PRECISION MADE PRACTICAL

USER MANUAL

Antibody Quantification Kit | Protein G (Human/Mouse IgG, Up to 320 Tests)

PRODUCT CODE: AK-Ab-005

VERSION 1.1
DATE OF ISSUE: 16 Oct. 2025

For research use only. Not for use in diagnostic procedures.

Introduction

The Antibody Quantification Kit | Protein G is designed for accurate measurement of antibody concentration in cell culture supernatants, with compatibility across a range of antibody isotypes and species, including commonly used human and mouse antibodies.

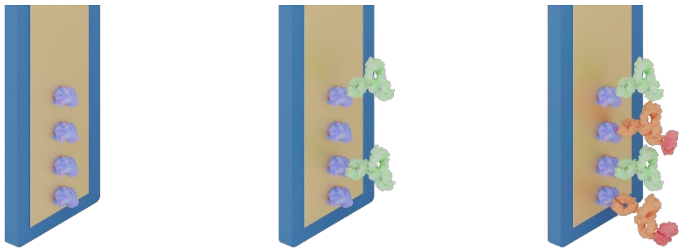
This kit is intended for use with the **Amperia™** Protein Quantification System which automates data acquisition and analysis to deliver reproducible and high-quality results.

The assay follows an **inverse occupancy immunoassay format (Figure 1)**, in which antibodies are captured by Protein G-coated sensors via Fc interaction. A horseradish peroxidase (HRP)-conjugated detection antibody is then applied to bind unoccupied Fc-binding sites, producing a quantifiable inverse signal upon substrate conversion.

The kit supports three concentration ranges*—High, Mid, and Low. Because Protein G capture depends on antibody affinity, species, and subclass, the effective working range is antibody dependent. Select the configuration that brackets the expected concentration and run each range as a separate assay in its own experiment for optimal performance. The example ranges provided in the protocol are for guidance only; the appropriate working range should be validated by the user for their specific antibodies.

Figure 1: Assay Workflow Schematic

1 2 3



1. Sensor surface coated with Protein G.
2. Analyte antibody binds to Protein G via Fc interaction.
3. Detection antibody (HRP-conjugated) binds unoccupied Fc-binding sites, generating an inverse signal upon substrate conversion

***NOTE:**

Detection range may vary depending on analyte affinity and assay conditions.

Table of Contents

1 Kit Components

Each Antibody Quantification Kit | Protein G (Product Code: AK-Ab-005) contains the following components:

- Protein G Sensor|Antibody Quantification (IgG) (SN-006): 5 Sensor Strips, Up to 320 Tests
- 25 mL Regeneration Buffer|Antibody Quantification (A1-8022)
- 125 mL Wash Buffer (A1-8023)
- 100 mL Sample Dilution Buffer|Antibody Quantification (A1-8024)
- 25 mL Detection Reagent|Antibody Quantification (A1-8025)
- 100 mL Substrate (A1-8026)
- 10 × 96-well Non-binding Plates (CN-001)

ADDITIONAL MATERIALS REQUIRED (NOT INCLUDED):

- Antibody reference material
- Deionised water or PBS

NOTE:

For best accuracy, prepare calibration standards using antibody reference materials that match the species, isotype, and buffer conditions of your samples.

2 Reagent Plates Preparation

Please allow all sensors and reagents to reach room temperature before use. Do not open the sensor bags until they have equilibrated.

Each sensor strip contains four independent probes and can be regenerated during the run. Sensor strips are not reusable across experiments.

Each pair of plates (Plate 1 and Plate 2) supports **up to 64** measurements. To support this, the kit includes 10 plates, allowing for multiple runs to reach the full test capacity of each assay range.

When preparing Plate 1 and Plate 2, ensure that:

- If a well is used for measurement (i.e., assigned to a sample, standard, or control), the corresponding well in the paired plate must be filled with Substrate.
- If a well is not used for measurement, the corresponding wells in both plates may be filled with PBS or deionised water.

You may prepare your experiment using one of the following options:

- Option 1: Calibration Curve + Samples – for generating a new calibration curve and quantifying samples in the same run.
- Option 2: Samples Only – for quantifying samples using a previously saved calibration curve.

2.1 OPTION 1: CALIBRATION CURVE + SAMPLES

This option allows you to generate a new calibration curve and quantify your samples in the same run. Each concentration range uses a dedicated assay setup and should be run in a separate experiment.

2.1.1 PLATE LAYOUT

Prepare Plate 1 and Plate 2 according to the layout shown in **Figure 2.1**.

Figure 2.1: Example plate layout for calibration curve and sample wells

PLATE 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std5	S	S	S	S	S	S	W	W	W	R
B	Std1	Std5	S	S	S	S	S	S	W	W	W	R
C	Std2	Std6	S	S	S	S	S	S	W	W	W	R
D	Std2	Std6	S	S	S	S	S	S	W	W	W	R
E	Std3	Std7	S	S	S	S	S	S	W	W	W	R
F	Std3	Std7	S	S	S	S	S	S	W	W	W	R
G	Std4	Std8	S	S	S	S	S	S	W	W	W	R
H	Std4	Std8	S	S	S	S	S	S	W	W	W	R

PLATE 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	T	T	T	T	T	T	T	T	W	W	D	D
B	T	T	T	T	T	T	T	T	W	W	D	D
C	T	T	T	T	T	T	T	T	W	W	D	D
D	T	T	T	T	T	T	T	T	W	W	D	D
E	T	T	T	T	T	T	T	T	W	W	D	D
F	T	T	T	T	T	T	T	T	W	W	D	D
G	T	T	T	T	T	T	T	T	W	W	D	D
H	T	T	T	T	T	T	T	T	W	W	D	D

Notation:

S: Measurement Wells
R: Regeneration Buffer|Antibody Quantification (A1-8022)
W: Wash Buffer (A1-8023)
Std1–Std8: Standards (see Section 2.1.2)
D: Detection Reagent|Antibody Quantification (A1-8025)
T: Substrate (A1-8026)

2.1.2 PREPARATION OF REAGENTS

Calibrator Preparation

Dilute the antibody calibrator using the Sample Dilution Buffer|Antibody Quantification (A1-8024) to prepare the standard concentrations listed in **Table 1**. The calibration curve is generated using an 8-point, 2× serial dilution. Use a calibrator that matches the species and isotype of your samples for best results.

NOTE:
For optimal performance, ensure the buffer composition matches that of your samples.

Table 1: Calibration Curve Concentrations

Standard ID	Low Range (µg /mL)	Mid Range (µg/mL)	High Range (µg/mL)
Std1	0.007	0.06	0.39
Std2	0.015	0.12	0.78
Std3	0.029	0.23	1.56
Std4	0.058	0.47	3.13
Std5	0.117	0.94	6.25
Std6	0.234	1.88	12.5
Std7	0.469	3.75	25.0
Std8	0.938	7.50	50.0

NOTE: The concentrations shown are example values for human IgG1. Actual ranges may vary depending on antibody affinity, species, and subclass.

Dispense 250 µL of each standard concentration into **Plate 1** as shown in the plate layout (see **Figure 2.1**).

Samples

Dilute your samples using the Sample Dilution Buffer|Antibody Quantification (A1-8024) to fall within the calibration range (low, mid, or high). Dispense 250 µL of each diluted sample into the appropriate wells (see **Figure 2.1**).

Other Reagents

Dispense 250 µL of each reagent into the corresponding wells according to the plate layout (see **Figure 2.1**).

2.1.3 EXPERIMENT SETUP

Once the calibration and sample plates are prepared, proceed to **Section 3: Instrument Setup** for details on creating the experiment and loading plates and sensors.

Ensure the correct template is selected for the assay range (Low, Mid, or High) when configuring the experiment.

2.2 OPTION 2: SAMPLES ONLY (USING A SAVED CALIBRATION CURVE)

This option allows you to quantify samples using a previously generated calibration curve stored on the instrument.

Each assay range (low, mid, or high) should be run in a separate experiment using the corresponding saved calibration curve.

2.2.1 PLATE LAYOUT

Prepare Plate 1 and Plate 2 according to the layout shown below.

Figure 2.2: Sample-Only Plate Layout

PLATE 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	S	S	S	S	S	S	S	S	W	W	W	R
B	S	S	S	S	S	S	S	S	W	W	W	R
C	S	S	S	S	S	S	S	S	W	W	W	R
D	S	S	S	S	S	S	S	S	W	W	W	R
E	S	S	S	S	S	S	S	S	W	W	W	R
F	S	S	S	S	S	S	S	S	W	W	W	R
G	S	S	S	S	S	S	S	S	W	W	W	R
H	S	S	S	S	S	S	S	S	W	W	W	R

PLATE 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	T	T	T	T	T	T	T	T	W	W	D	D
B	T	T	T	T	T	T	T	T	W	W	D	D
C	T	T	T	T	T	T	T	T	W	W	D	D
D	T	T	T	T	T	T	T	T	W	W	D	D
E	T	T	T	T	T	T	T	T	W	W	D	D
F	T	T	T	T	T	T	T	T	W	W	D	D
G	T	T	T	T	T	T	T	T	W	W	D	D
H	T	T	T	T	T	T	T	T	W	W	D	D

Notation:

S: Measurement Wells
R: Regeneration Buffer|Antibody Quantification (A1-8022)
W: Wash Buffer (A1-8023)
D: Detection Reagent|Antibody Quantification (A1-8025)
T: Substrate (A1-8026)

2.2.2 PREPARATION OF REAGENTS

Samples

Dilute your samples using the Sample Dilution Buffer|Antibody Quantification (A1-8024) to fall within the appropriate concentration range (low, mid, or high). Dispense 250 µL into each **S** well.

Other Reagents

Dispense 250 µL of each reagent into the appropriate wells according to the plate layout, see **Figure 2.2**.

2.2.3 EXPERIMENT SETUP

Proceed to **Section 3: Instrument Setup** for instructions on creating the experiment, assigning wells, and loading plates and sensors.

When configuring the run, select the appropriate **assay range template** (Low, Mid, or High) and ensure the correct **previously saved calibration curve** is selected for quantification.

2.3 OTHER INFORMATION

2.3.1 CONTROLS DURING QUANTIFICATION

When using a previously saved calibration curve, the Amperia™ system allows you to quantify samples without including new standards in the same run. However, to maintain quantification accuracy, it is recommended to include **internal controls**.

These controls can be standards or samples with a known concentration within the relevant assay range, placed in two or more wells.

Including controls allows the system to compensate for potential variations in environmental or plate-specific conditions.

TIP: To include controls, replace one or more sample wells with standards or known material, and adjust your plate layout accordingly.

3 Instrument Setup

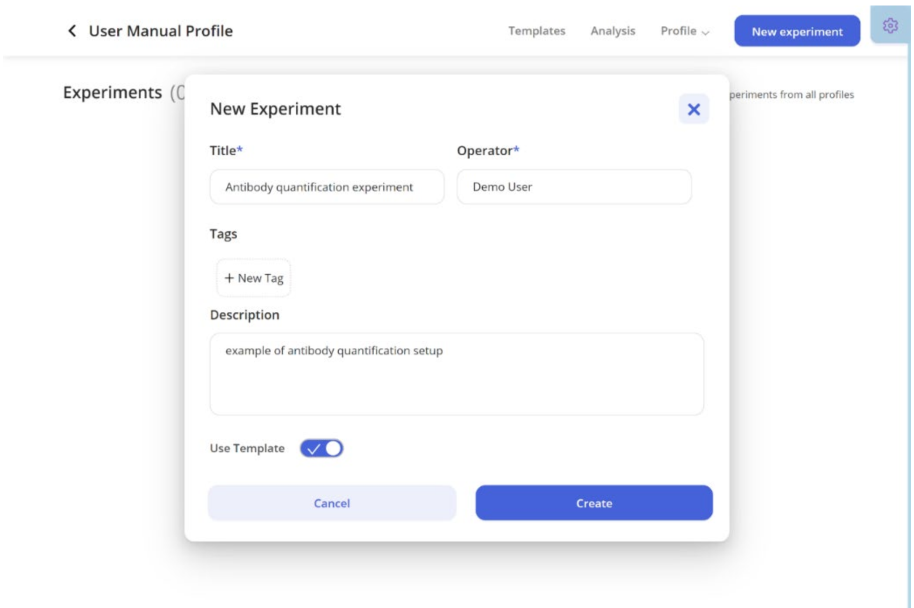
This section describes how to set up an experiment in the Amperia™ system using a predefined template. The workflow includes creating the experiment, assigning wells, loading plates and sensors, and starting the run.

3.1 CREATE A NEW EXPERIMENT

From the **Experiments** page:

- Tap **New Experiment**
- Enter a unique title and optional description
- Ensure **Use Template** is selected
- Tap **Create** → See **Figure 3.1**

Figure 3.1. Creating a new experiment from the Experiments page.

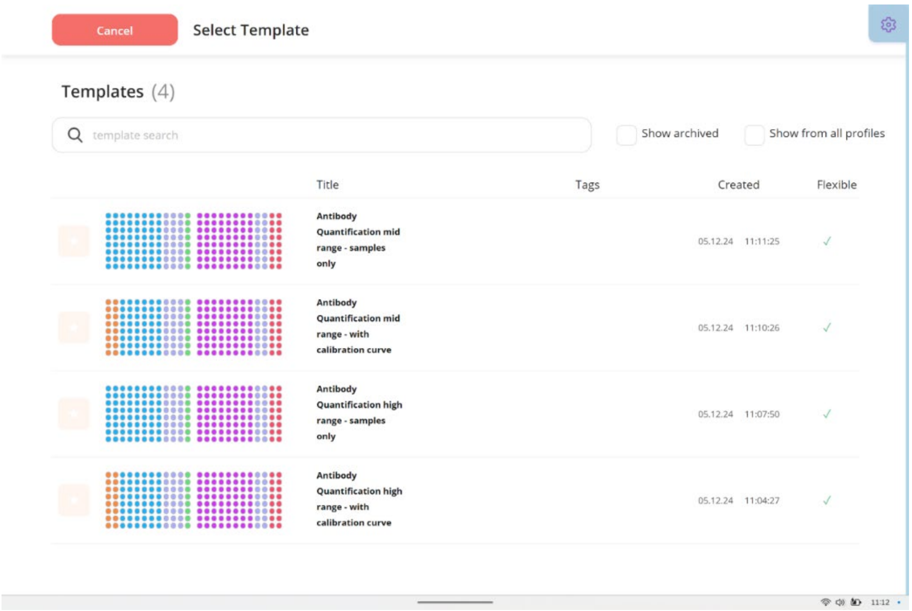


3.2 RUNNING THE EXPERIMENT

Select Template

- Choose **Antibody Quantification – Calibration Curve + Samples**
- Tap **Create** again to confirm → See **Figure 3.2**

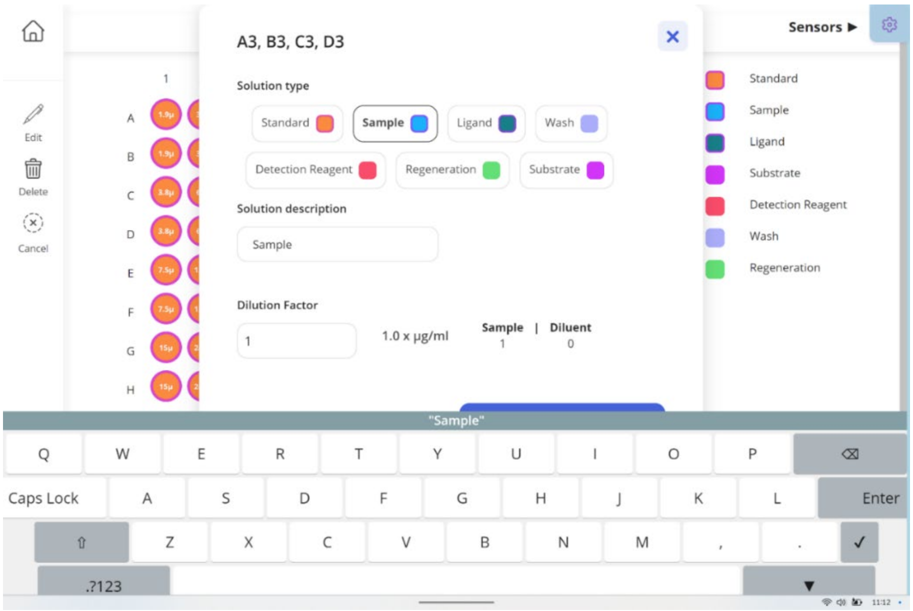
Figure 3.2. Selecting the appropriate template for Antibody quantification.



Assign Wells

- Tap the **Timeline** button, then navigate to: **Sequence** → **Sensors** → **Plate**
- In the **Plate** screen, tap any well to edit
- Use the **Solution Description** field to assign each well (e.g. as Sample, Standard, or other relevant solution types) → See **Figure 3.3**

Figure 3.3 Assign wells.



Review Layout

- Use Sensors → Layout →Review to confirm assignments

Start Run and Load Materials

- Tap Start Experiment → See **Figure 3.4**
- Open the front door when prompted
- Load Plate 1 with holder → See **Figure 3.5**
- Load Plate 2 with holder → See **Figure 3.5**
- Insert required number of sensor strips

Figure 3.4 Tap to start experiment

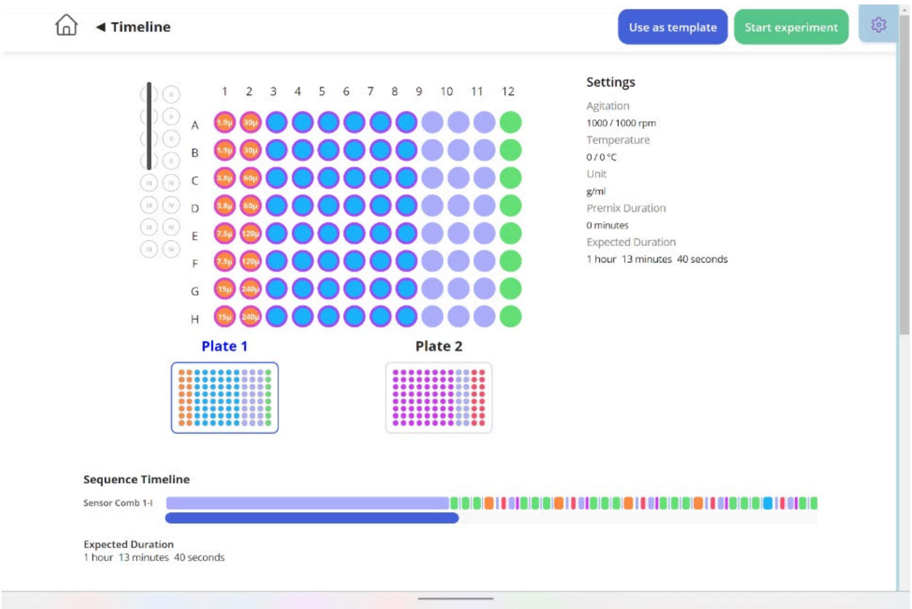
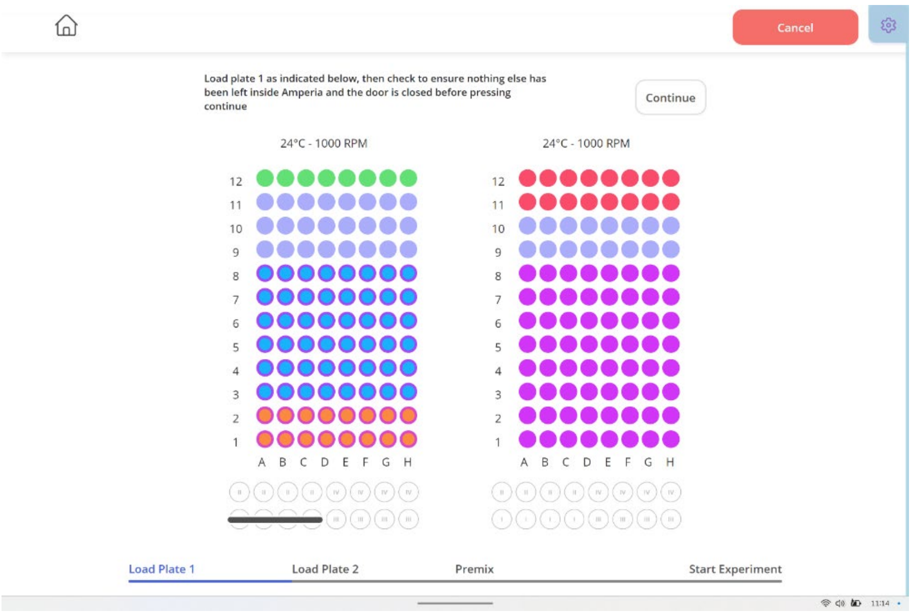


Figure 3.5 Load Plate



4 Data Analysis

This section covers two key workflows: generating a calibration curve and using it to quantify antibody samples. All steps are performed through the Amperia™ system's analysis interface.

4.1 CALIBRATION CURVE

You can generate a calibration curve using a completed experiment containing standard measurements. This curve will be saved and available for future quantification.

- From the **Experiments** page, tap the experiment used to measure the standards.
  → See **Figure 4.1**
- A summary window will appear. Tap **Details** to proceed.
  → See **Figure 4.2**
- On the experiment summary screen, tap **Analysis**.
  → See **Figure 4.3**
- If no analysis has been created yet, the list will be empty. Tap **New Analysis** to start.
  → See **Figure 4.4**
- Enter a unique name for your new analysis.
  → See **Figure 4.5**
- A summary of all measurements in the experiment will be shown, grouped by solution type. Standards will be clearly labelled. Tap **New Chart**.
  → See **Figure 4.6**
- Select **Generate standard curve** to begin.
  → See **Figure 4.7**
- The software will automatically fit a curve to all valid standard measurements.
  → See **Figure 4.8**
- To exclude individual points, tap the pen icon and uncheck them. You can also rename the curve. Tap the wave icon to save.
  → See **Figure 4.9**
- Once complete, tap **Finalize** to save the analysis. This calibration curve can now be used for sample quantification.

Figure 4.1 Select experiment from the Experiments page

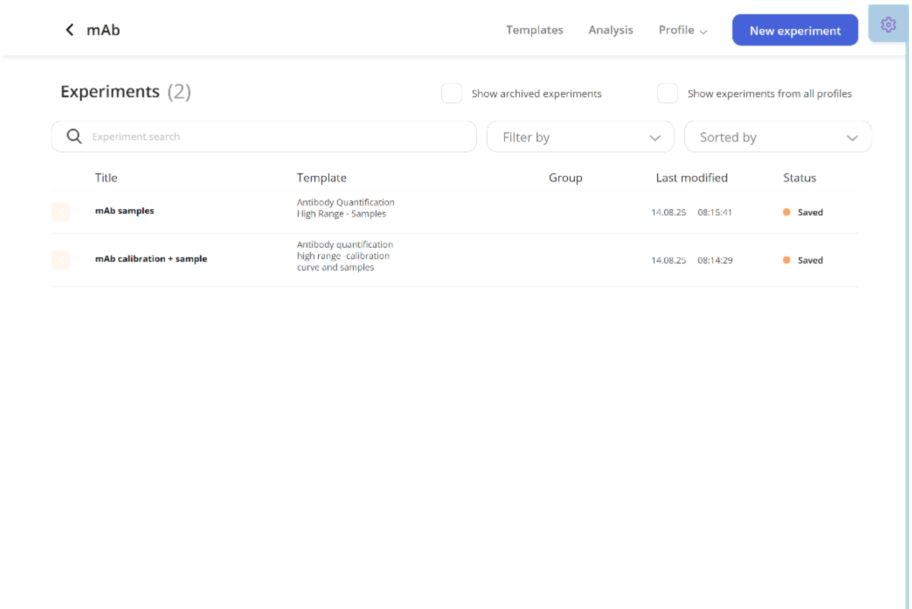


Figure 4.2 Experiment summary window

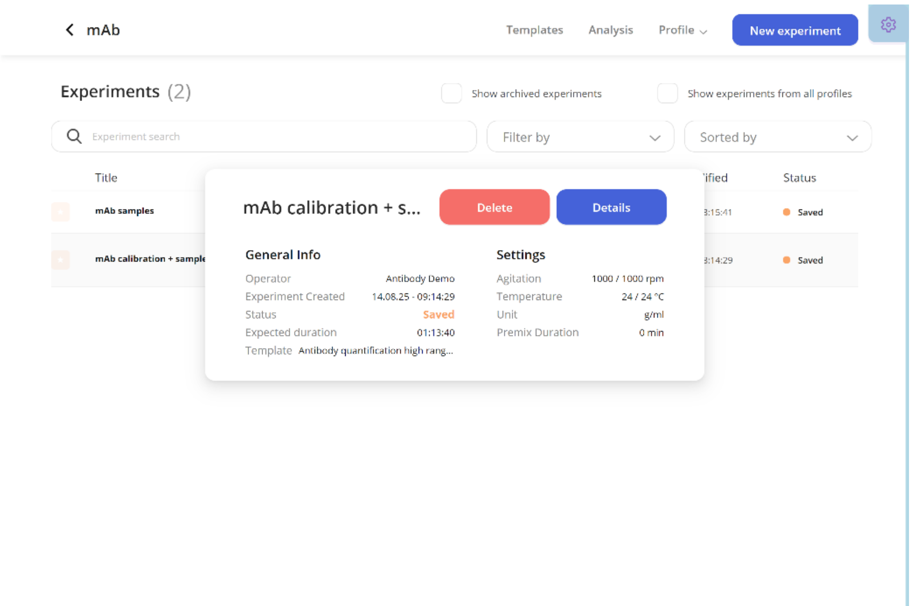


Figure 4.3 Tap Analysis to view existing or create new analysis

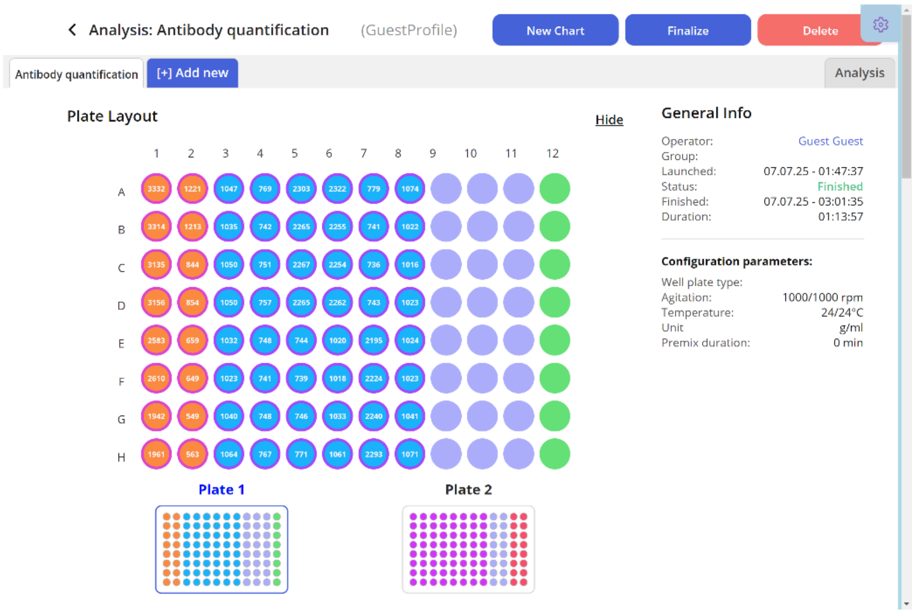


Figure 4.4 Tap on New Analysis to start

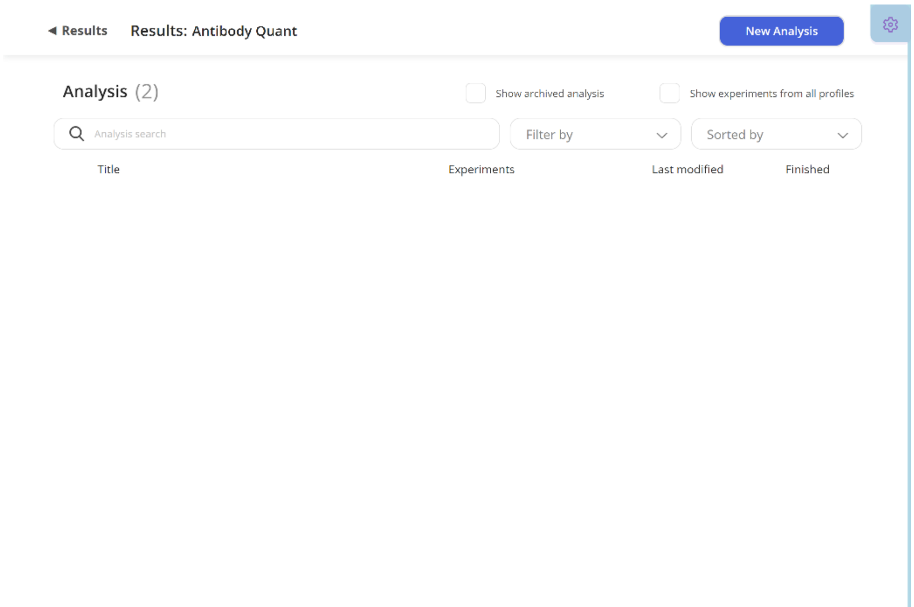


Figure 4.5 Name your new analysis

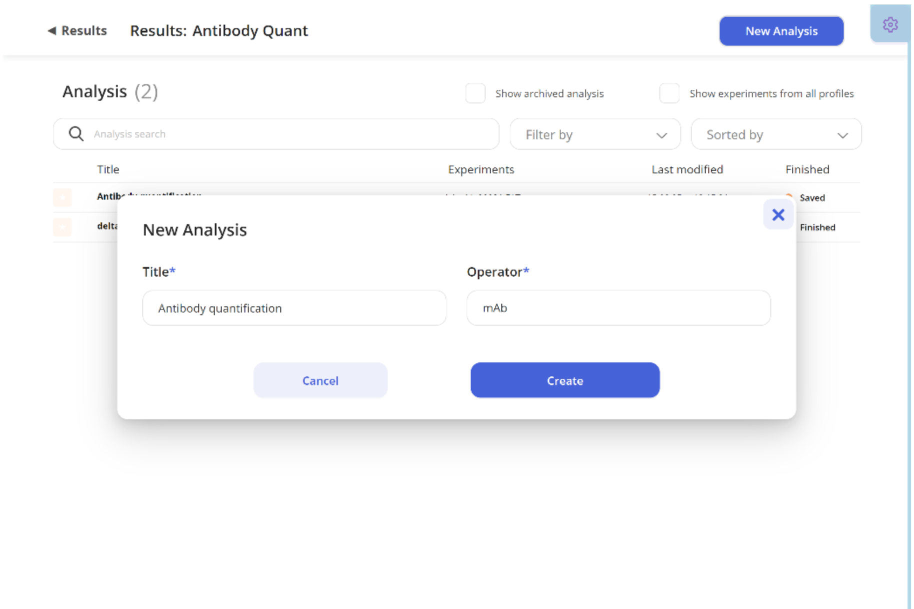
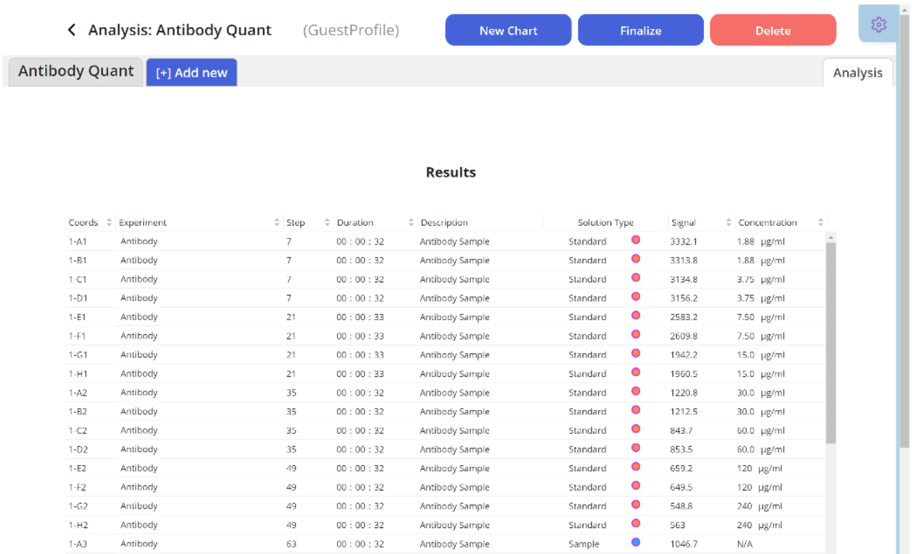


Figure 4.6 Overview of standard measurements grouped by type



Coords	Experiment	Step	Duration	Description	Solution Type	Signal	Concentration
1-A1	Antibody	7	00 : 00 : 32	Antibody Sample	Standard	3332.1	1.88 µg/ml
1-B1	Antibody	7	00 : 00 : 32	Antibody Sample	Standard	3313.8	1.88 µg/ml
1-C1	Antibody	7	00 : 00 : 32	Antibody Sample	Standard	3134.8	3.75 µg/ml
1-D1	Antibody	7	00 : 00 : 32	Antibody Sample	Standard	3156.2	3.75 µg/ml
1-E1	Antibody	21	00 : 00 : 33	Antibody Sample	Standard	2583.2	7.50 µg/ml
1-F1	Antibody	21	00 : 00 : 33	Antibody Sample	Standard	2609.8	7.50 µg/ml
1-G1	Antibody	21	00 : 00 : 33	Antibody Sample	Standard	1942.2	15.0 µg/ml
1-H1	Antibody	21	00 : 00 : 33	Antibody Sample	Standard	1960.5	15.0 µg/ml
1-A2	Antibody	35	00 : 00 : 32	Antibody Sample	Standard	1220.8	30.0 µg/ml
1-B2	Antibody	35	00 : 00 : 32	Antibody Sample	Standard	1212.5	30.0 µg/ml
1-C2	Antibody	35	00 : 00 : 32	Antibody Sample	Standard	843.7	60.0 µg/ml
1-D2	Antibody	35	00 : 00 : 32	Antibody Sample	Standard	853.5	60.0 µg/ml
1-E2	Antibody	49	00 : 00 : 32	Antibody Sample	Standard	659.2	120 µg/ml
1-F2	Antibody	49	00 : 00 : 32	Antibody Sample	Standard	649.5	120 µg/ml
1-G2	Antibody	49	00 : 00 : 32	Antibody Sample	Standard	548.8	240 µg/ml
1-H2	Antibody	49	00 : 00 : 32	Antibody Sample	Standard	563	240 µg/ml
1-A3	Antibody	63	00 : 00 : 32	Antibody Sample	Sample	1046.7	N/A

Figure 4.7 Tap to generate a standard curve

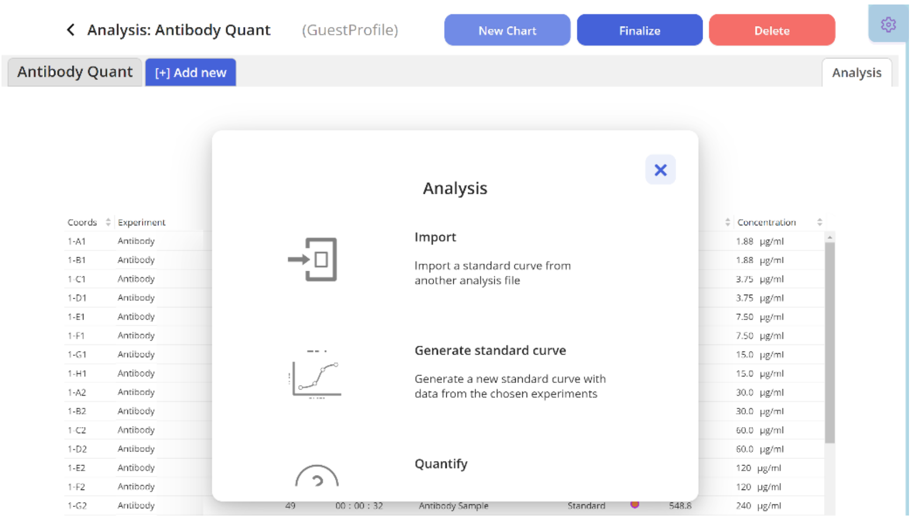


Figure 4.8 Auto-generated standard curve

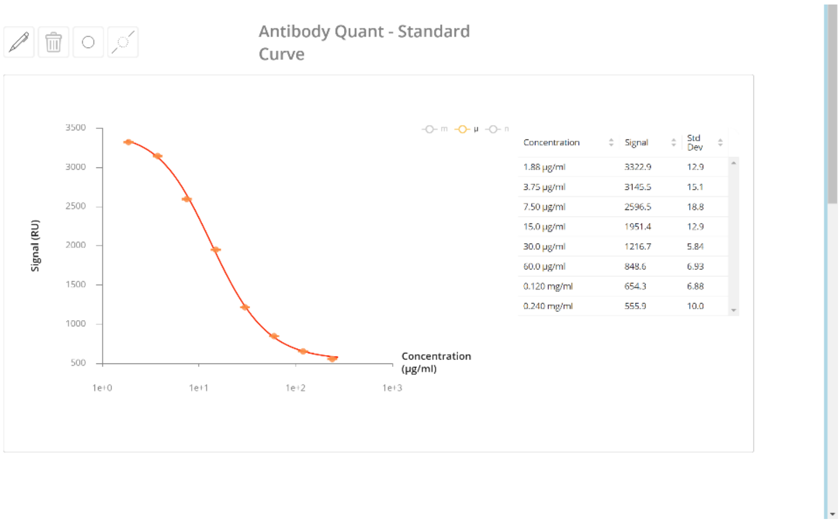
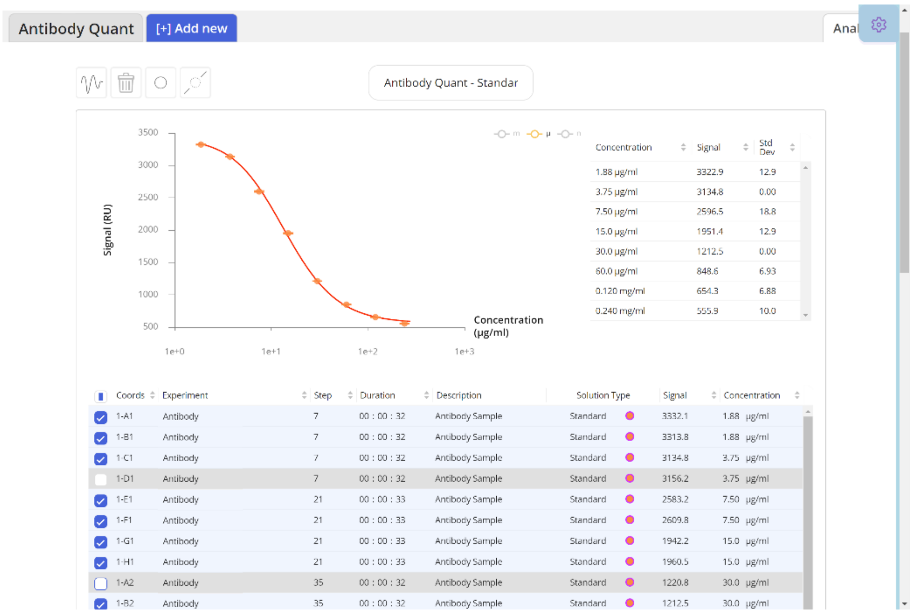


Figure 4.9 Edit or exclude standard curve points



4.2 SAMPLE QUANTIFICATION

- You can quantify antibody samples using a previously saved calibration curve. For best accuracy, use a calibration antibody with the same species origin and isotype as the samples.
- From **the Experiments** page, tap the experiment containing the sample measurements.
  → See **Figure 4.10**
- On the experiment summary screen, tap **Analysis**.
  → See **Figure 4.11**
- A summary of measurements will appear, grouped by solution type. Note: All the measurements labelled as **Sample** will be used for quantification. Measurements labelled as **Standards** (if present) can also be used to **compensate for experimental variation** between the sample and calibration curve runs, improving quantification accuracy. Tap **New Chart.**
  → See **Figure 4.12**
- Tap **Import** to add a previously saved calibration curve to this analysis.
  → See **Figure 4.13**
- Select the analysis that contains the calibration curve you wish to use.
  → See **Figure 4.14**
- All curves associated with that analysis will appear. Tap the desired curve to select it.
  → See **Figure 4.15**
- Tap **New Chart**, then select **Quantify** to begin.
  → See **Figure 4.16**
- All sample wells will be quantified against the selected calibration curve. A plot will show the curve (orange line) and sample positions (blue dots).
  → See **Figure 4.17**
- A summary table will display for each sample:
  - Signal Compensation
  - Raw Concentration
  - Adjusted Concentration
    Tap Finalize to save the analysis.
    → See **Figure 4.18**
- To export the analysis, insert a USB drive and tap **Export**, then choose the desired format and location.
  → See **Figure 4.19**

Figure 4.10 Select experiment from the Experiments page

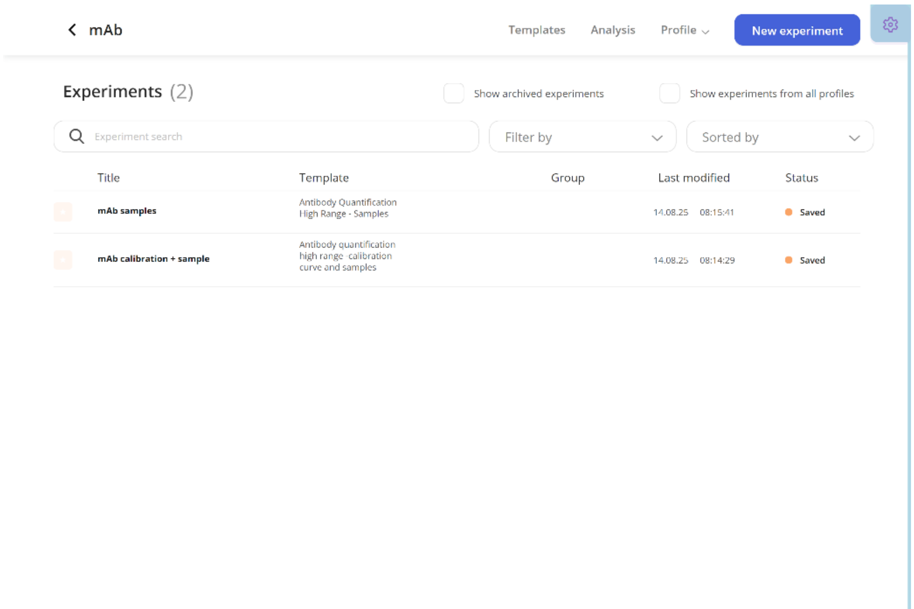


Figure 4.11 Tap Analysis from the experiment summary

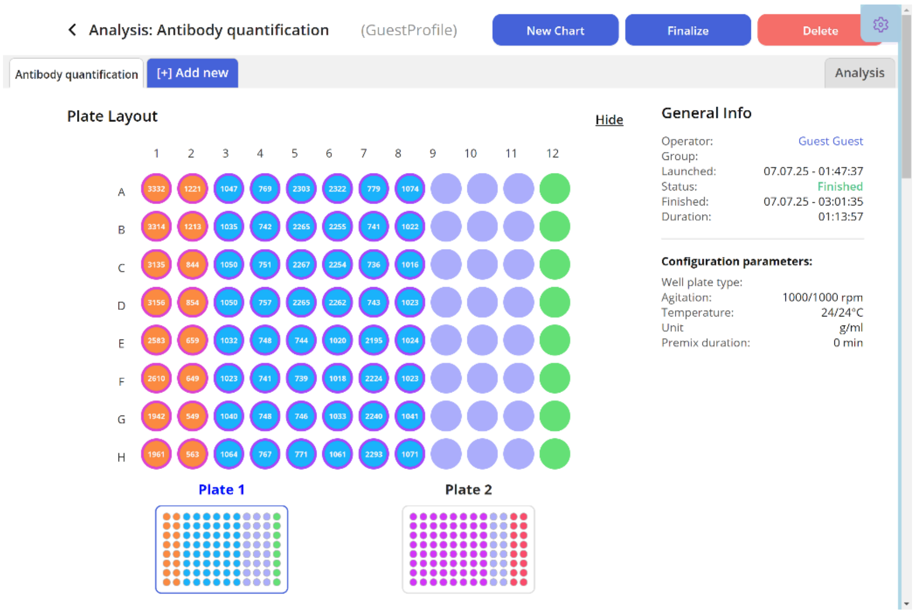


Figure 4.12 Tap New Chart to start

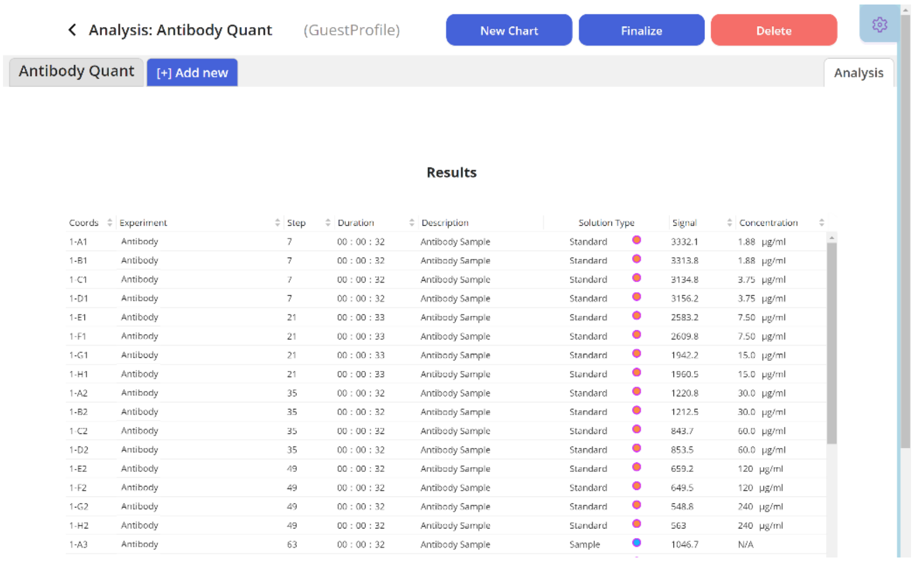


Figure 4.13 Tap Import to load a saved calibration curve

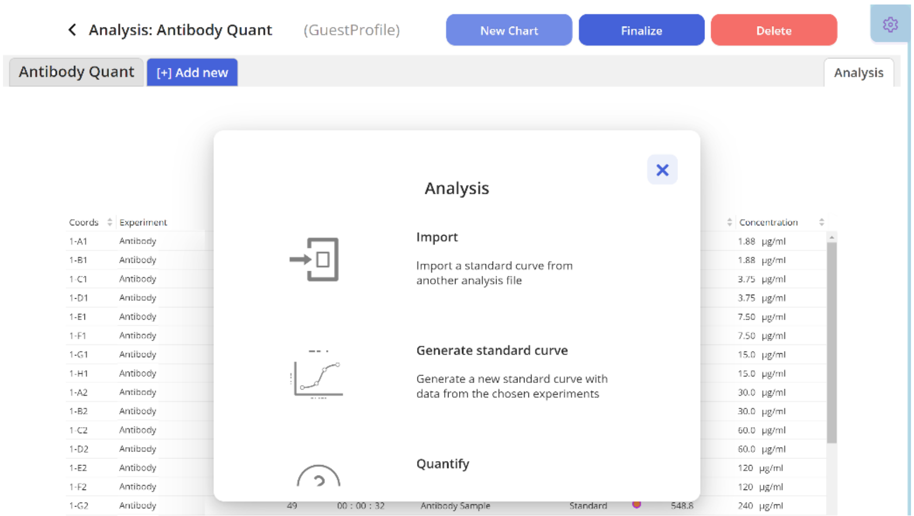


Figure 4.14 Select the analysis containing the desired curve

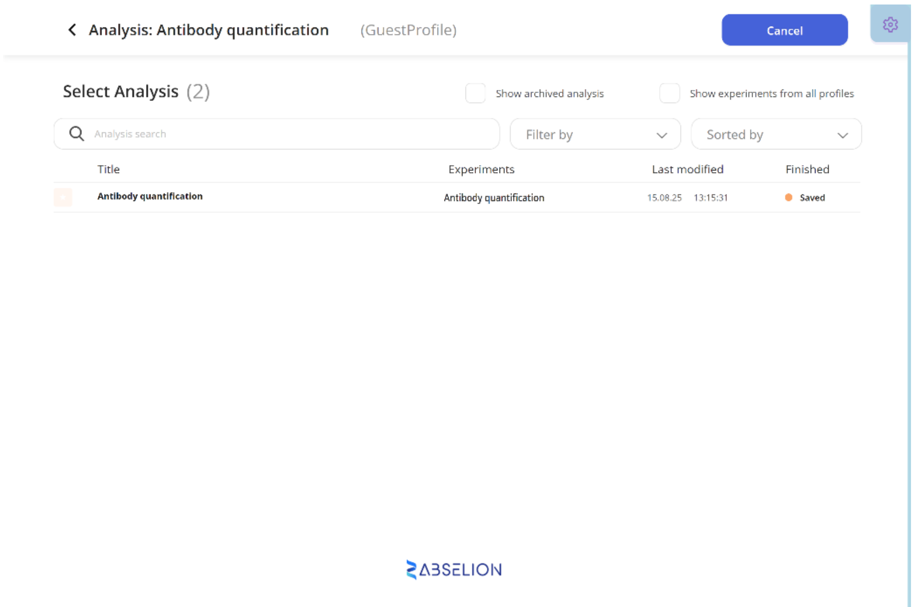


Figure 4.15 Choose a specific curve from the selected analysis

Figure 4.16 Tap Quantify to apply the curve to sample data

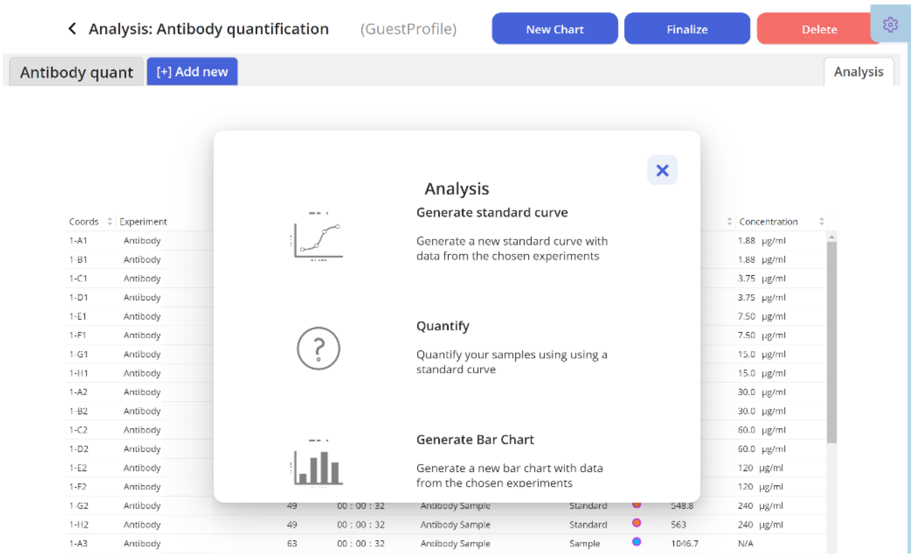


Figure 4.17 Samples plotted against the calibration curve

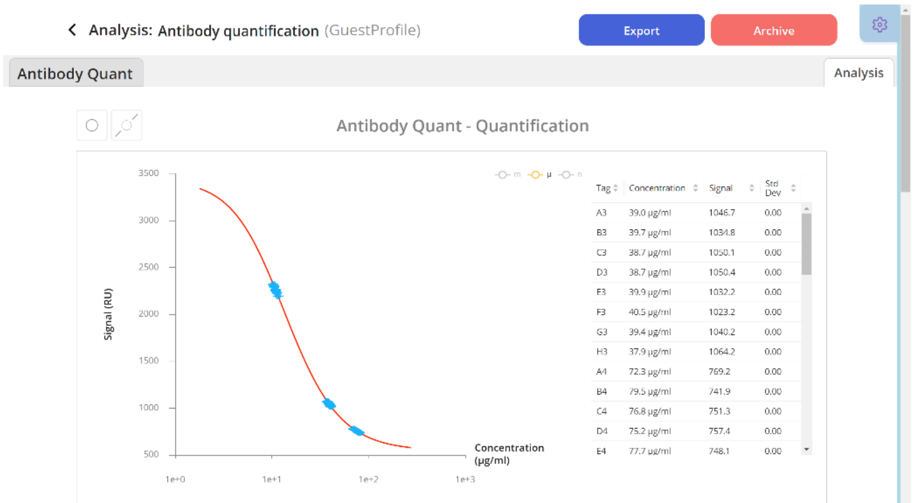


Figure 4.18 Summary of quantified sample results with compensation

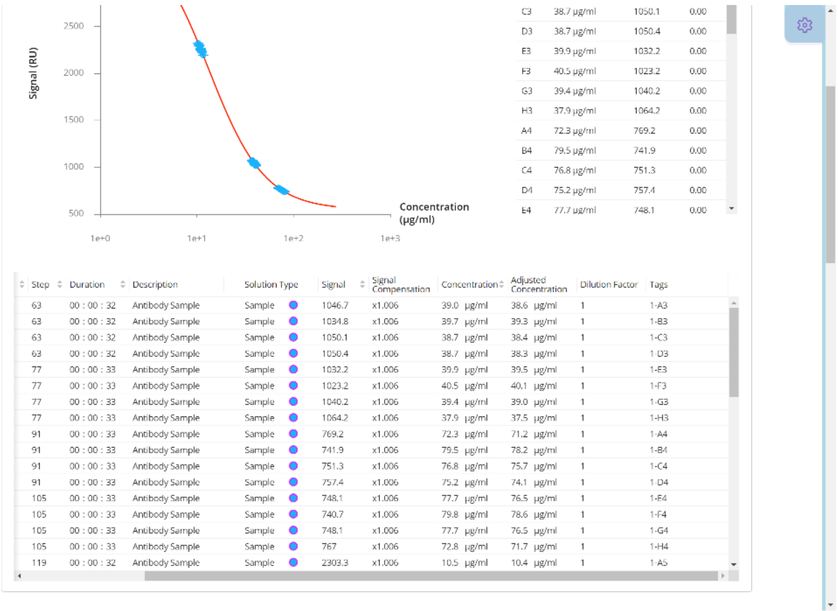
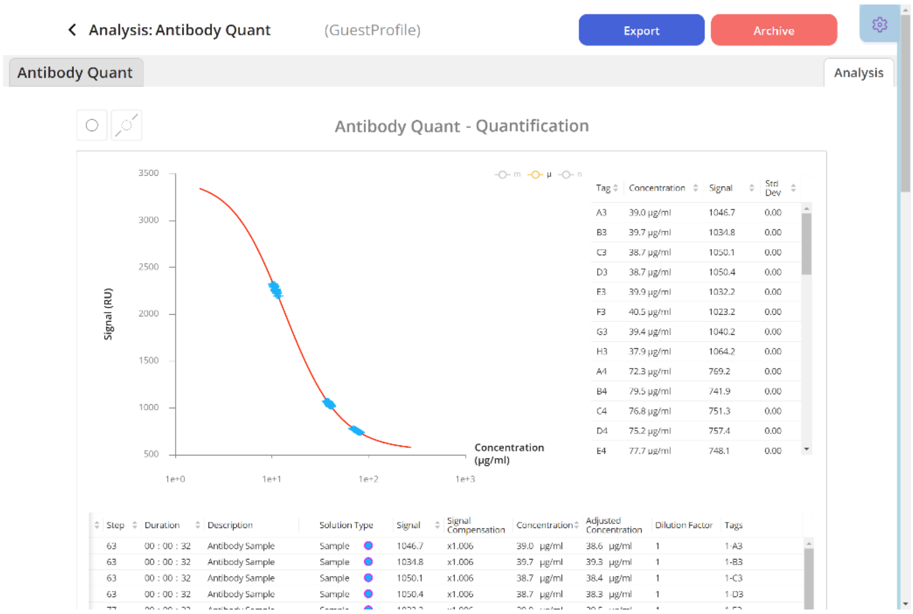


Figure 4.19 Export options for saving analysis data



5. Appendix

CUSTOMISING QUANTIFICATION UNITS

Amperia assay templates can display calibration curves using either **molar units** (e.g., µM) or **mass concentration units** (e.g., µg/mL) on the x-axis.

By default, **His-tagged protein assays** use molar units, while **antibody assays** use mass concentration units.

To change the x-axis unit, duplicate the desired assay template and open the **Settings** section under **Sequence**. Select your preferred unit from the **Default Units** dropdown, then save the template. The modified template will appear as a new option for future assays without altering the original.

STORAGE AND STABILITY

- Store kit components at 2–8 °C.
- Do not freeze reagents.
- Sensors should remain sealed in their original foil pouch until use.
- Kit is stable until the expiration date indicated on the label.

ABSELION™

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SAFETY INFORMATION

This kit is intended for **research use only**. Not for diagnostic or therapeutic use.

Handle all samples and reagents according to your institution's biosafety guidelines.

TRADEMARKS

MANUFACTURER

HexagonFab Ltd. (trading as **Abselion™**)
Unit 1, Cambridge House
Camboro Business Park
Oakington Road, Girton
Cambridge, CB3 0QH
United Kingdom

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For product information or technical support, please contact: **support@abselion.com**