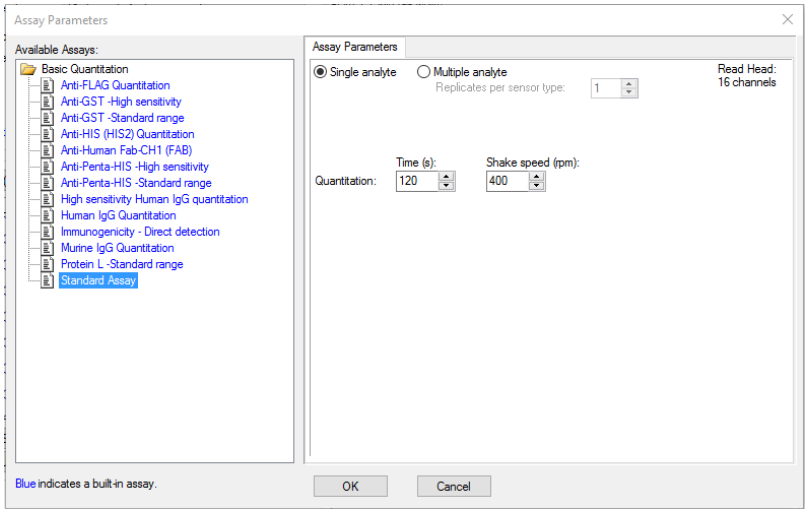


Select Number of Read Heads

(8, 16, 32, 48, or 96; instrument dependent)

If desired, click here to modify the Acquisition Rate

Select Modify to adjust Step Time & Shake Speed



Click on Modify to select 96 or 384 wells for Plate 1 & 2. In HTX model, the two plate positions are equivalent. In 384 model, Plate 1 is "Sample Plate" and Plate 2 is "Reagent Plate"

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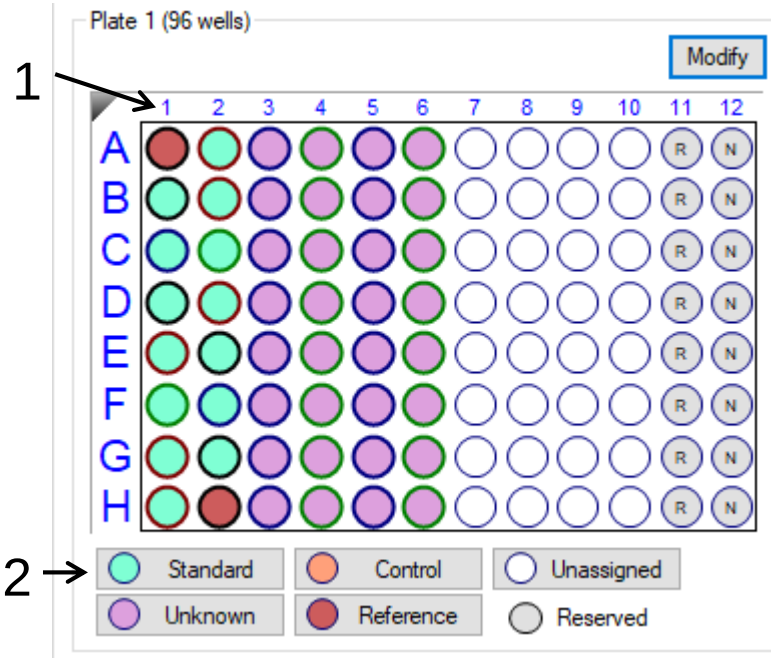
Tab 1. Plate Definition (96-well Plate)

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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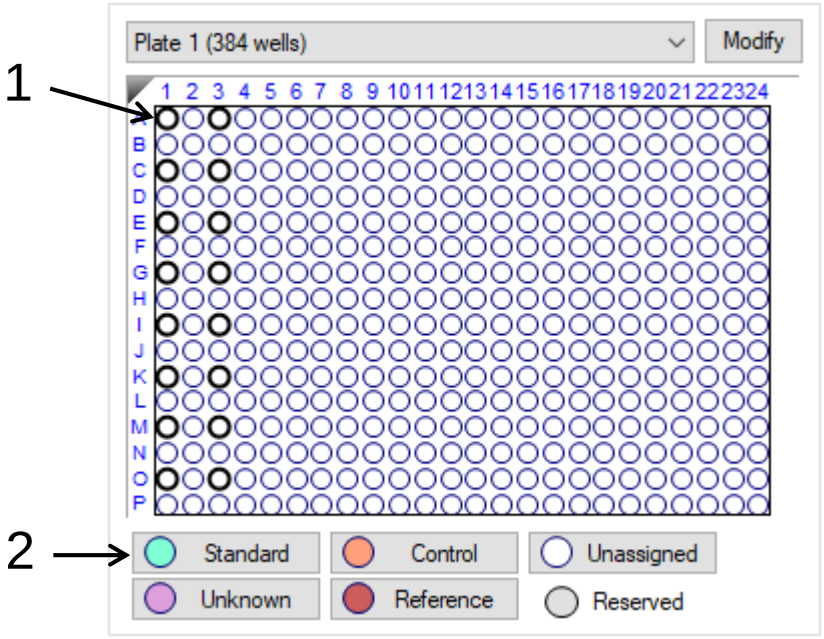
Tab 1. Plate Definition (384-well Plate)

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



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Tab 1. Plate Definition (96 well Plate)

3. Make sure that units are correct for the assay

2. Add concentration

1. Label Sample ID →

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

Plate 1 Table (96 wells)

Concentration units: µg/ml | Export... | Import... | Print...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	hIgG in Sample Diluent	0	Reference	n/a	n/a	
B1	hIgG in Sample Diluent	3	Standard	3	n/a	
C1	hIgG in Sample Diluent	10	Standard	10	n/a	
D1	hIgG in Sample Diluent	30	Standard	30	n/a	
E1	hIgG in Sample Diluent	100	Standard	100	n/a	
F1	hIgG in Sample Diluent	300	Standard	300	n/a	
G1	hIgG in Sample Diluent	500	Standard	500	n/a	
H1	hIgG in Sample Diluent	700	Standard	700	n/a	
A2	hIgG in Sample Diluent	700	Standard	700	n/a	
B2	hIgG in Sample Diluent	500	Standard	500	n/a	
C2	hIgG in Sample Diluent	300	Standard	300	n/a	
D2	hIgG in Sample Diluent	100	Standard	100	n/a	
E2	hIgG in Sample Diluent	30	Standard	30	n/a	
F2	hIgG in Sample Diluent	10	Standard	10	n/a	
G2	hIgG in Sample Diluent	3	Standard	3	n/a	
H2	hIgG in Sample Diluent	0	Reference	n/a	n/a	
A3	hIgG in Sample Diluent	3a	Unknown	n/a		3
B3	hIgG in Sample Diluent	3a	Unknown	n/a		3
C3	hIgG in Sample Diluent	3a	Unknown	n/a		3
D3	hIgG in Sample Diluent	3a	Unknown	n/a		3
E3	hIgG in Sample Diluent	10a	Unknown	n/a		10
F3	hIgG in Sample Diluent	10a	Unknown	n/a		10
G3	hIgG in Sample Diluent	10a	Unknown	n/a		10
H3	hIgG in Sample Diluent	10a	Unknown	n/a		10
A4	hIgG in Sample Diluent	100a	Unknown	n/a		100
B4	hIgG in Sample Diluent	100a	Unknown	n/a		100
C4	hIgG in Sample Diluent	100a	Unknown	n/a		100
D4	hIgG in Sample Diluent	100a	Unknown	n/a		100
E4	hIgG in Sample Diluent	500a	Unknown	n/a		500
F4	hIgG in Sample Diluent	500a	Unknown	n/a		500
G4	hIgG in Sample Diluent	500a	Unknown	n/a		500
H4	hIgG in Sample Diluent	500a	Unknown	n/a		500

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

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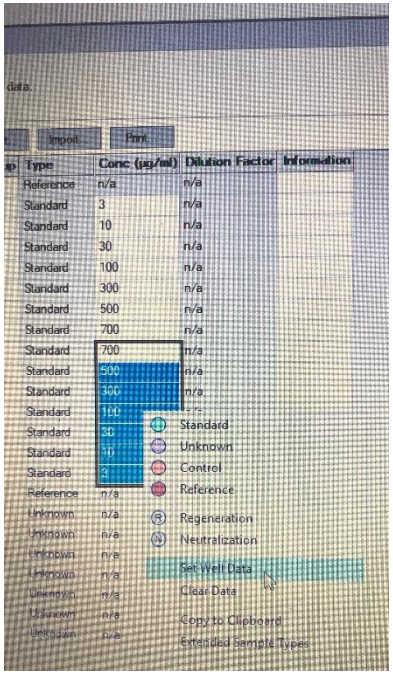
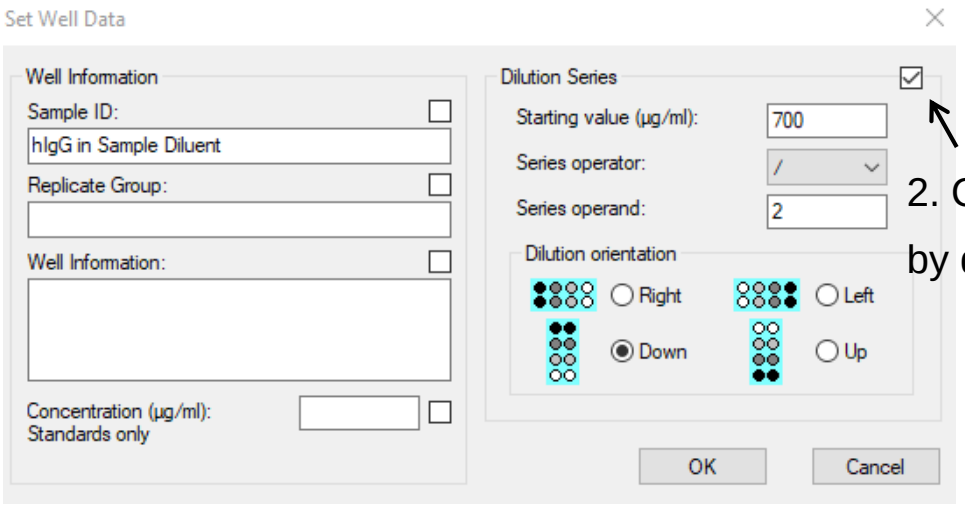
Tab 1. Plate Definition

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





2. Check the box by dilution series

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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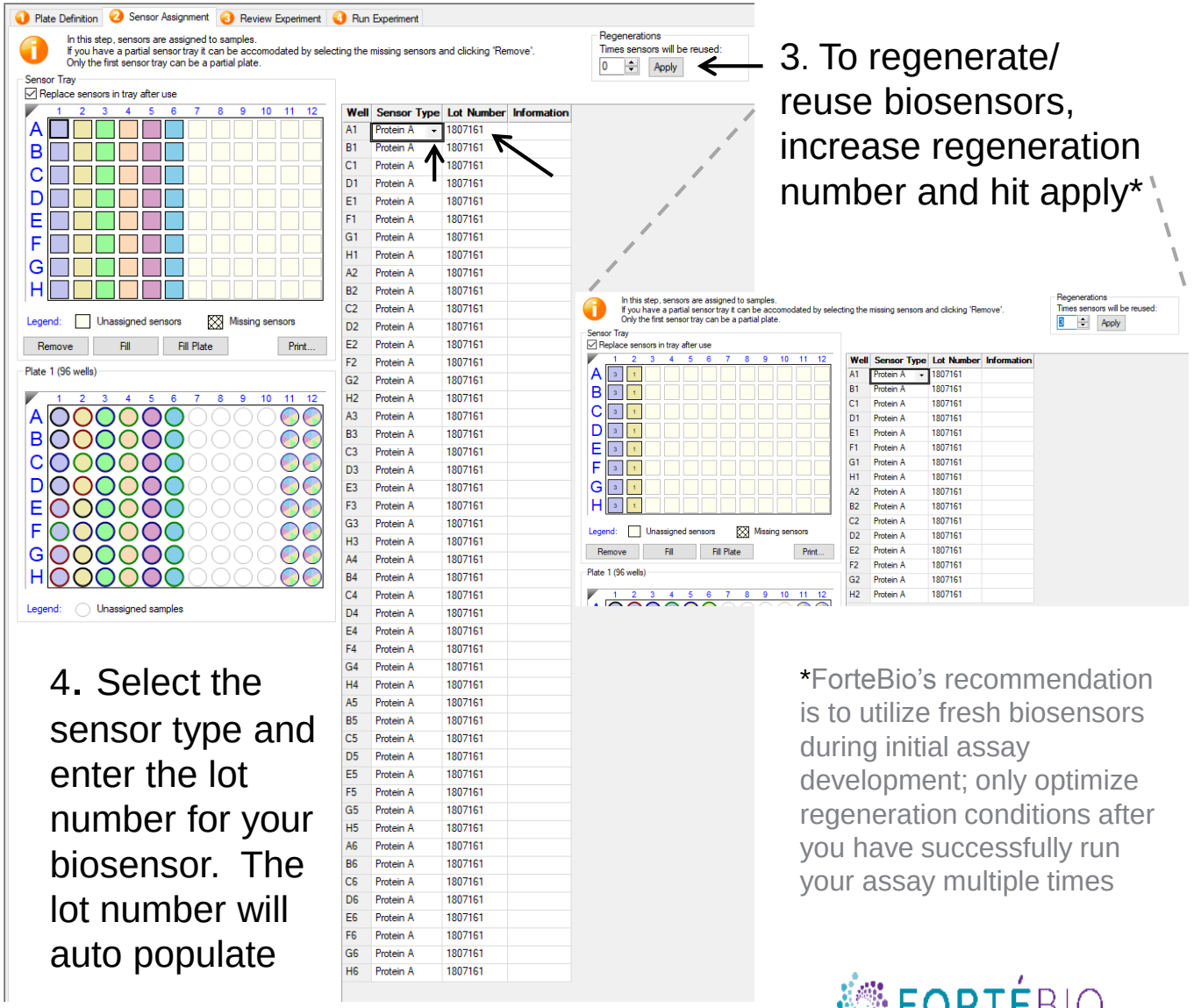
Tab 2. Sensor Assignment

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



3. To regenerate/ reuse biosensors, increase regeneration number and hit apply*

4. Select the sensor type and enter the lot number for your biosensor. The lot number will auto populate

*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

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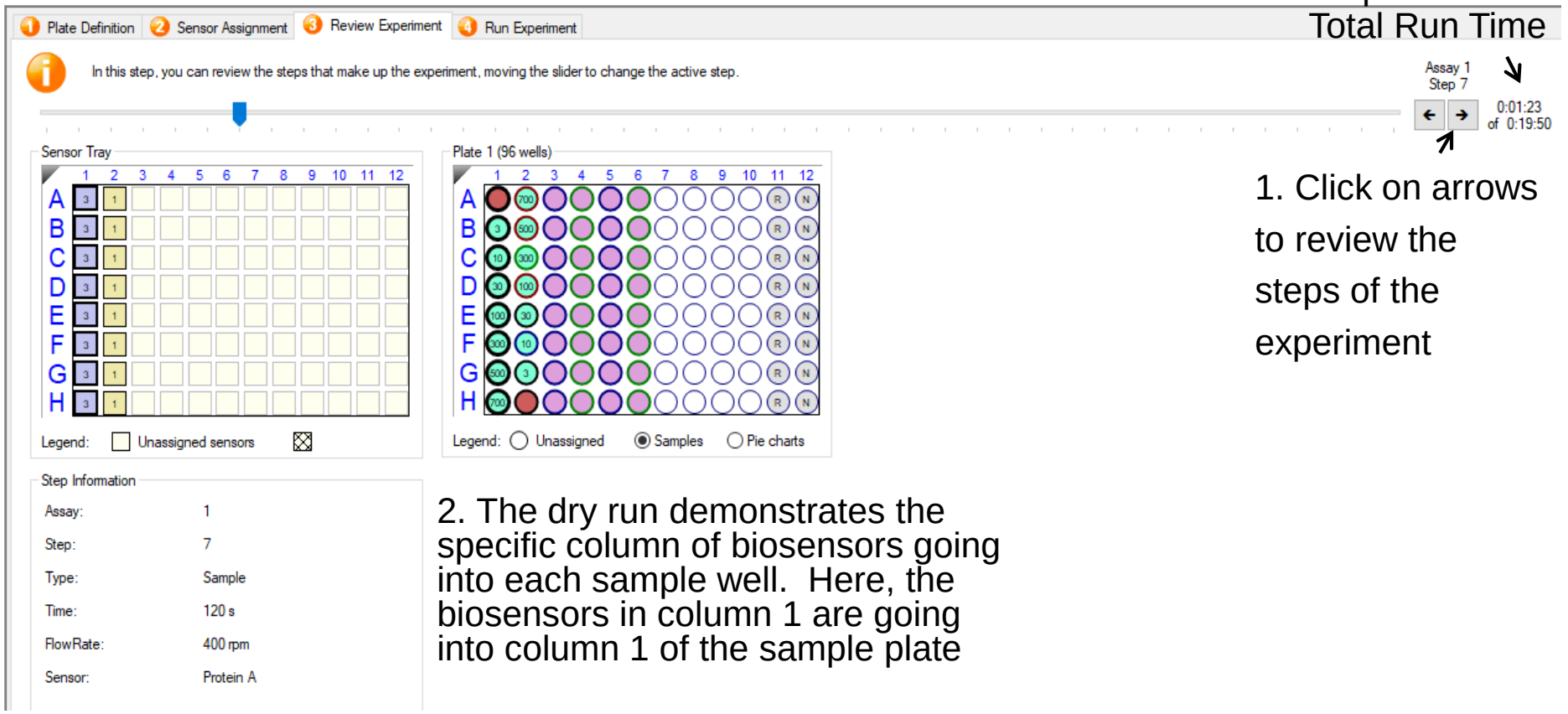
Tab 3. Review Experiment



1. Click on arrows to review the steps of the experiment

2. The dry run demonstrates the specific column of biosensors going into each sample well. Here, the biosensors in column 1 are going into column 1 of the sample plate

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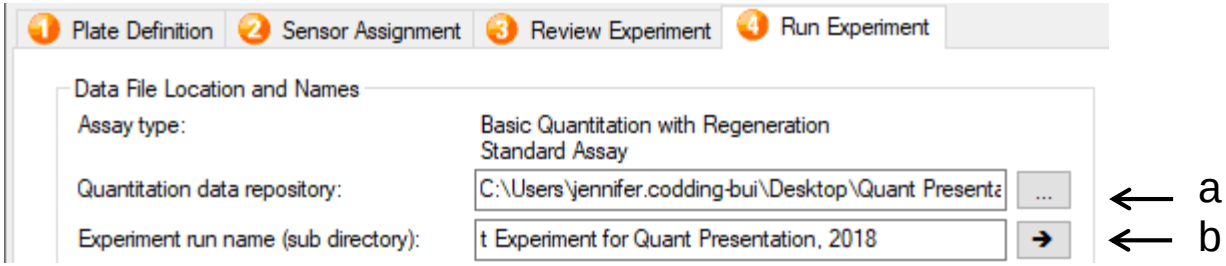
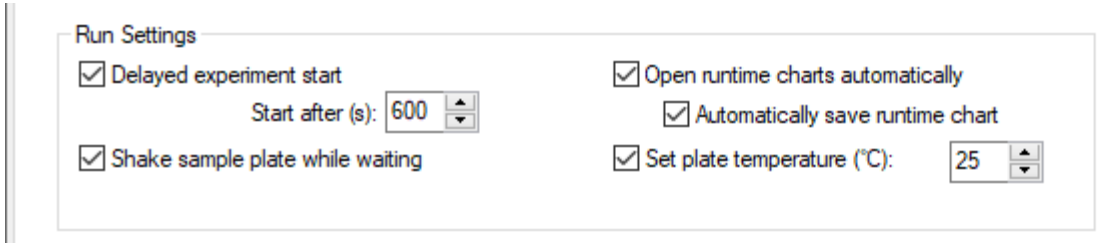
Tab 4. Run Experiment

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.



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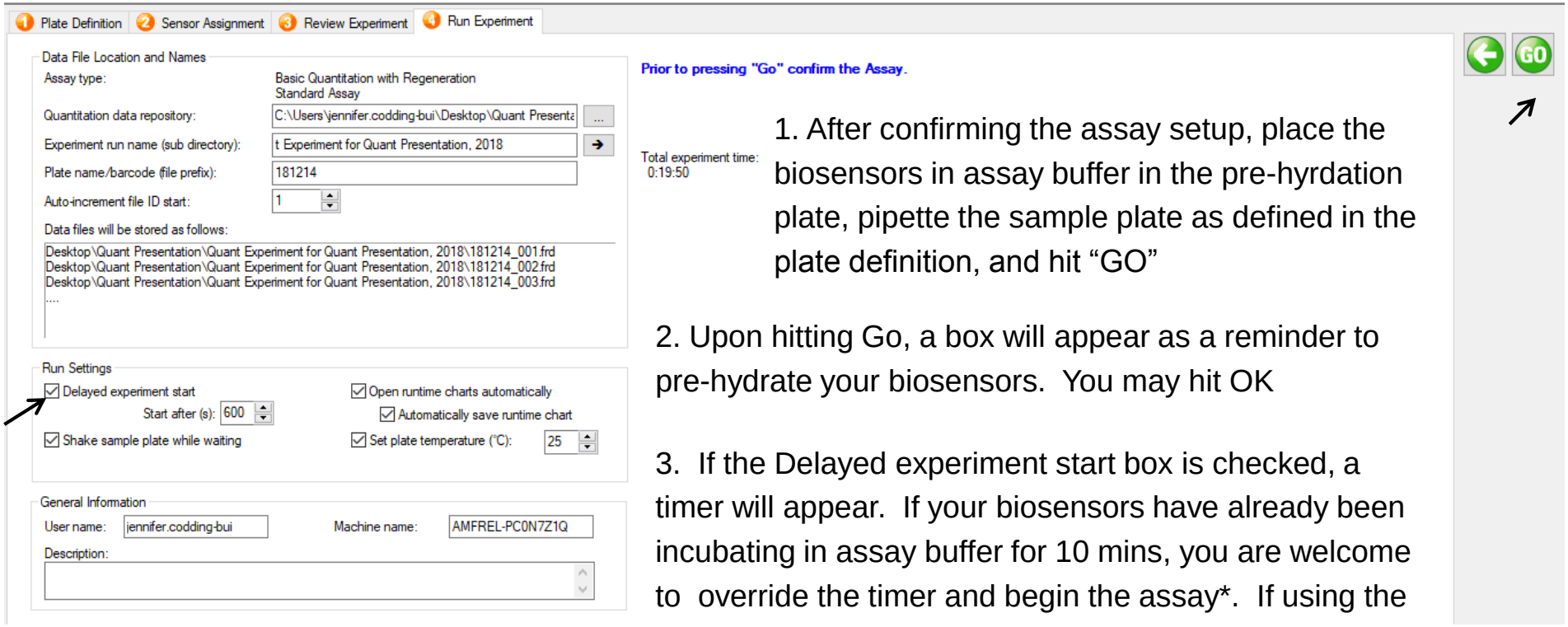
Tab 4. Run Experiment



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.

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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

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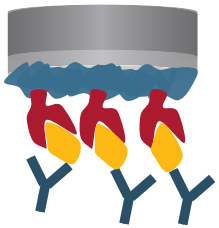
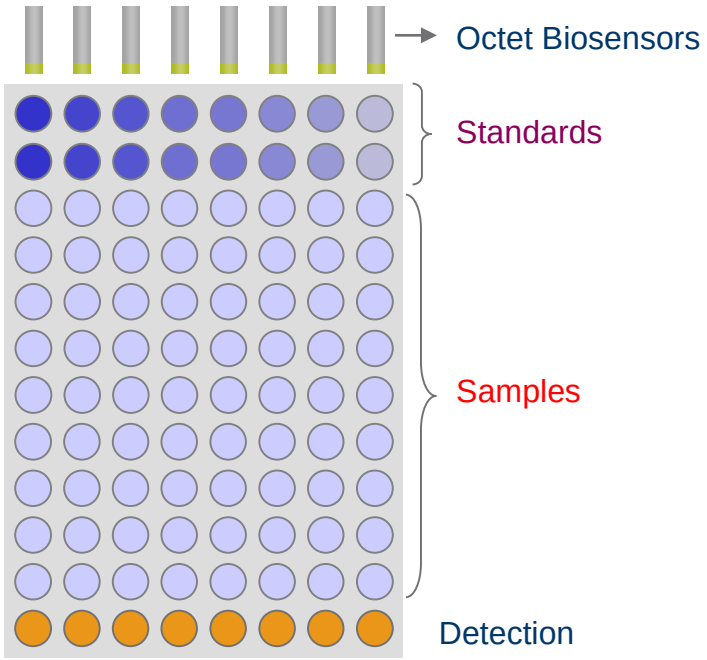
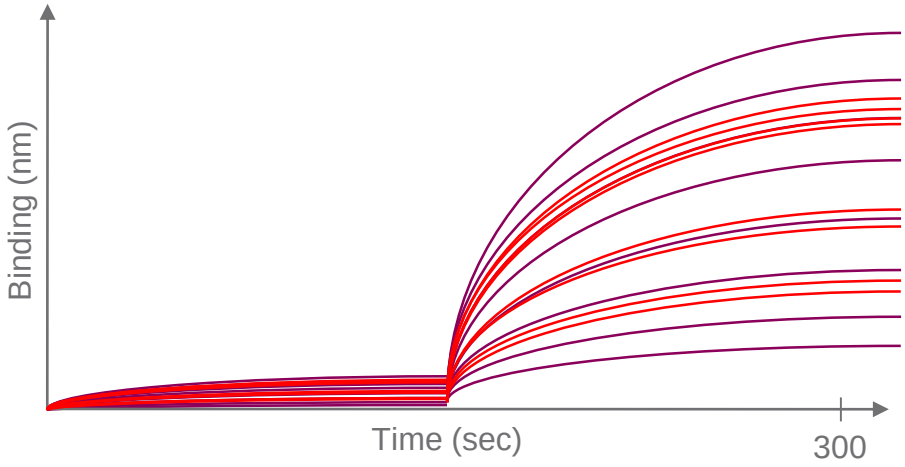
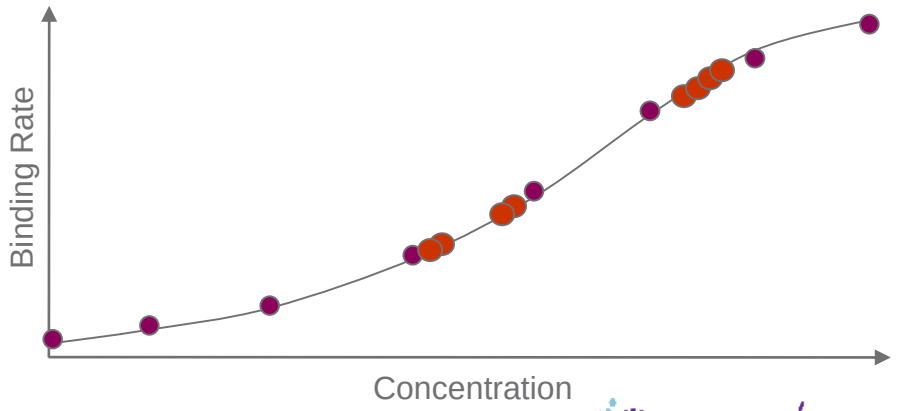
Octet Automated Workflow for 2-Steps Sandwich-style Quantitation









- Reuse detection reagent, which bind and amplify the initial analyte binding signal
- No plate washing needed

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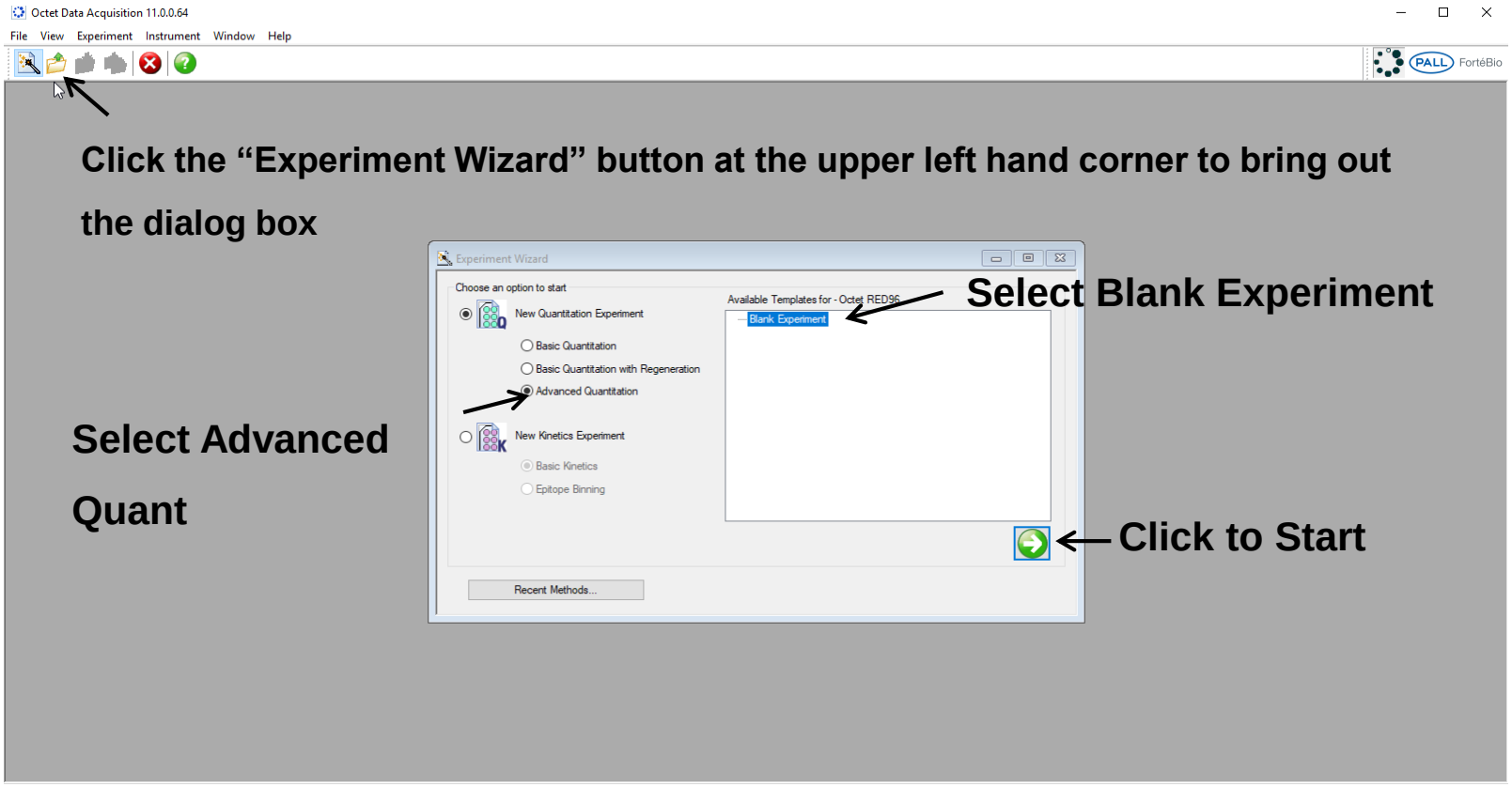
Select Assay Type



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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.

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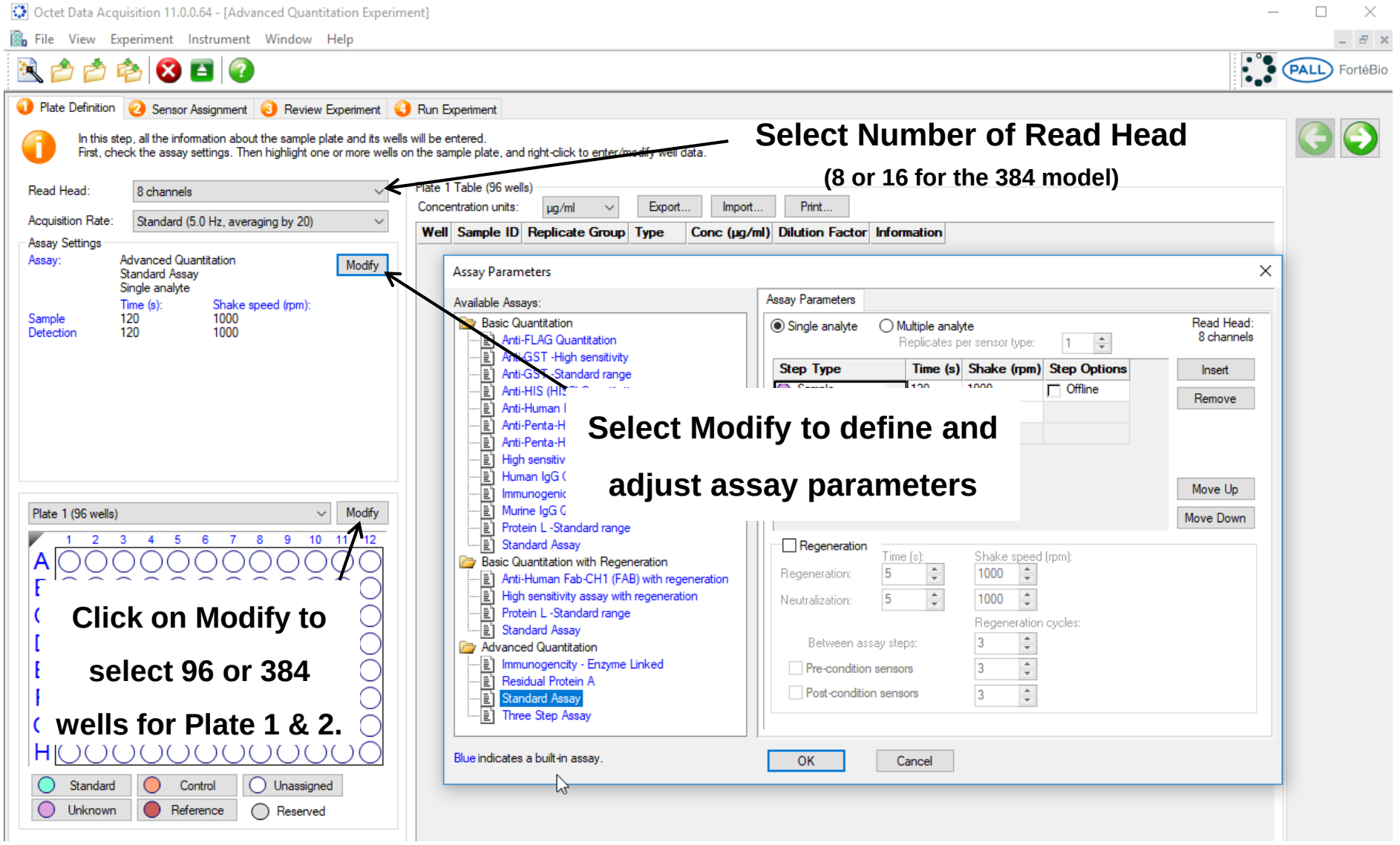
Tab 1. Plate Definition



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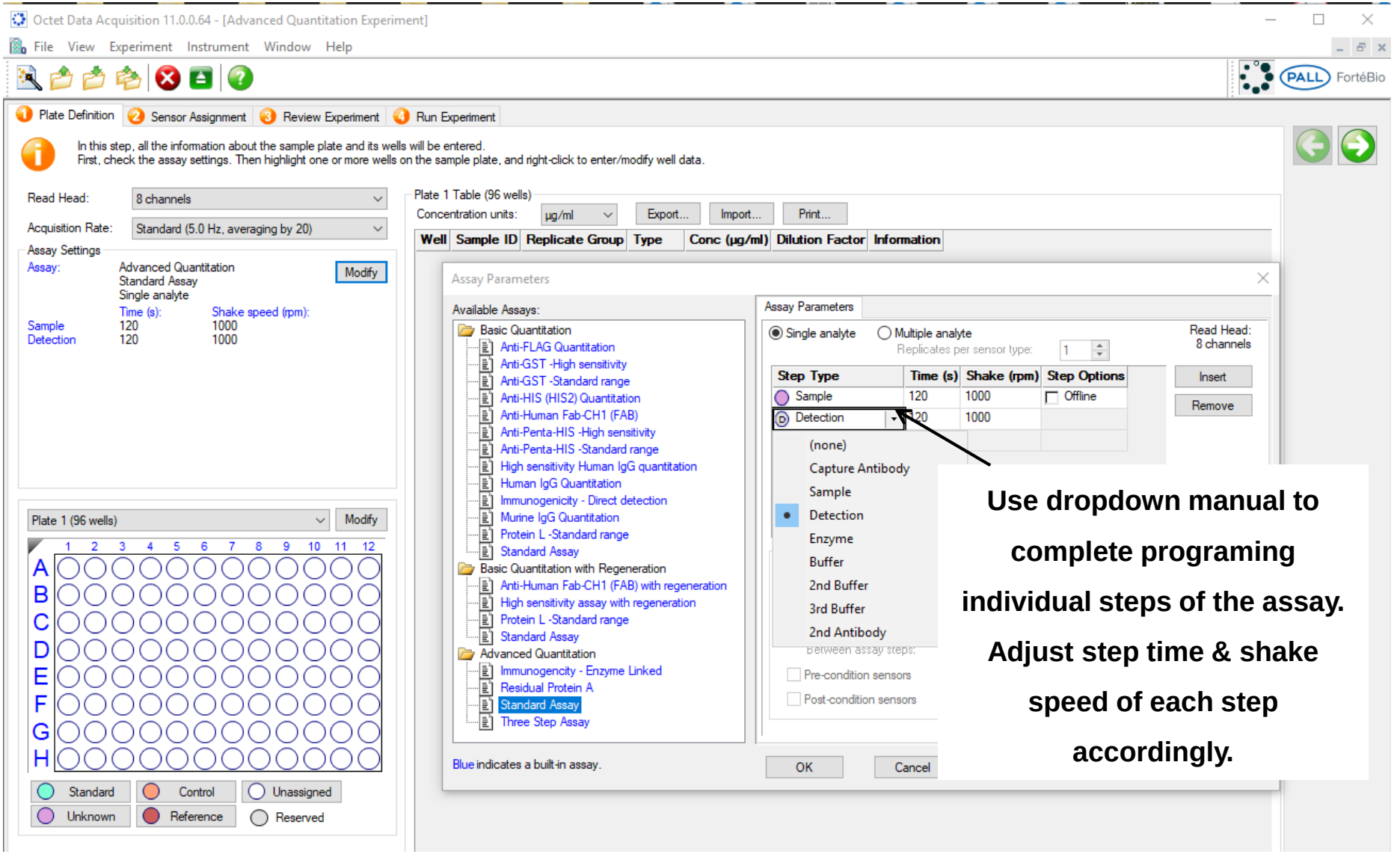
Tab 1. Plate Definition



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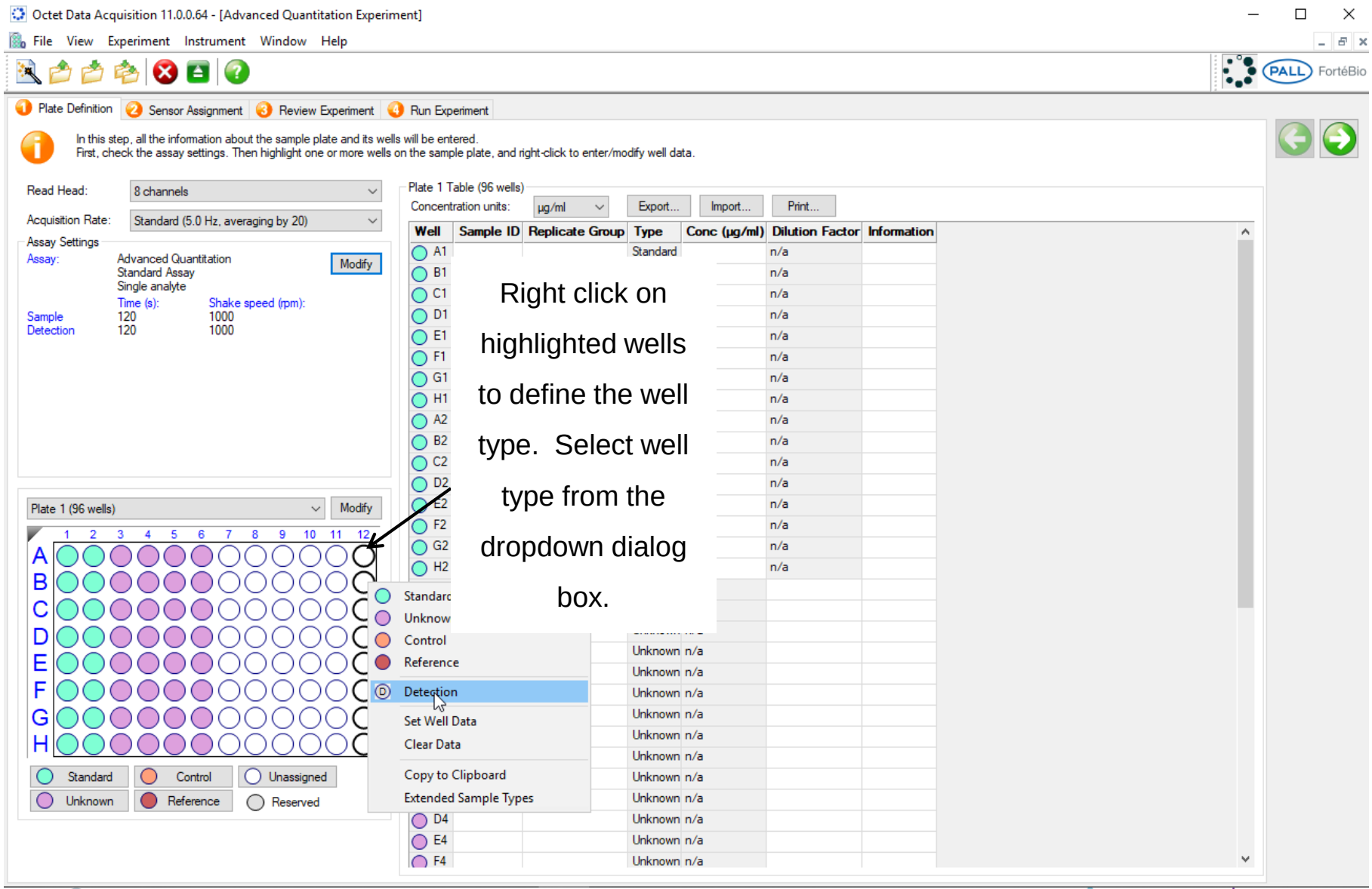
Tab 1. Plate Definition



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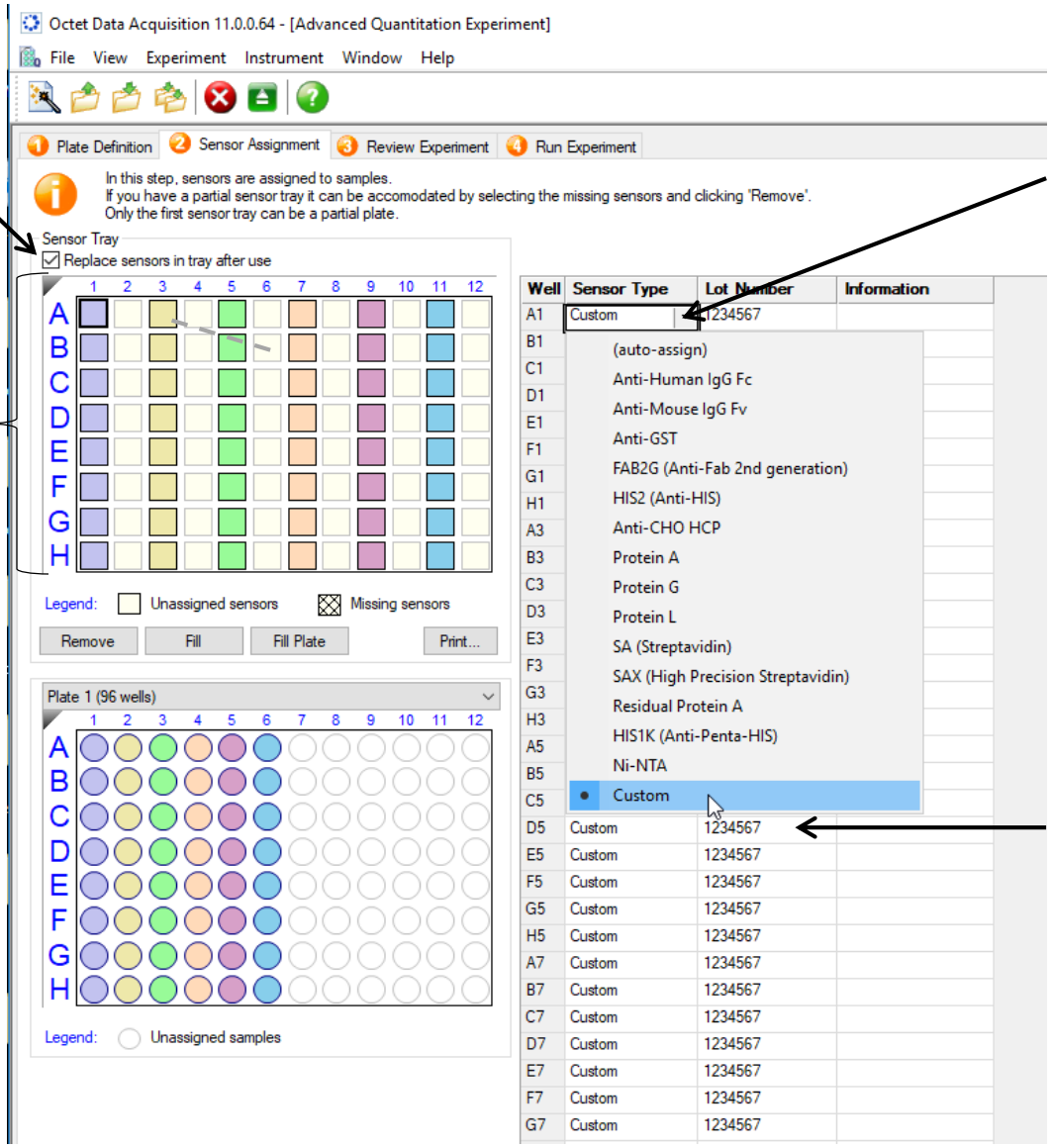
Tab 2. Sensor Assignment

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.

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Setting Up

Tab 3. Review Experiment &

Tab 4. Run Experiment

are the same as example shown for basic quantitation.

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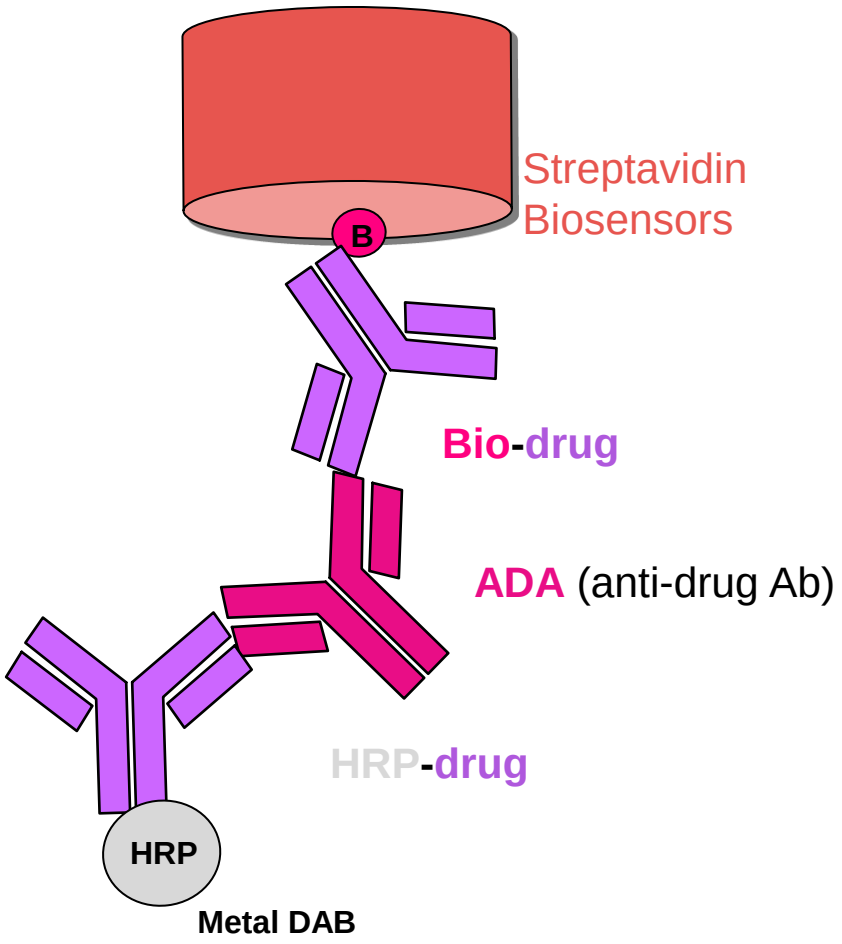
Multi-Step, Enzyme-linked Quantitation Assay



- 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

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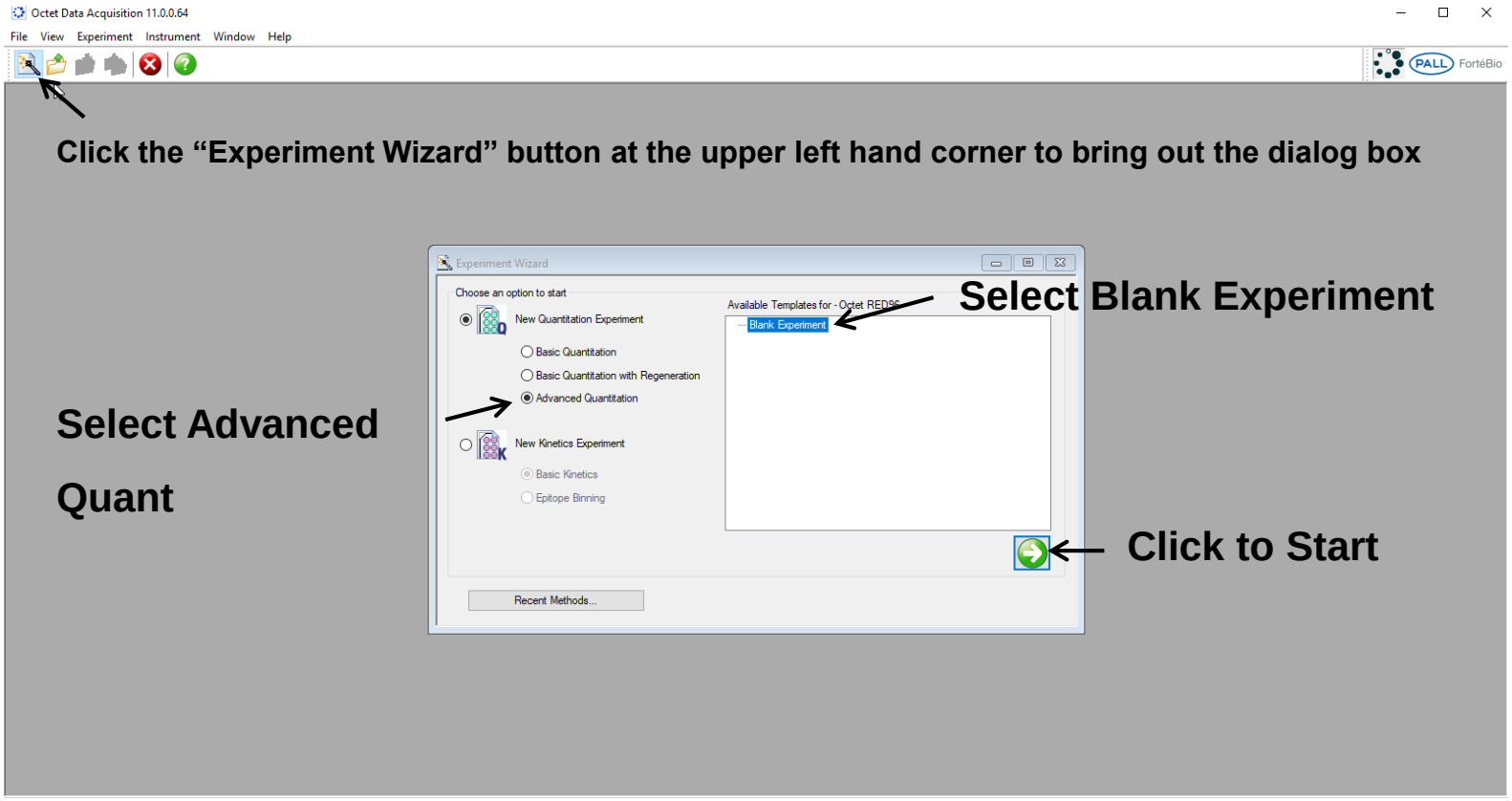
Select Assay Type



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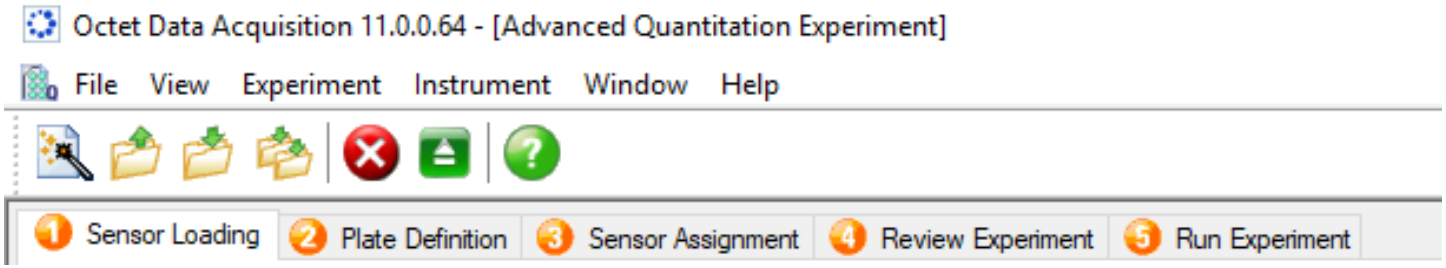
Acquisition Steps for Advanced Quantitation HTX Model

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.

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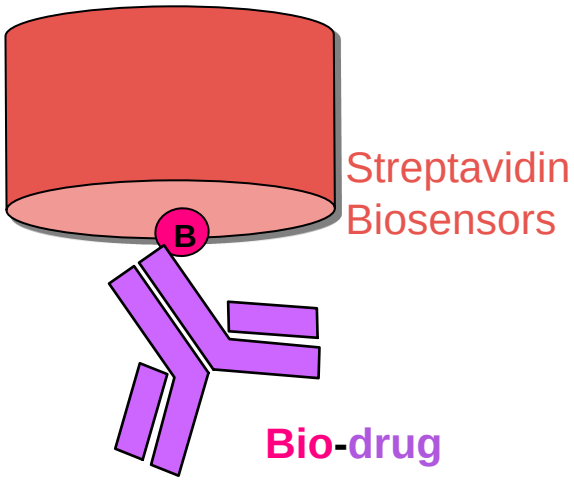
Creating Custom Biosensors



- 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

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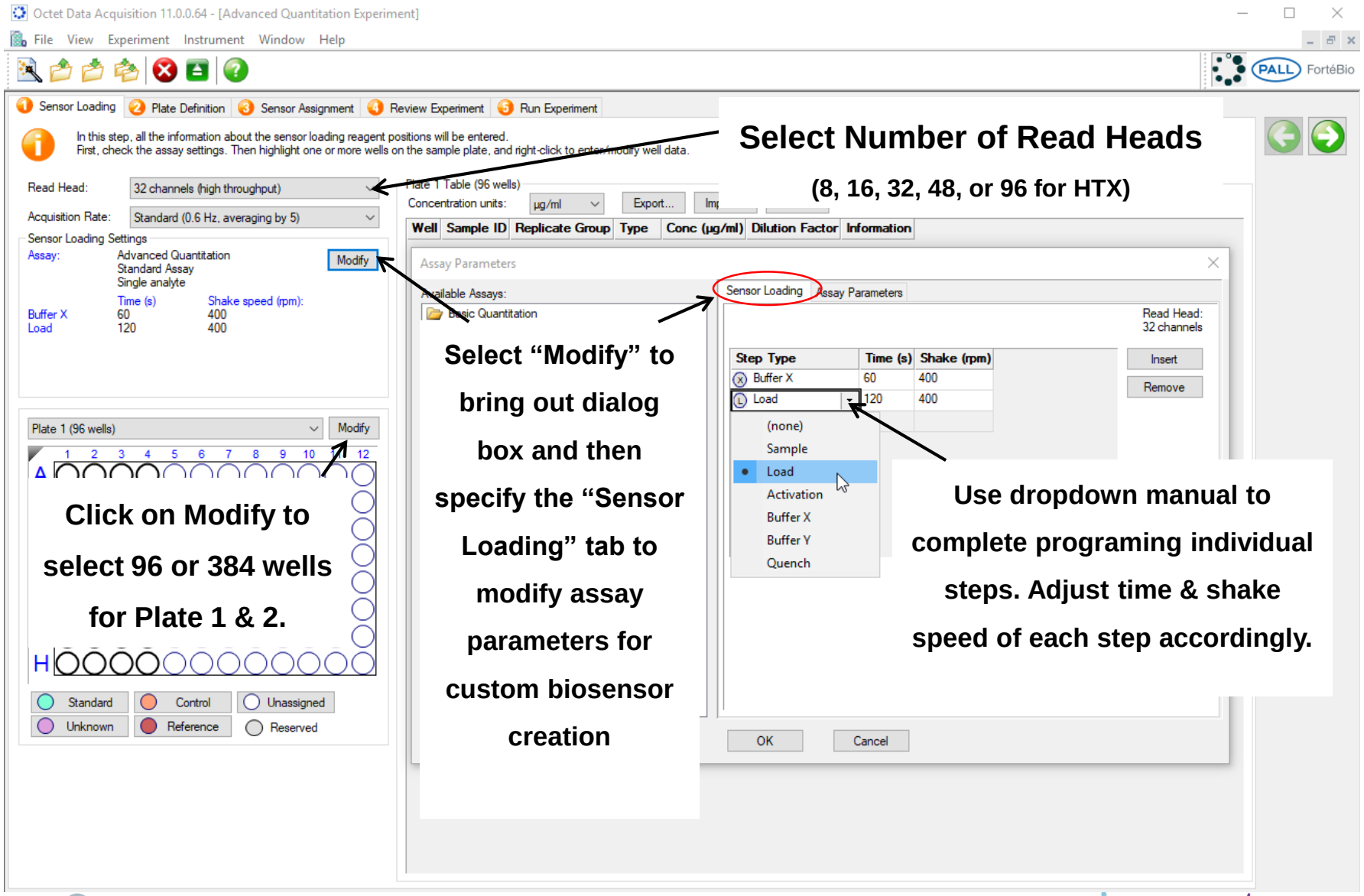
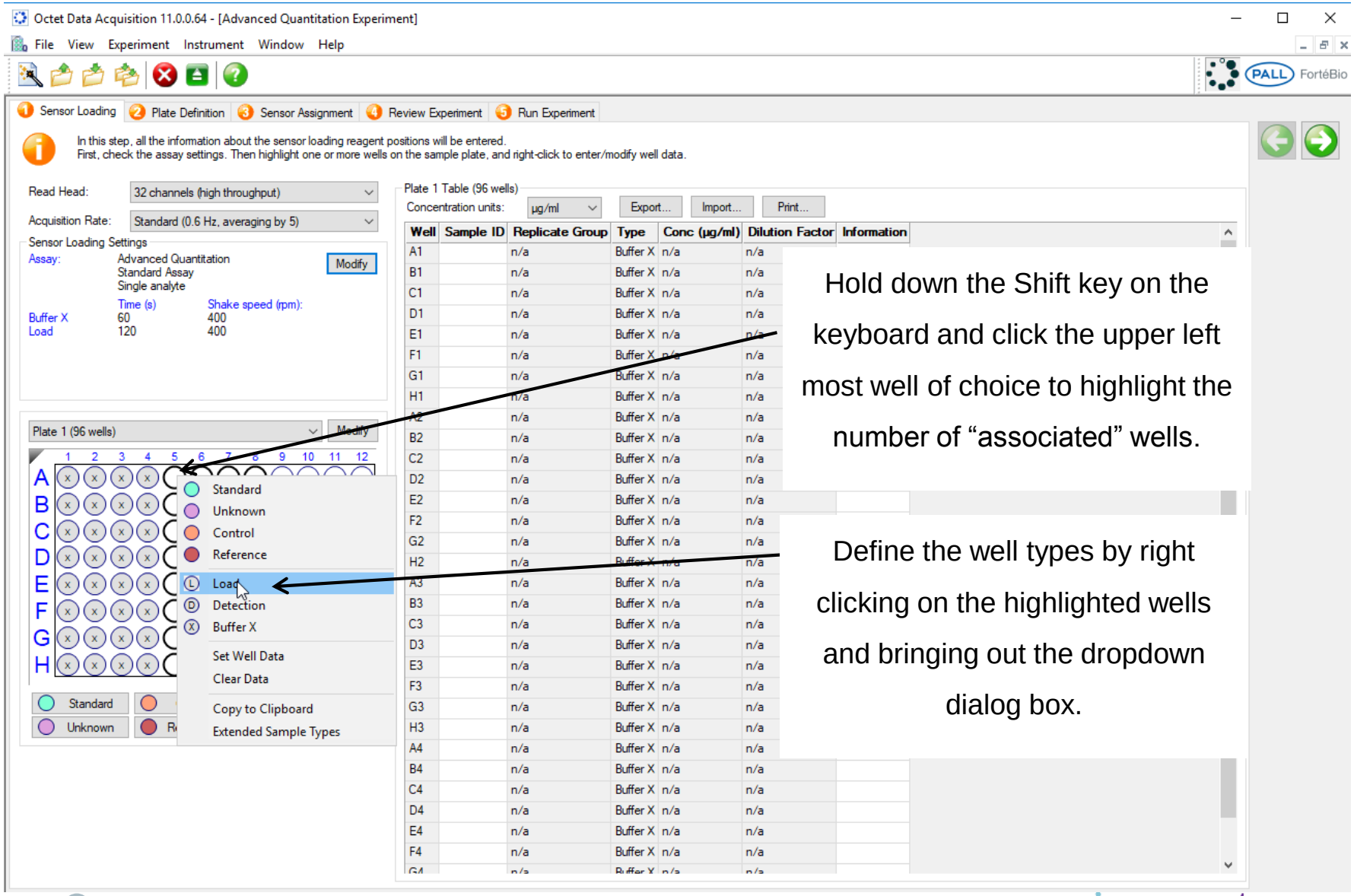
Tab 1. Sensor Loading



CONFIDENTIAL

Tab 1. Sensor Loading



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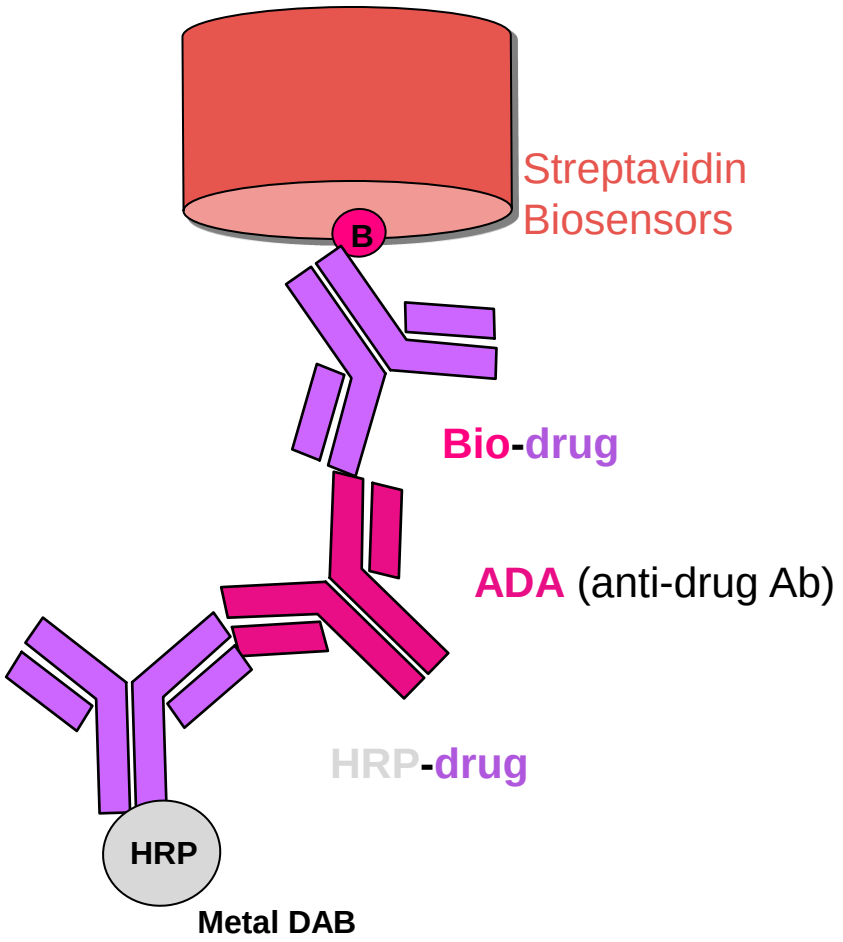
Multi-Steps Enzyme-linked Quantitation Assay



- 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

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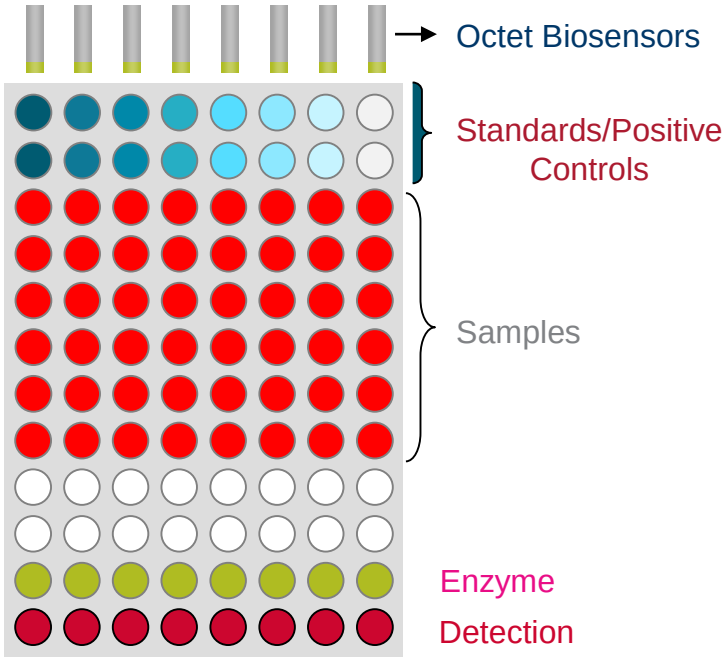
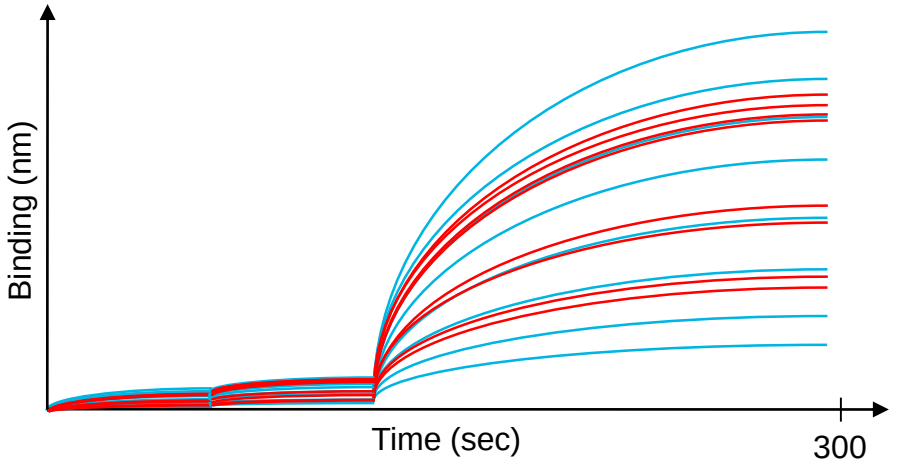
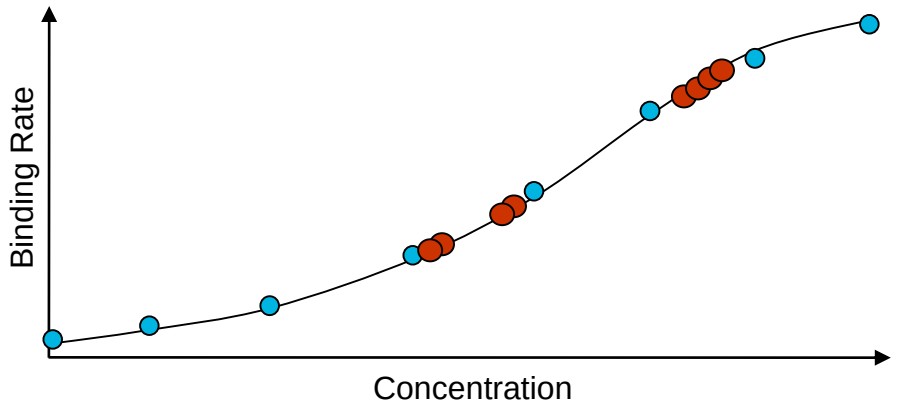
Octet Automated Workflow for Multi-Steps Enzyme-linked Quantitation Assay







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

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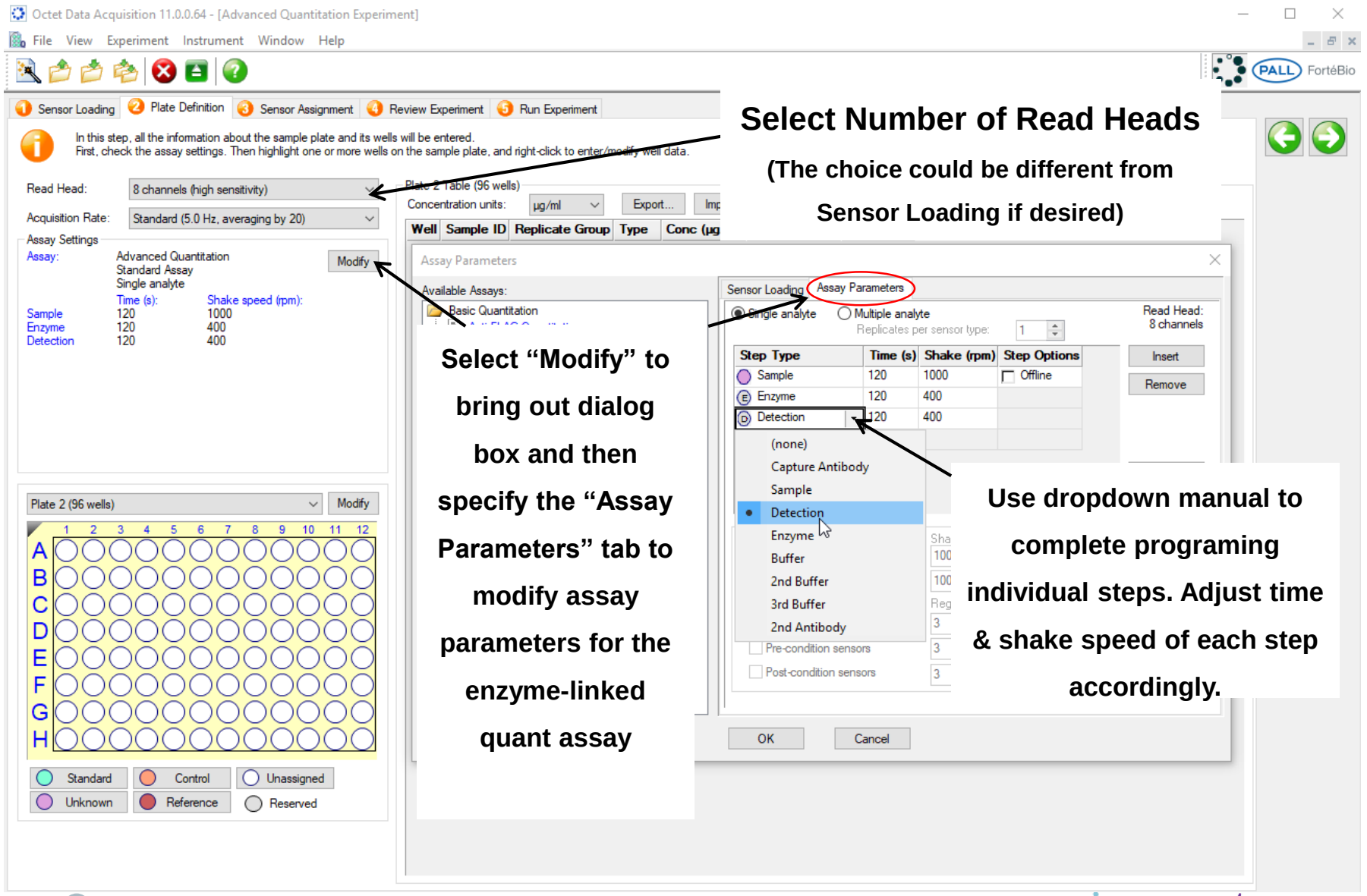
Tab 2. Plate Definition



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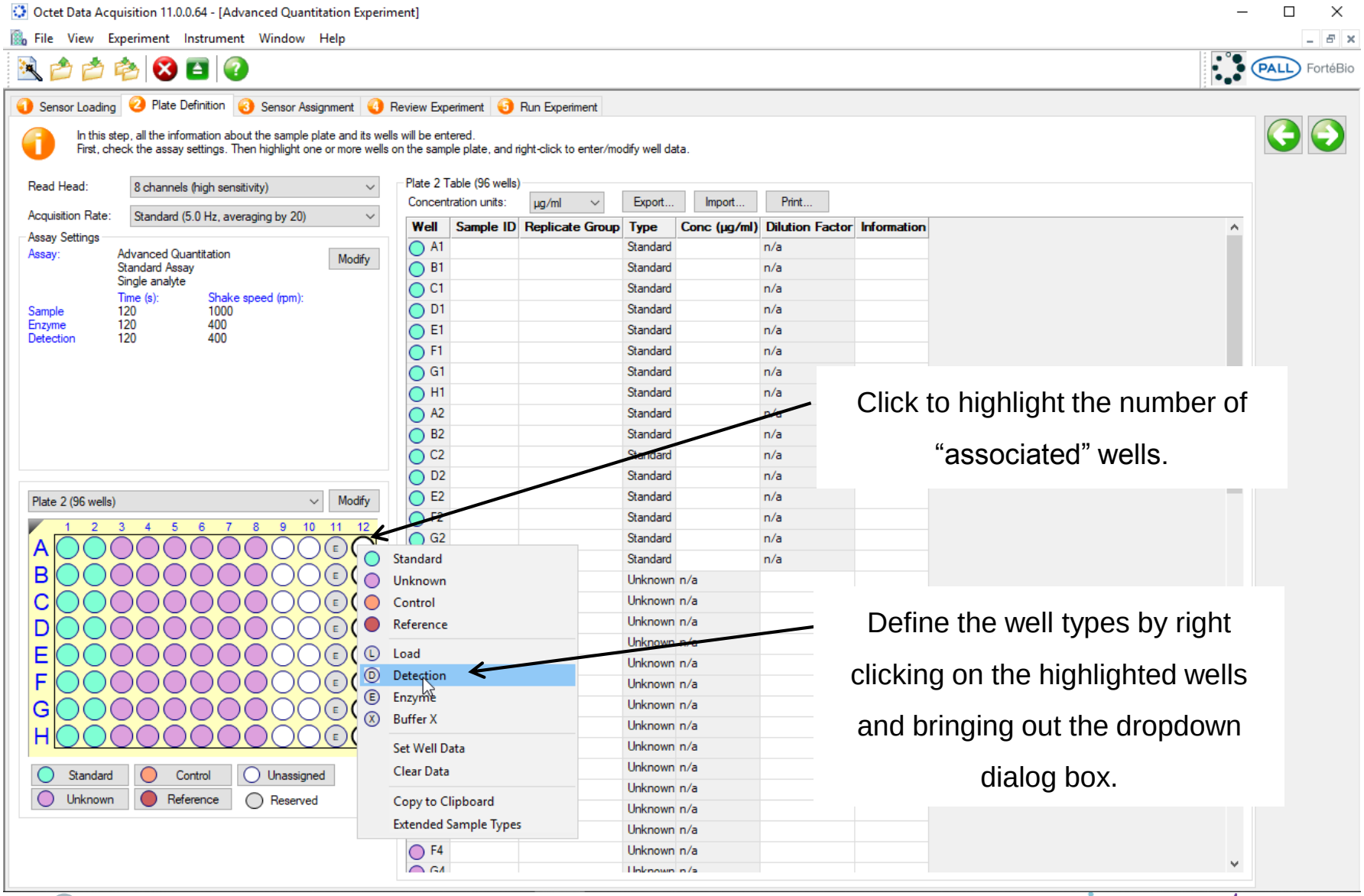
Tab 2. Plate Definition



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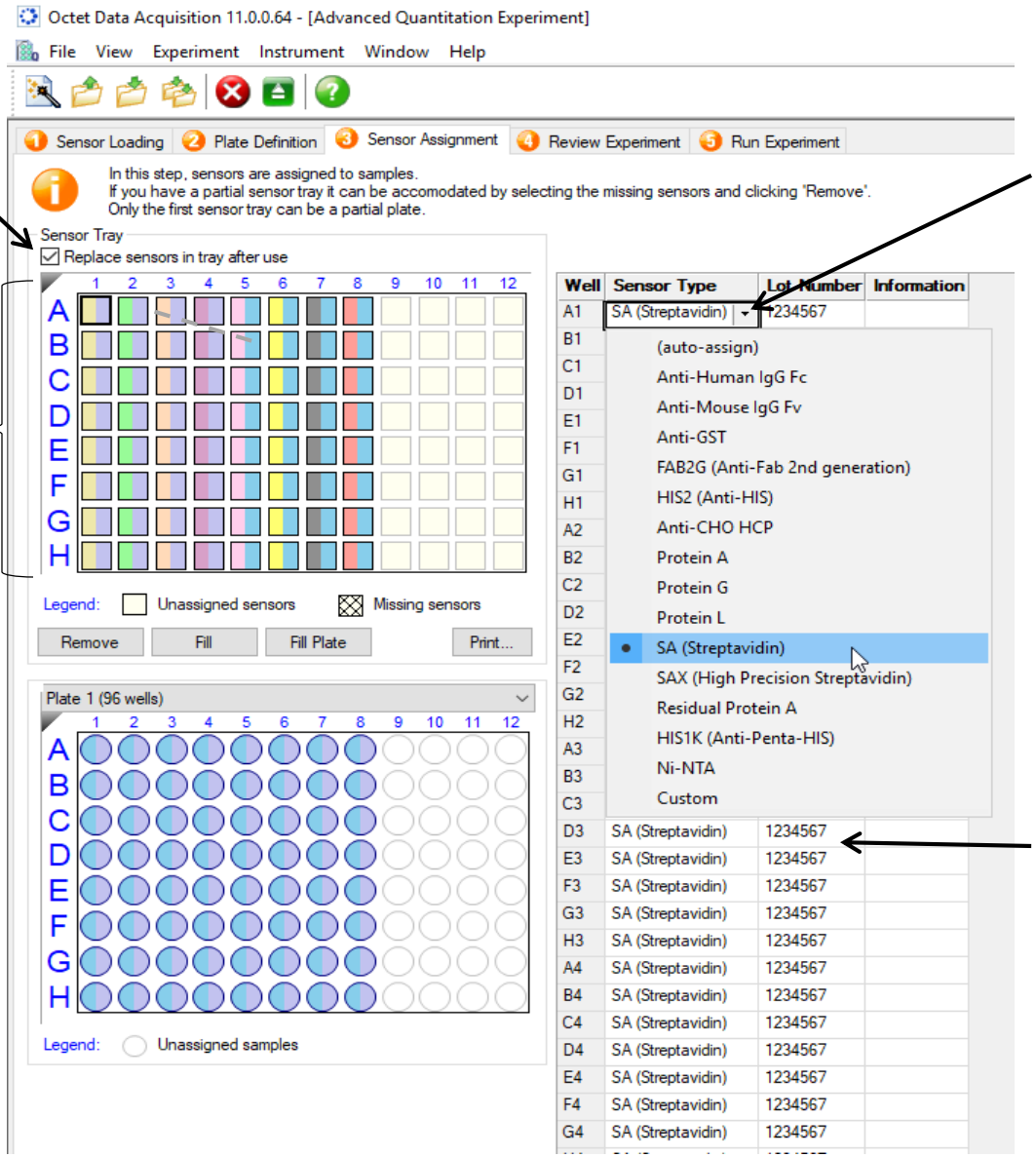
Tab 3. Sensor Assignment

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.

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Setting Up

Tab 4. Review Experiment &

Tab 5. Run Experiment

are the same as example shown for basic quantitation.

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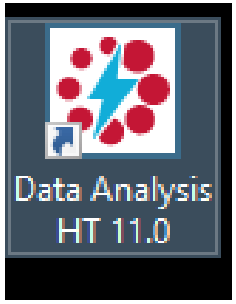
Advanced Quantitation Workflow with Data Analysis HT V11



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

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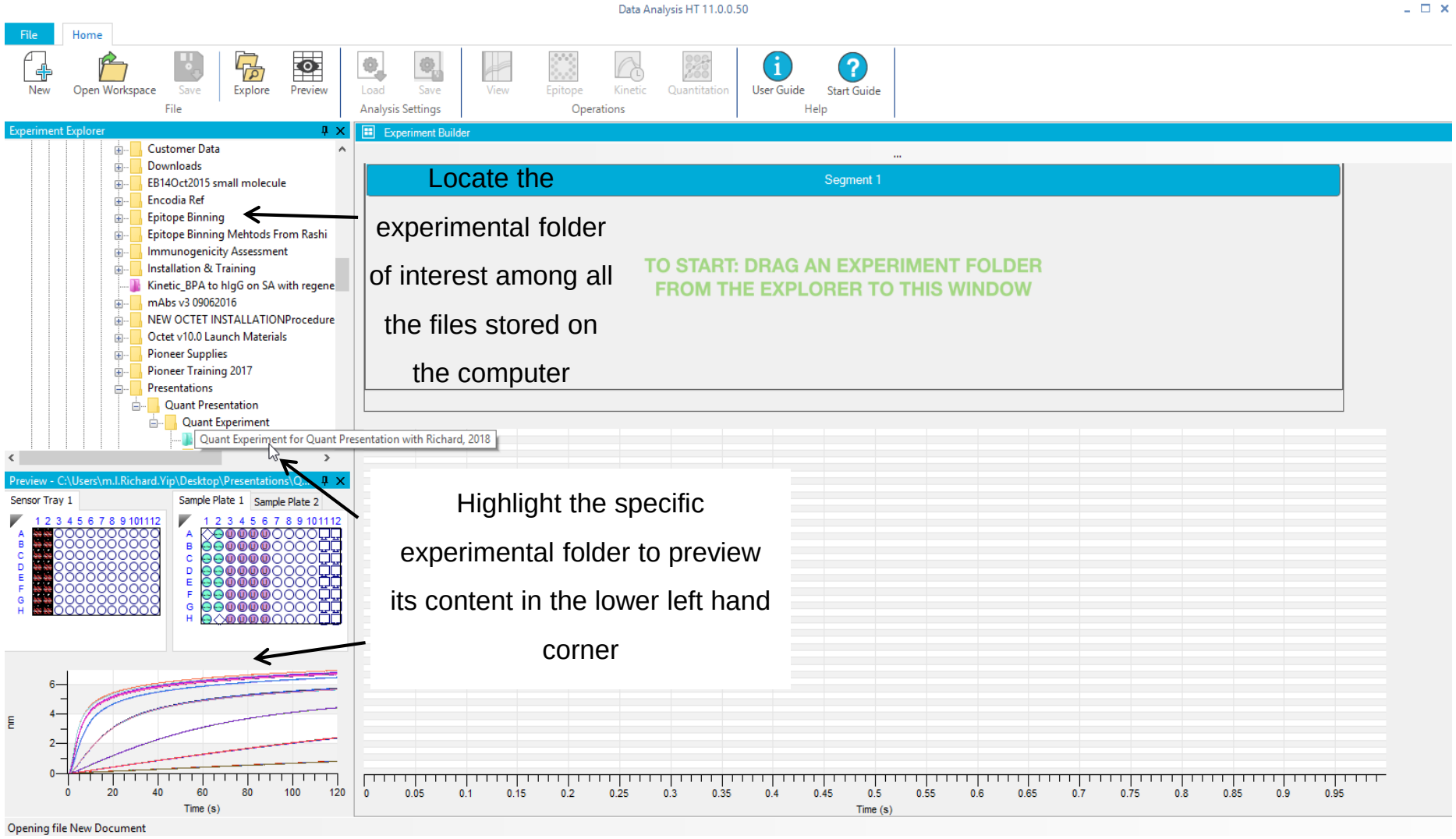
Data Analysis HT V11



Locate the experimental folder of interest among all the files stored on the computer

Highlight the specific experimental folder to preview its content in the lower left hand corner

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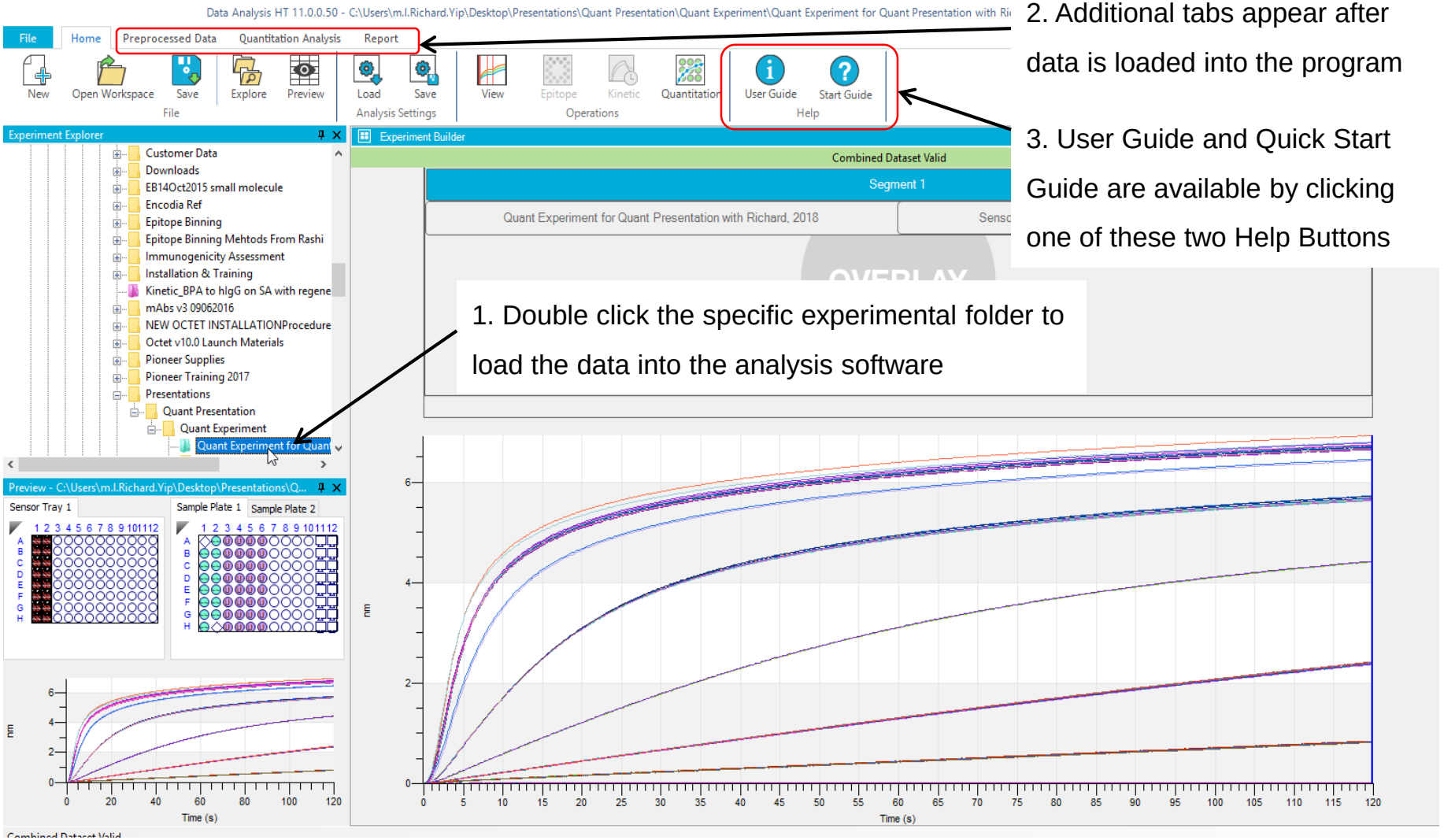
Loading Data into the Analysis Software



2. Additional tabs appear after data is loaded into the program

3. User Guide and Quick Start Guide are available by clicking one of these two Help Buttons

1. Double click the specific experimental folder to load the data into the analysis software

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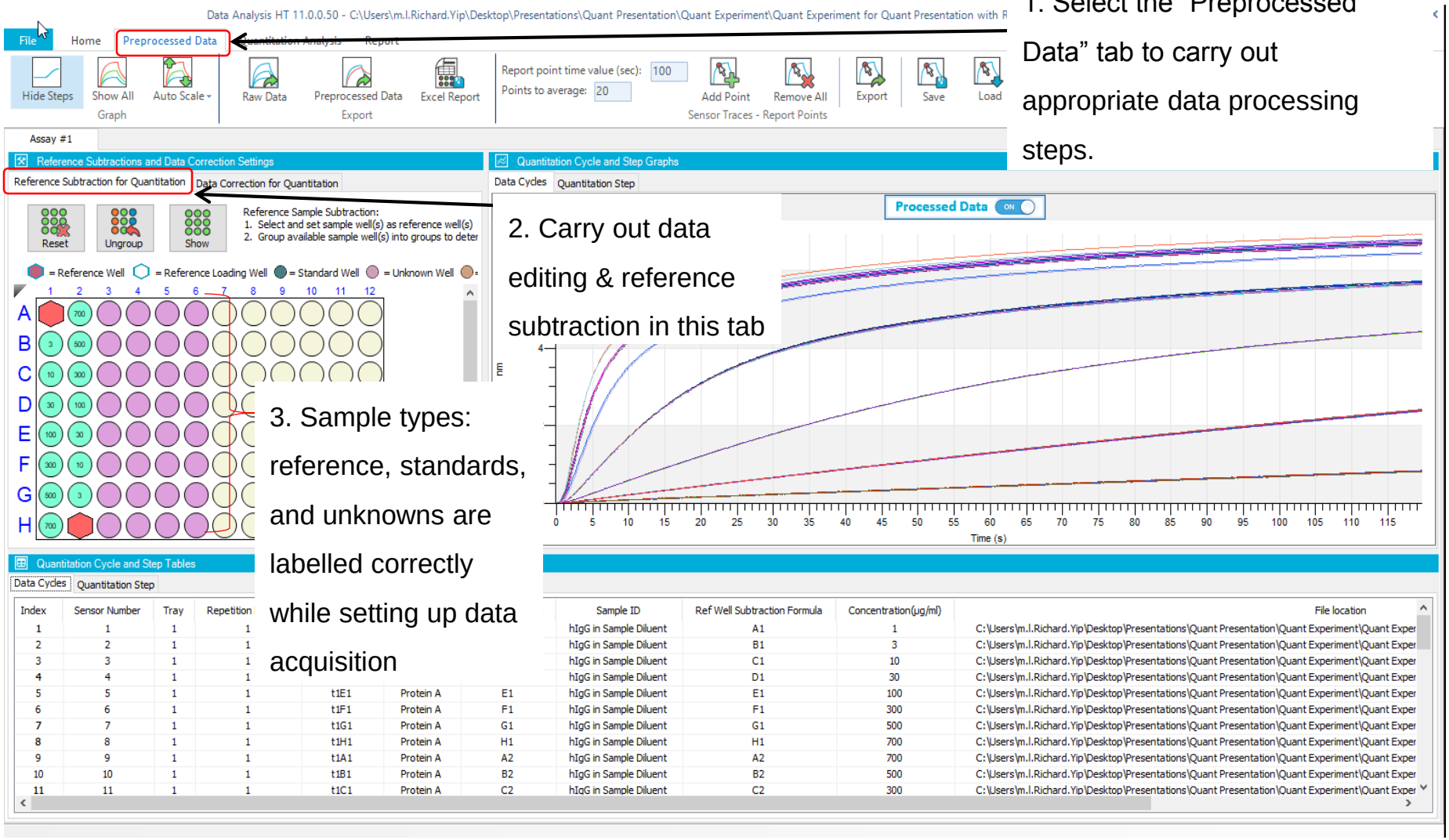
Processing Experimental Data

1. Select the “Preprocessed Data” tab to carry out appropriate data processing steps.

2. Carry out data editing & reference subtraction in this tab

3. Sample types: reference, standards, and unknowns are labelled correctly while setting up data acquisition



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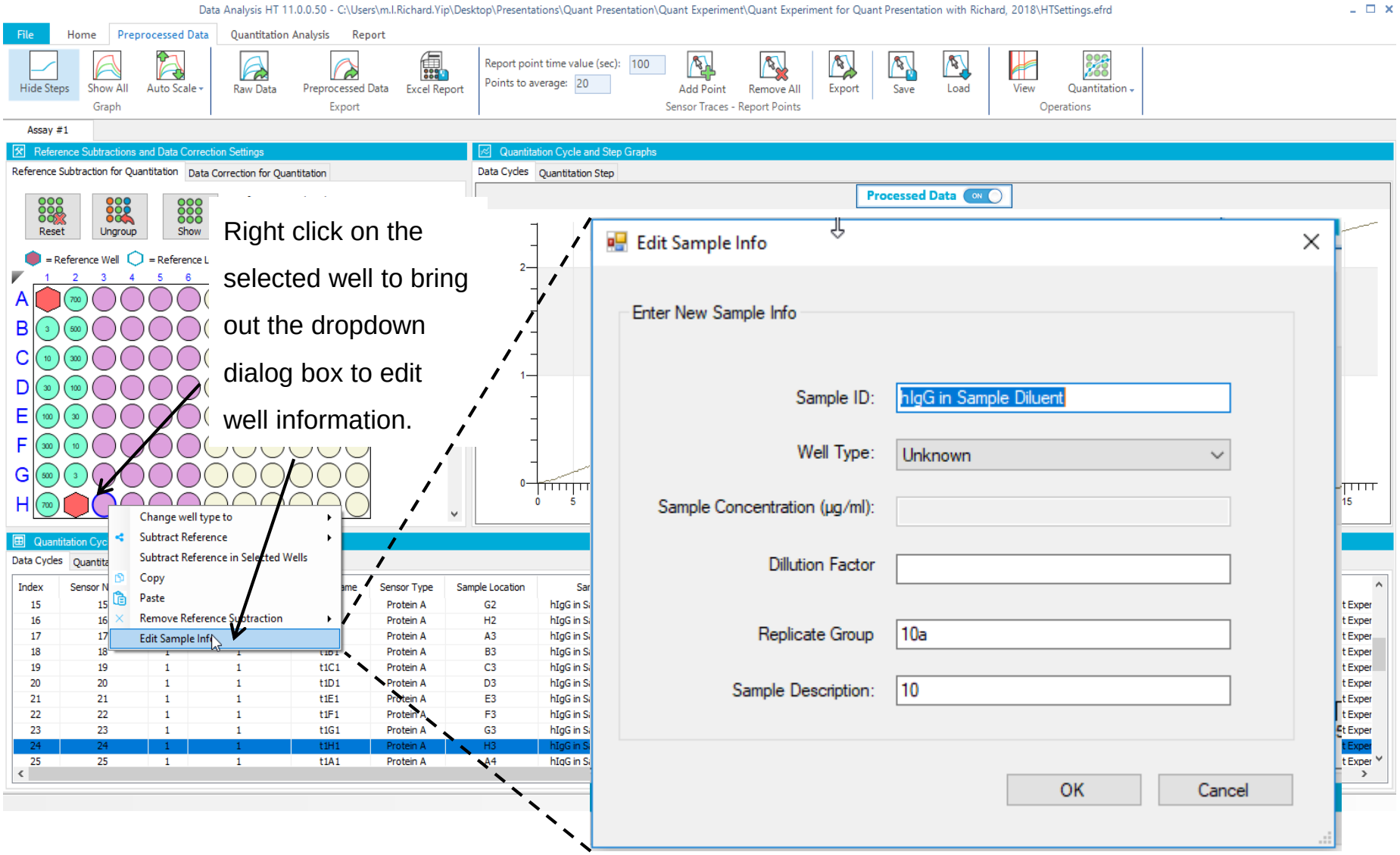
Processing Experimental Data



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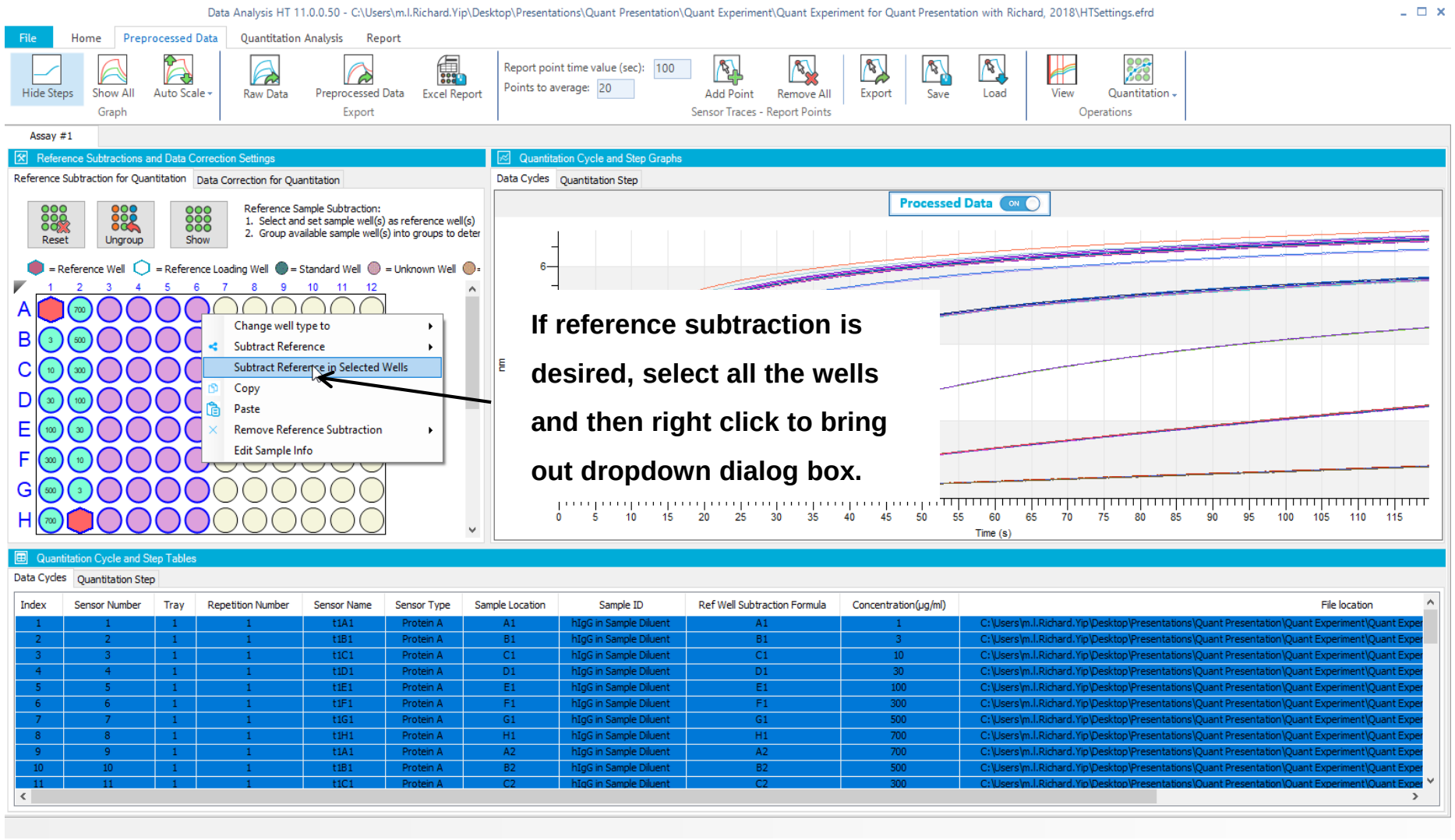
Processing Experimental Data



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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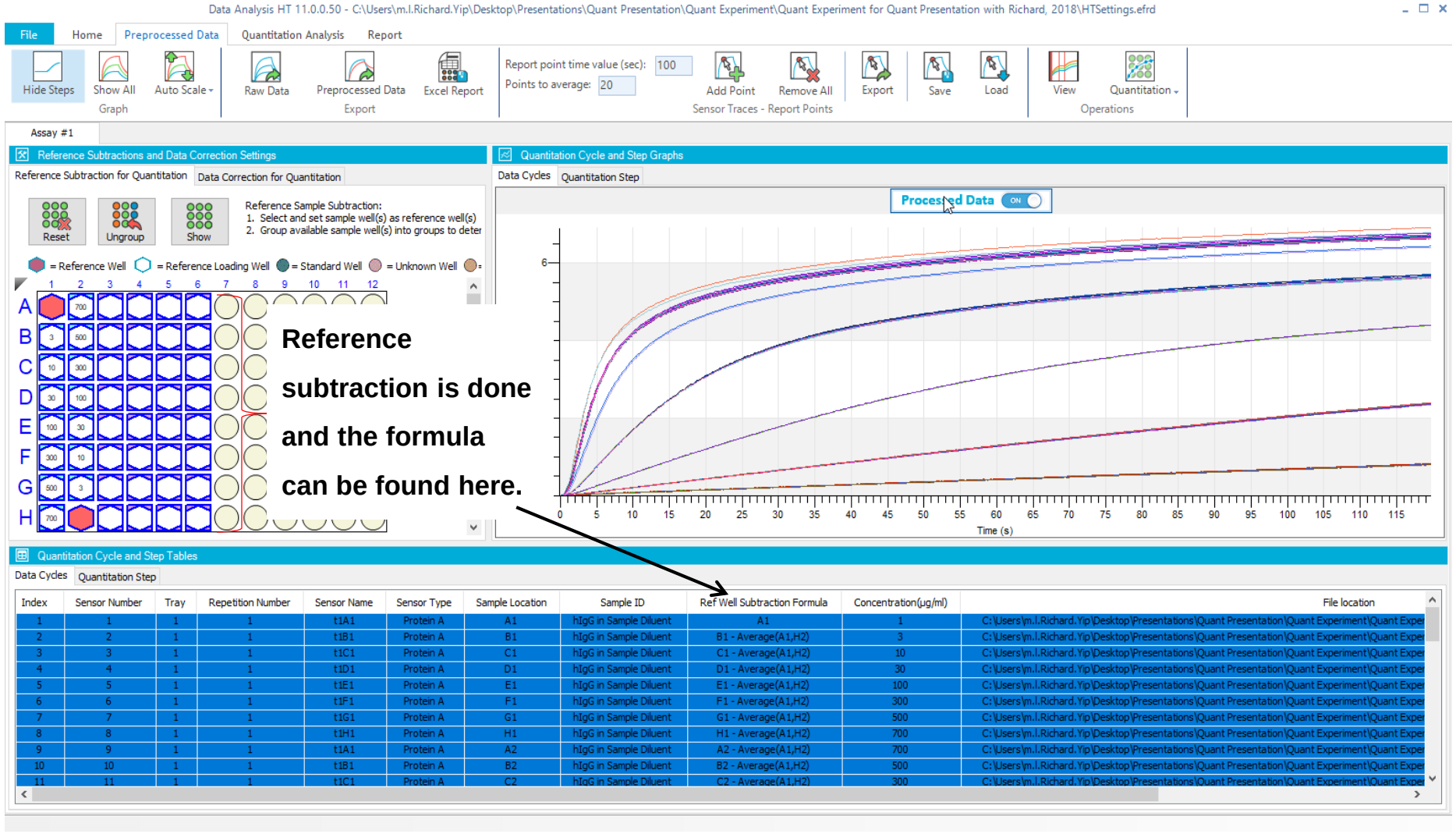
Processing Experimental Data



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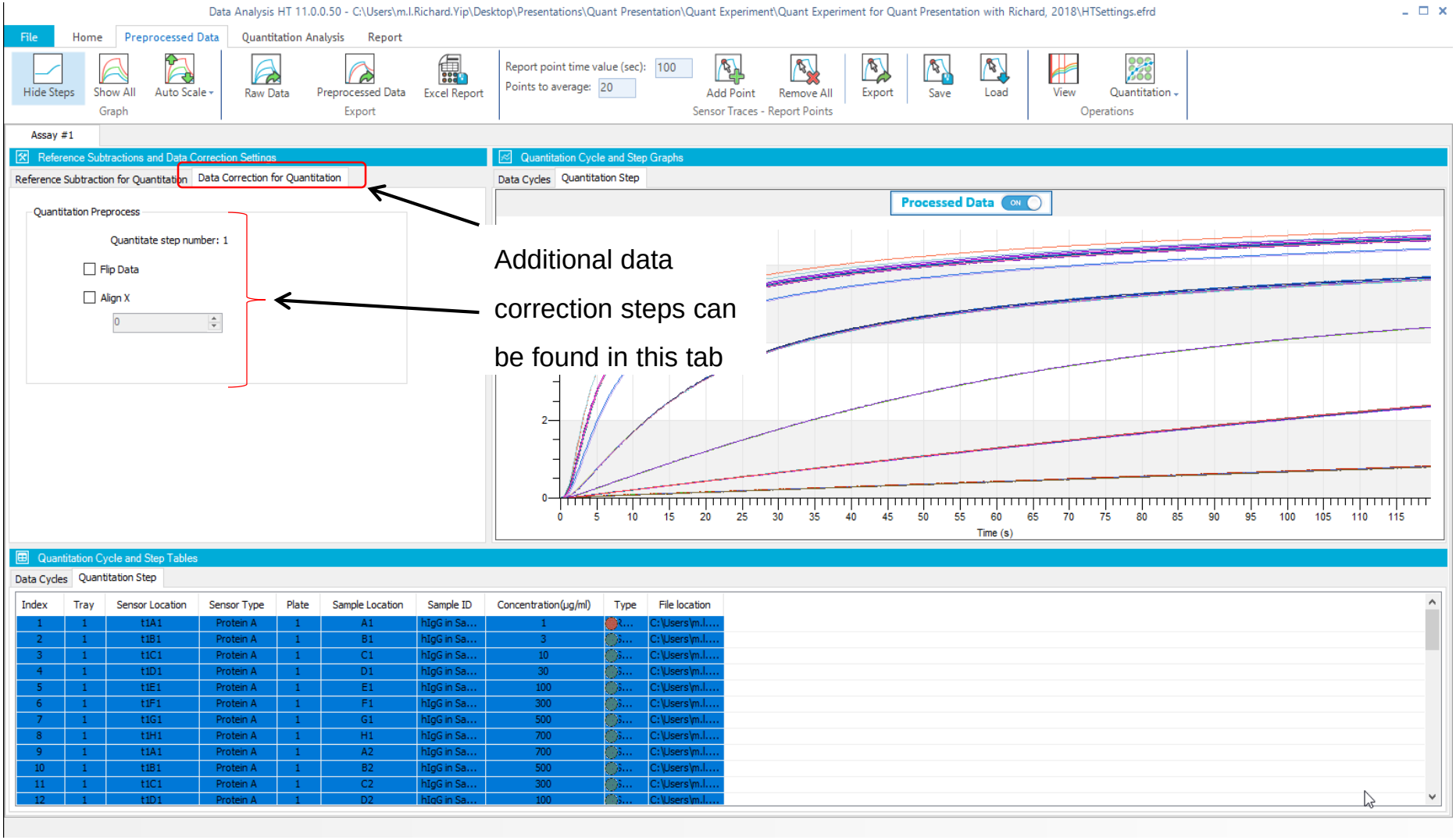
Processing Experimental Data



Additional data correction steps can be found in this tab

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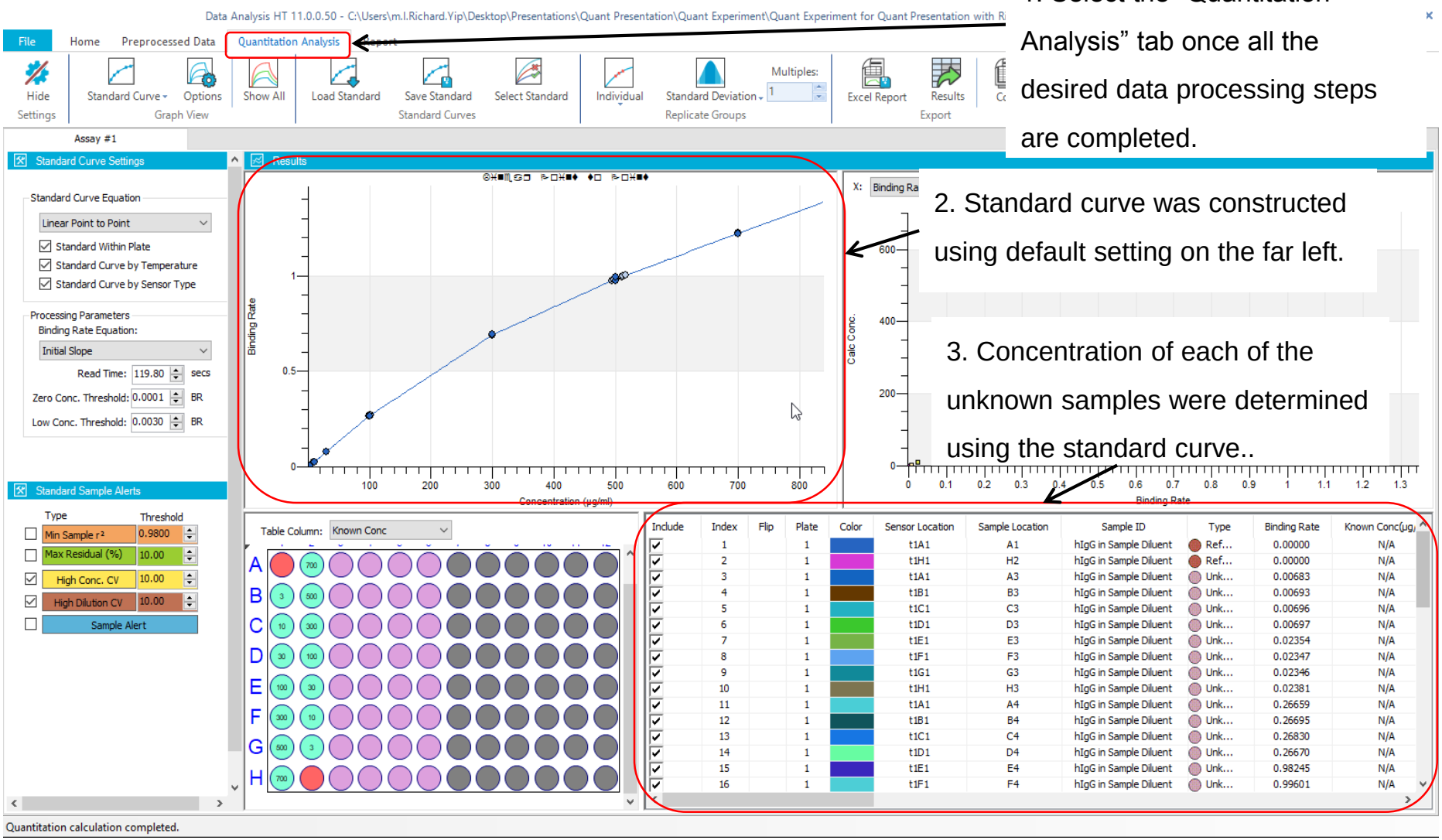
Quantitation Analysis



1. Select the “Quantitation Analysis” tab once all the desired data processing steps are completed.

2. Standard curve was constructed using default setting on the far left.

3. Concentration of each of the unknown samples were determined using the standard curve..

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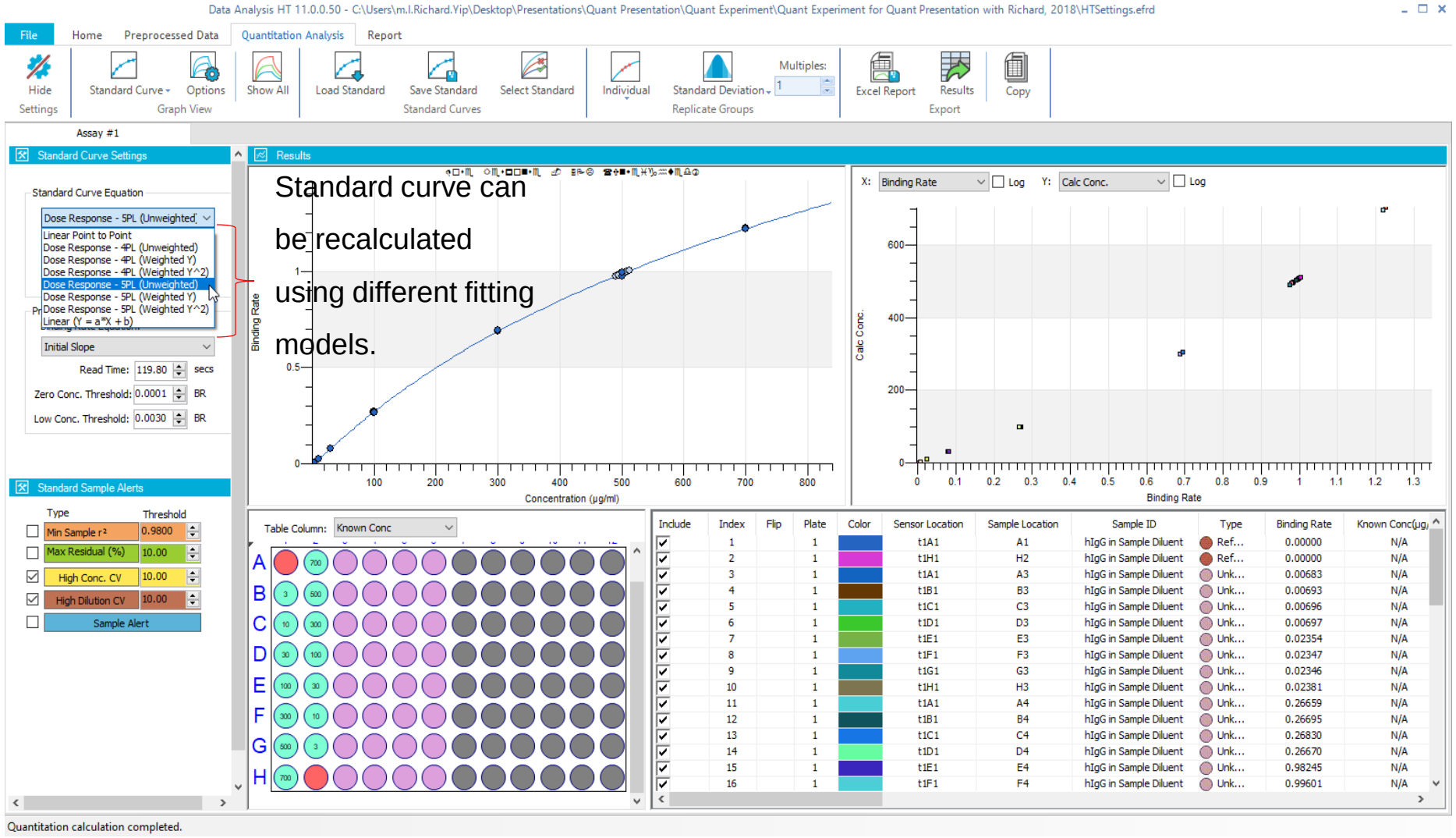
Quantitation Analysis



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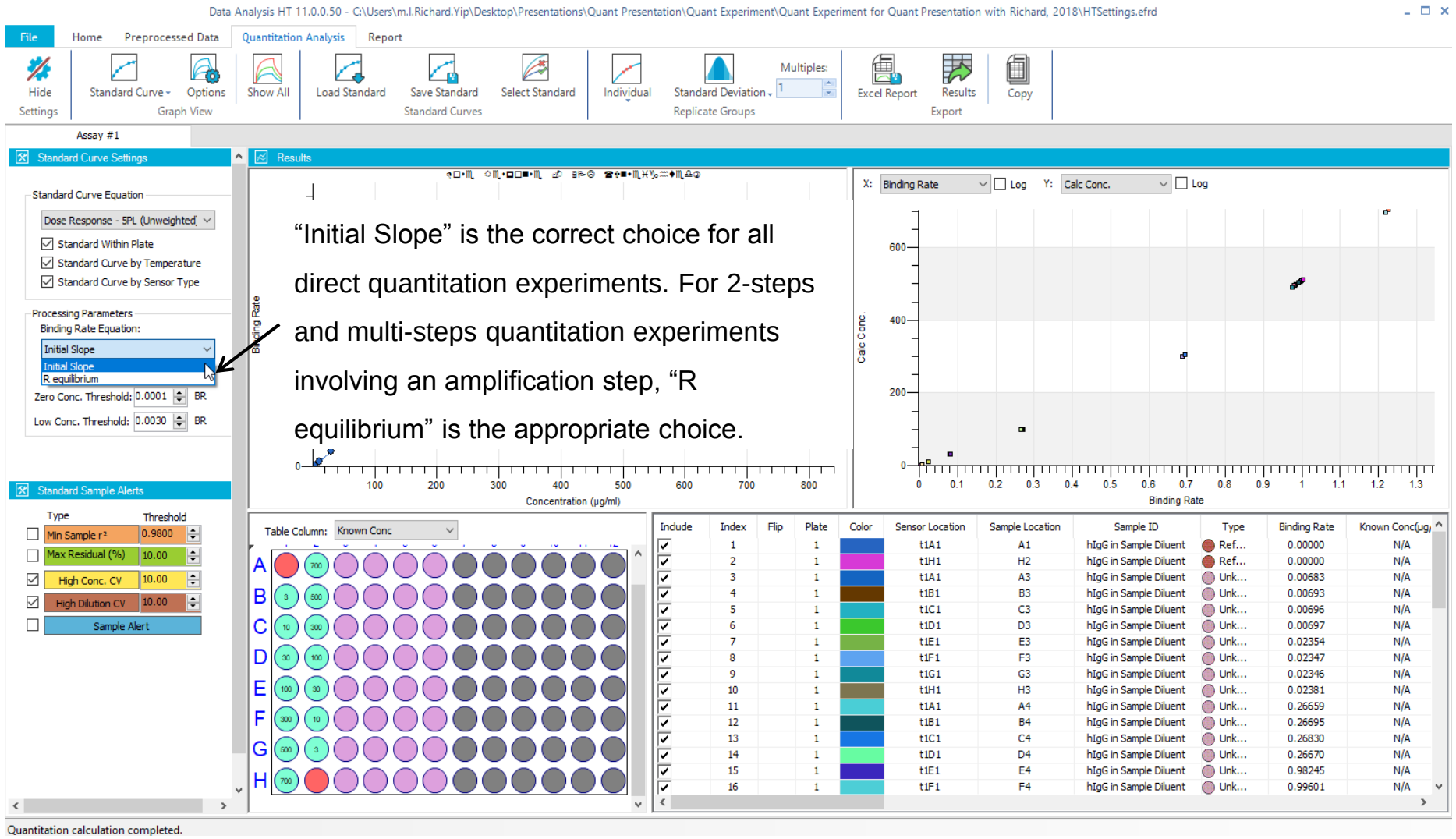
Quantitation Analysis



"Initial Slope" is the correct choice for all direct quantitation experiments. For 2-steps and multi-steps quantitation experiments involving an amplification step, "R equilibrium" is the appropriate choice.

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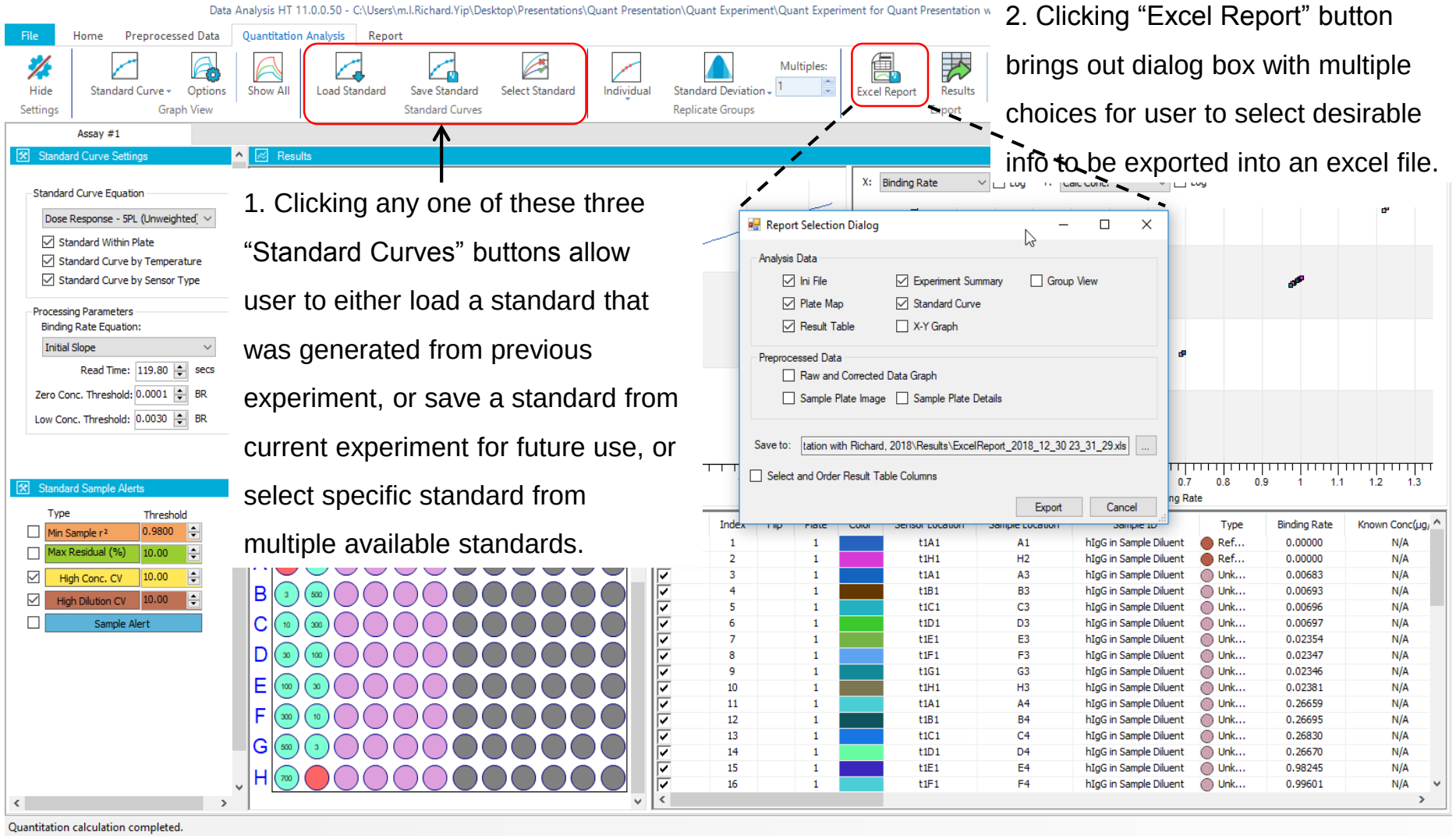
Quantitation Analysis



1. Clicking any one of these three “Standard Curves” buttons allow user to either load a standard that was generated from previous experiment, or save a standard from current experiment for future use, or select specific standard from multiple available standards.

2. Clicking “Excel Report” button brings out dialog box with multiple choices for user to select desirable info to be exported into an excel file.

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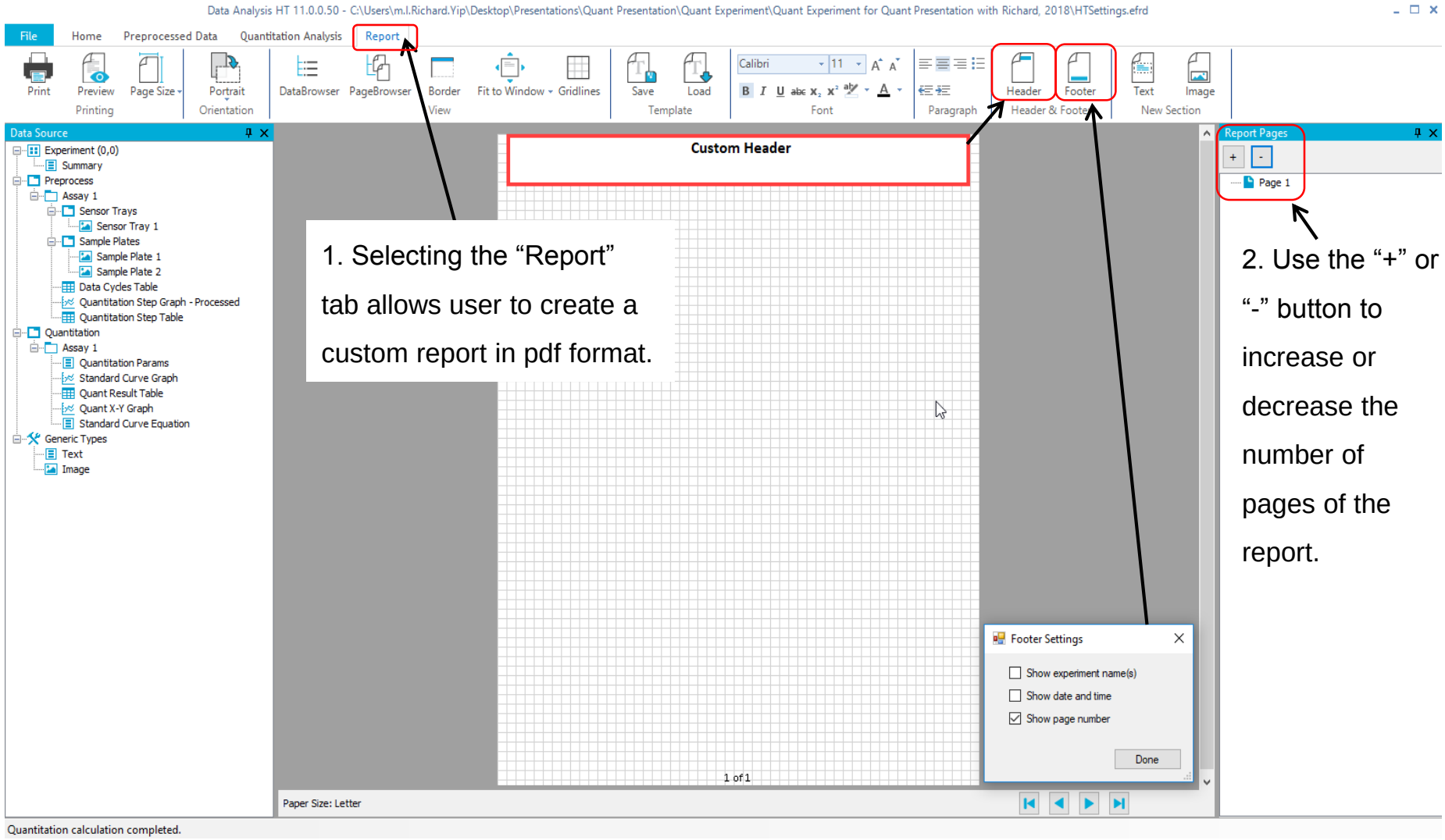
Generating Custom Report



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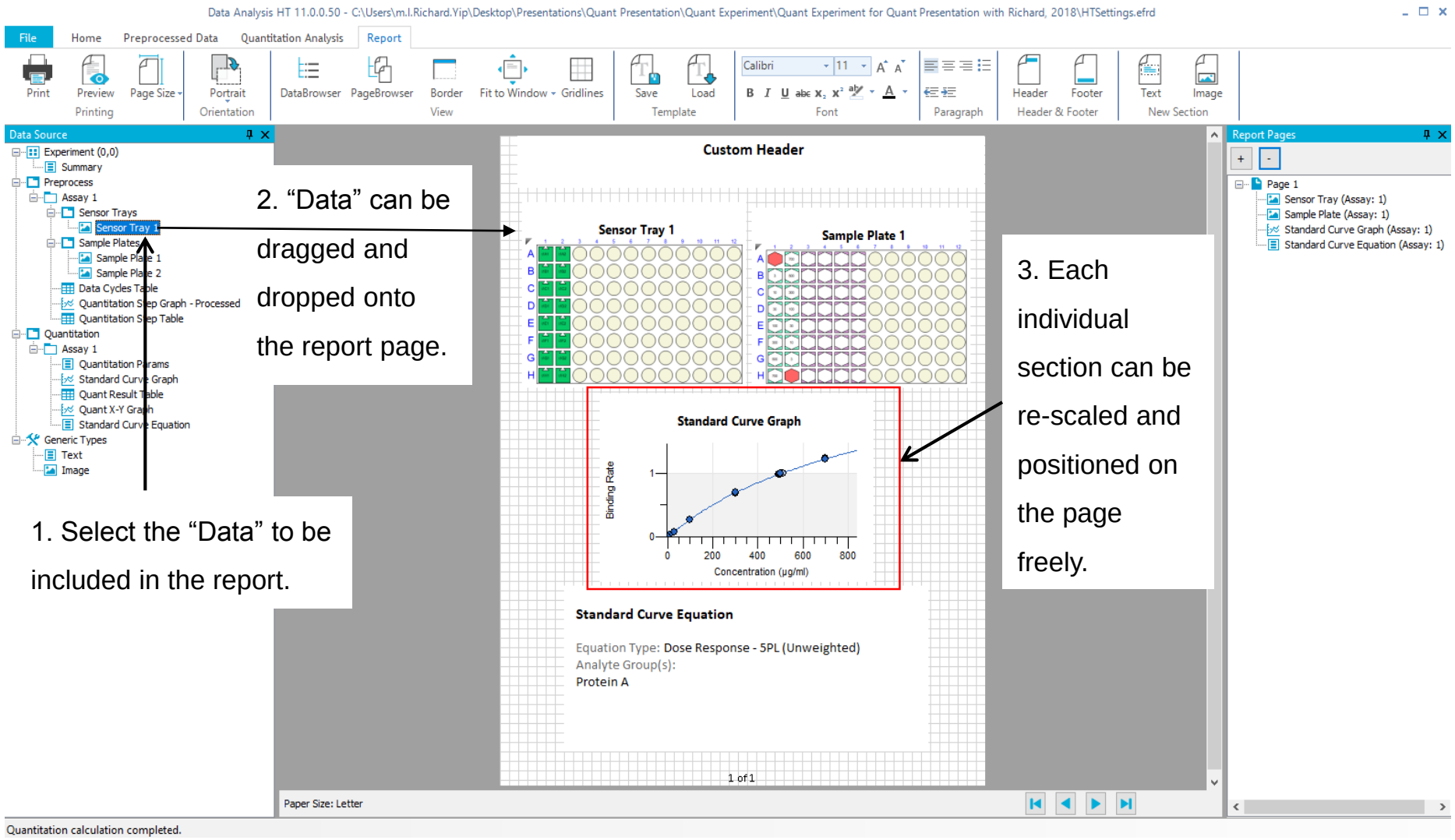
Generating Custom Report



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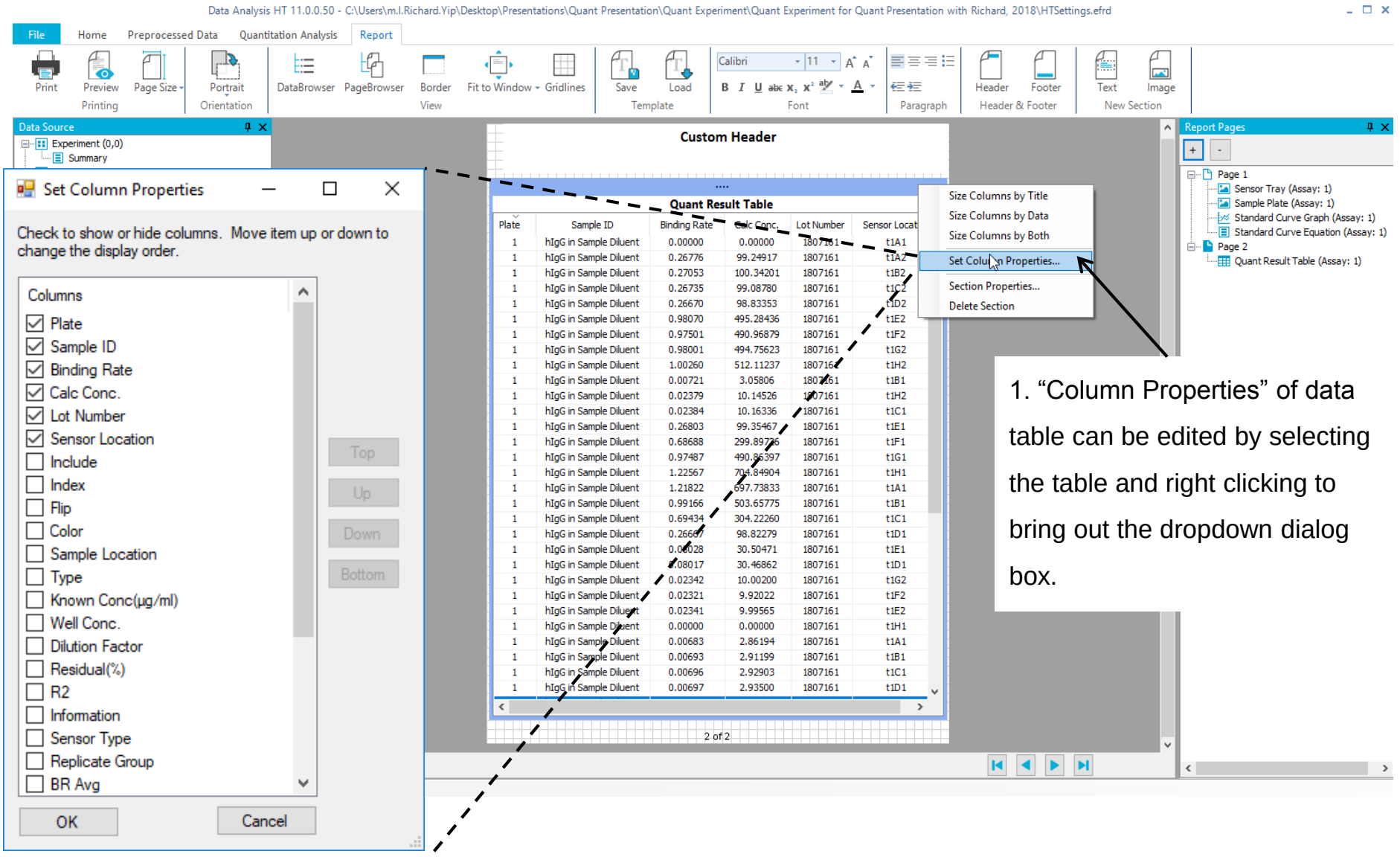
Generating Custom Report



1. “Column Properties” of data table can be edited by selecting the table and right clicking to bring out the dropdown dialog box.

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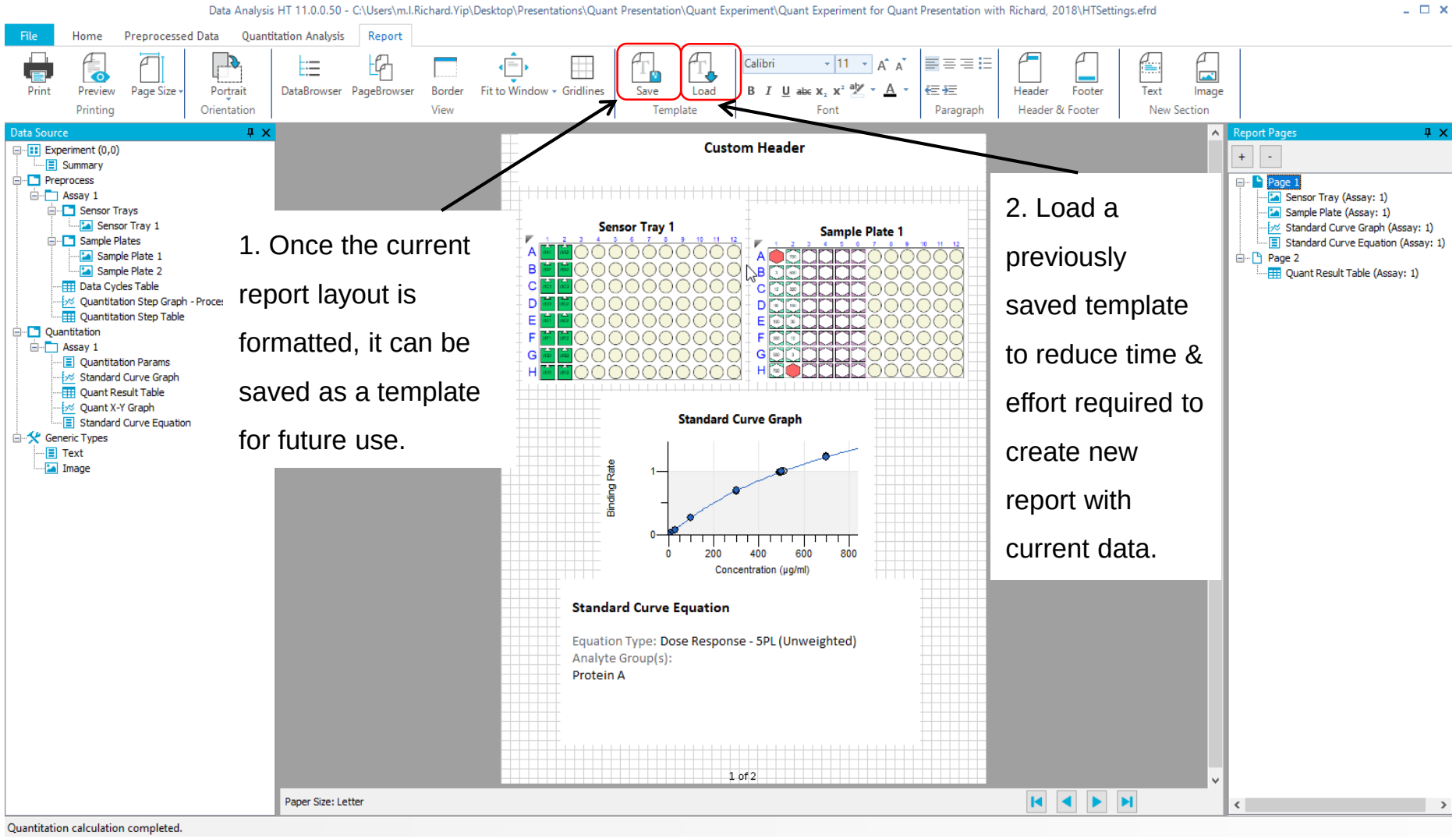
Generating Custom Report



1. Once the current report layout is formatted, it can be saved as a template for future use.

2. Load a previously saved template to reduce time & effort required to create new report with current data.

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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: www.fortebio.com/kb

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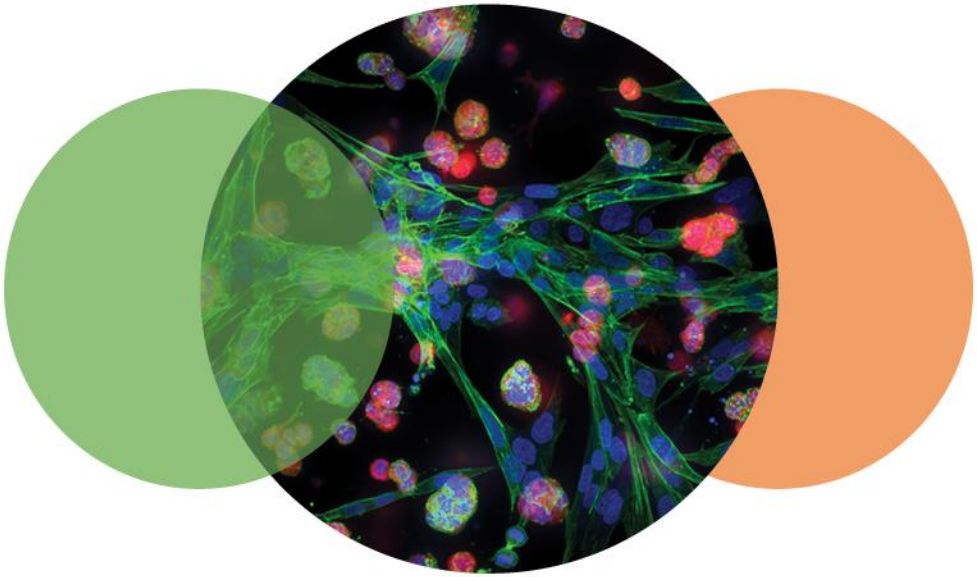

MOLECULAR DEVICES

ADVANCING DISCOVERY

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