SPLASH™ Singleplex Kit

USER GUIDE

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Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

The information in this guide is subject to change without notice.

Revision history:

Revision	Date	Details
A.0	Nov 10, 2026	New user guide for SPLASH singleplex assay kits

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Product Information

Product Description

SPLASH™ (Solid Phase Ligation Assay with Single wasH) assays unlock ultrasensitive protein detection through a streamlined proximity ligation workflow and standard benchtop qPCR (quantitative Polymerase Chain Reaction) instrumentation. The assay achieves sub-picogram per milliliter (pg/mL) sensitivity with small sample volumes, no specialized equipment, and minimal hands-on time.

Kit Contents and Storage

SPLASH kits ship in one shipment on gel pack. Box 1 contains lyophilized standards, AmPLAmix and the 20x AmPLAmix additive and should be stored at -20 °C upon receipt. Box 2 contains buffers for sample, probe, and wash, the magnetic beads, and the probes for the target protein and should be stored at 4 °C upon receipt. The kits contain sufficient material to run a total of 96 wells across two runs.



Important: Do not store the magnetic beads (in box 2) in the freezer. They should be stored at 4 °C or the effectiveness of the kit will be compromised.

Box #	Item	P/N	Quantity	Storage
1	Lyophilized Standard	Kit-specific	2 vials	-20 °C
	Lyophilized CheckPoint	Kit-specific	2 vials	
	20x AmPLAmix Additive	Kit-specific	300 µL	
	AmPLAmix	SPL-00055	6 mL	
2	Sample Buffer	SPL-00013	8 mL	4 °C
	Probe Buffer	SPL-00014	6.5 mL	
	Wash Buffer	SPL-00015	50 mL	
	Magnetic Beads	SPL-00016	5 mL	
	Target Protein Probe A	Target-specific	60 µL	
	Target Protein Probe B	Target-specific	60 µL	

Resources

Each SPLASH assay kit has an associated Quick Guide, Product Sheet, qPCR template, and a TallyPro™ Analysis Template, which can be found at https://taudia.com/splash-ultrasensitive-biomarker-kits/ in the Documentation section under each kit.

Methods

Workflow Overview

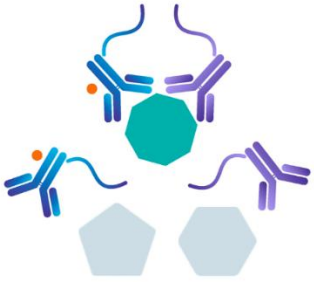
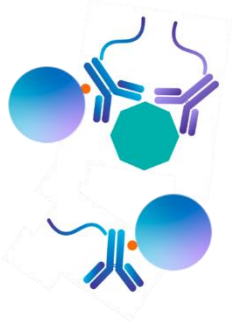
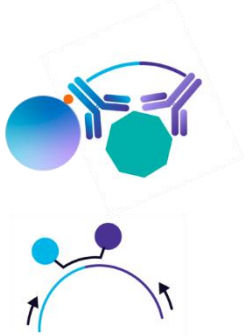
SPLASH assay kits enable ultrasensitive protein detection for specific protein targets. The workflow consists of four distinct steps: (1) Binding, (2) Washing, (3) qPCR Readout, and (4) Analysis. Protein binding, wash, and qPCR readout occur within the same 96-well consumable plate.

During binding, the sample is incubated with two different antibody probes, specific for different regions on the target protein. The antibodies bind to the target protein, forming immunocomplexes. Each antibody is functionalized with an oligonucleotide sequence, but only one of the antibodies is biotinylated. After immunocomplex formation, the biotinylated antibodies are captured by streptavidin-coated magnetic beads.

The washing step removes background material from the sample, while retaining the target protein immunocomplexes bound to magnetic beads. A standard magnetic rack secures the beads within the 96-well plate during the wash.

After the non-specific background is washed away, qPCR readout quantifies the amount of target protein in solution (pg/mL). The AmPLAmix solution, containing all reagents required for ligation and qPCR, is added to the washed magnetic beads. The plate is placed directly in the qPCR instrument, where oligonucleotide ligation and amplification of the ligated product can proceed.

In the final analysis step, the Cq output from the qPCR instrument is converted to absolute pg/mL in the TallyPro software. The conversion utilizes an in-plate standard curve prepared using the lyophilized protein standard provided in each kit. With the recommended two replicates of the standard curve, and two replicates of CheckPoint, 78 wells remain on each plate for samples.

Step 1	Step 2	Step 3	Step 4
Binding	*Washing*	*Ligation & qPCR*	*Analysis*
			
Form immunocomplexes	Wash to reduce background	Ligation and Quantification	Convert Cq to pg/mL with TallyPro software

Guidelines

General Guidelines

- If preparing samples with a multichannel pipette, ensure that each tip fits securely for uniform pipetting, and use low-dead-volume reservoirs with at least 500 µL overage.
- Use the appropriately sized pipette for the desired volume and only use calibrated pipettes.
- Avoid bubbles by following best pipetting practices.
- Do not use expired reagents.
- Do not freeze magnetic beads.
- Do not vortex plates.
- Do not vortex Protein Standards or CheckPoint.

Lyophilized Protein Reconstitution and Standard Curve Guidelines

- Careful preparation of the standard curve is vital for precise and accurate sample quantitation.
- When reconstituting the protein standard vial, ensure that the lyophilized pellet is at the bottom of the vial by spinning the tube before opening.
- After reconstituting the protein standard, allow the vial to sit at room temperature for 10 minutes before creating the standard curve.
- Ensure complete mixing when creating the standard curve by slowly pipetting up and down 15–20 times before moving to the next standard point.

Plating Standards and Samples Guidelines

- Minimize the time between plating standards and samples. Standards can be prepared and left capped and on ice or at 4 °C while preparing samples.
- After plating standards and samples, visually confirm that the liquid level is uniform between relevant wells.

Plasma Storage and Handling Guidelines

- Carefully handle plasma samples to maintain protein integrity and ensure that the sample is homogeneous.
- Upon thawing bulk plasma samples, clarify the samples by centrifuging at 10,000 – 14,000 rcf for 10 minutes at 4 °C. Aliquot small volumes (e.g., 200 µL), avoiding any pelleted debris or lipids.
- Store plasma samples at -80 °C as single-use aliquots in polypropylene tubes.
- Minimize freeze-thaw cycles for plasma samples (ideally <2) where possible. Many proteins degrade significantly after 2-3 freeze-thaw cycles.
- Ensure samples are completely thawed before use. Thaw them on ice just prior to the assay or at room temperature, depending on the analyte's stability. Once thawed, vortex or invert the samples gently but thoroughly to ensure they are homogeneous. This prevents variations in analyte concentration due to stratification during freezing.

PCR Guidelines

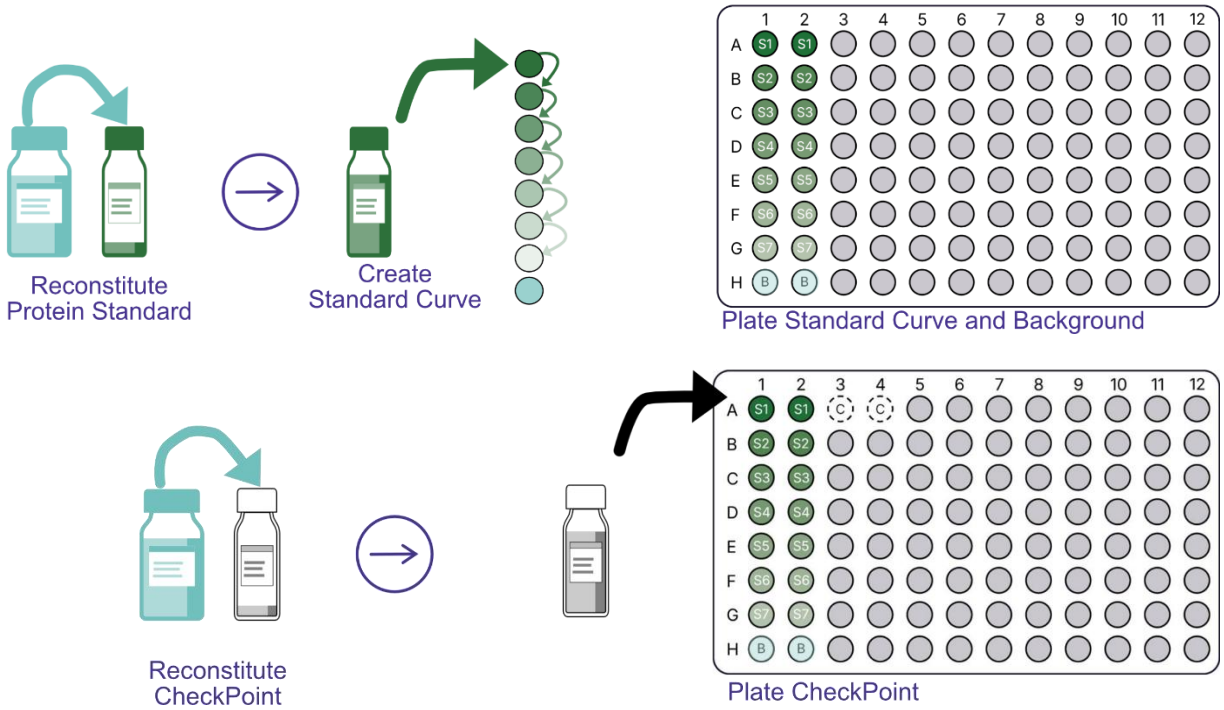
- SPLASH assays leverage qPCR for signal readout, so performance will be compromised in the presence of PCR inhibitors or amplicon contamination.
- Follow best lab practices for DNA and protein assays: wear gloves, use low-bind and DNase/RNase-free polypropylene plastics, clean the laboratory bench before initiating work, and use low-retention filter tips.
- Follow the appropriate user guide for the qPCR system.
- Ensure the qPCR instrument is calibrated and functioning correctly.
- Use 96-well consumables appropriate for the qPCR instrument.
- Utilize the plate sealer tool to seal 96-well plates before starting the PCR run. The optical seal on the top of the plate should be free of wrinkles.
- Before starting the PCR run, check that the liquid-fill level is above the magnetic beads and is uniform between all plated wells.

Required Materials Not Provided

- 96-well qPCR instrument with FAM channel
- 96-well plate magnetic bead separation rack
    - Recommended: Taudia's 96-well Ring Magnet
- 96-well qPCR plate suitable for the instrument with at least 0.2 mL well volume
- Microtiter plate adhesive seal, optical grade, suitable for the qPCR instrument
- Calibrated pipettes and appropriate low-retention filter tips
- Tubes and plastics for dilutions
- 15 mL tube
- Paper towels
- *Optional:* Multichannel pipettes
- *Optional:* Reagent reservoirs for multichannel pipetting
- *Optional:* Ice and ice bucket
- *Optional:* Vortex
- *Optional:* Microtiter plate adhesive seal or aluminum foil seal to cover the plate during the immunocomplex incubation

Step 1: Binding

Prepare and Plate Standard Curve and CheckPoint



Reconstitute Protein Standard and CheckPoint

1. Perform a quick spin of the Protein Standard and CheckPoint tubes to ensure that the lyophilized pellet is at the bottom of the tube before removing the lid.
2. Reconstitute the Protein Standard by adding 500 µL of Sample Buffer to 1 vial of Lyophilized Protein Standard.
3. *Optional:* Reconstitute the CheckPoint by adding 500 µL of Sample Buffer to 1 vial of CheckPoint.
    a. CheckPoint is an optional calibrator between Standards 4 and 5, which can help confirm the quality of the Standard Curve.
4. Cap and invert the tube 5 times; do not vortex.
5. Allow the vial to reconstitute at room temperature for 10 minutes before use.
6. Once reconstituted, keep on ice or at 4 °C.

> Important: Do not pipette-mix the lyophilized standard as the powder could stick to the pipette tip. Instead, reconstitute by gentle inversion.

Prepare Standard Curve

1. Label a tube "Blank" and add 120 µL of Sample Buffer.

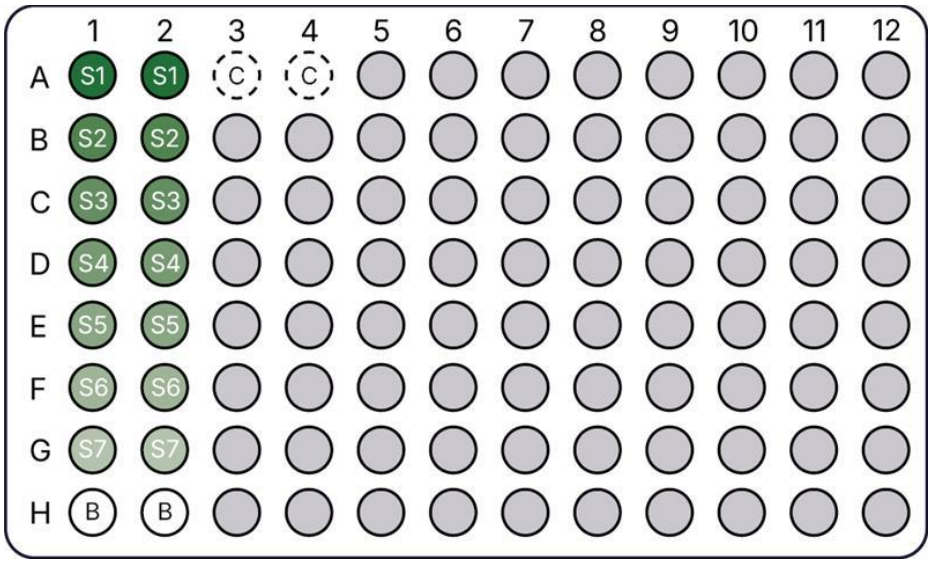
2. Label tubes for 7 dilution points (S1, S2, S3, S4, S5, S6, S7).
3. Add 120 µL of Sample Buffer to tubes S1 to S7.
4. Prepare S1 by adding 40 µL of Protein Standard to the S1 tube. Gently pipette up and down at least 15-20 times to mix.
5. Prepare a 4x dilution of S1 by adding 40 µL of S1 to the S2 tube. Gently pipette up and down at least 15-20 times to mix.
6. Continue the 4x serial dilution sequentially down to S7, each time adding 40 µL of the prior tube to the following tube and pipetting up and down at least 15-20 times to mix.

Plate Standard Curve and CheckPoint

1. Plate 50 µL of each standard and the blank into the 96-well plate as duplicates in columns 1 and 2, as shown in the plate map below.
2. *Optional:* Plate 50 µL of the reconstituted CheckPoint into wells A3 and A4.
3. After adding Standards and CheckPoint (optional), visually confirm that the liquid level is uniform between all plated wells.

Note: Standard Curve and CheckPoint locations can be changed in TallyPro, the Taudia Analysis Software, as desired when performing the analysis.

Important: Minimize time between plating standards and samples by ensuring the samples are thawed and ready to be added to the plate immediately after plating standards.



Prepare and Plate Samples

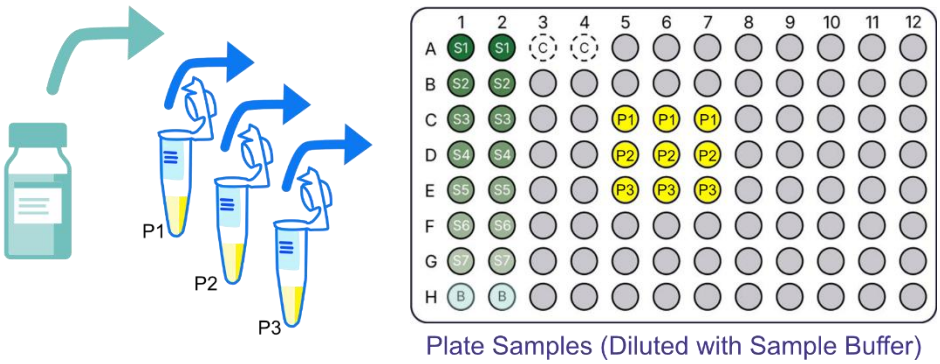


Plate Samples (Diluted with Sample Buffer)

Plasma Samples

1. Dilute plasma samples 4x into Sample Buffer for a total volume of 50 µL.
    a. Option A (Recommended for 1-2 replicates): Perform the dilution directly in the 96-well plate by slowly adding 37.5 µL of Sample Buffer into an empty well, followed by 12.5 µL of plasma sample. Pipette up and down a few times to mix.
    b. Option B (Recommended for >2 replicates): Create a bulk 4x dilution in a separate tube with overage, then transfer 50 µL volumes to the plate.
        i. Example: For 3 replicates of plasma, add 135 µL of Sample Buffer to a tube, followed by 45 µL of plasma, for a total volume of 180 µL. Mix by gently inverting the tube 3-5 times, then add 50 µL to each well on the plate.
2. After adding samples, visually confirm that the liquid levels are uniform between all plated wells.

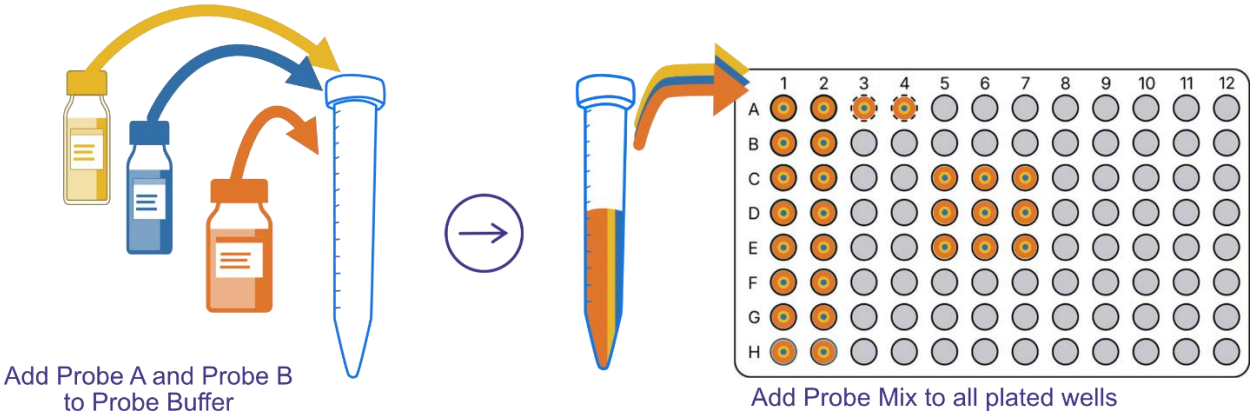
Cerebrospinal Fluid (CSF) Samples

1. Dilute CSF samples 10-50x into Sample Buffer and plate 50 µL of diluted sample into each well (singlets) or wells (2+ replicates).
    a. Recommended Dilution:
        i. Create bulk 10x-50x dilution in a separate low-bind tube with at least 20% overage.
        ii. Pipette volumes that are reliable for the pipettes used (recommended minimum volume of 5 µL CSF).
        iii. Mix by inverting the tube 3-5 times, then transfer 50 µL volumes to the plate.

⚠ Important: The SPLASH kits have 8 mL of sample buffer. Check the required volumes of sample buffer for your application before preparing the plate.

2. After adding samples, visually confirm that the liquid levels are uniform between all plated wells.

Prepare Probe Mix



1. Create the Probe Mix by adding Probe A and Probe B to Probe Buffer. Probe A and Probe B are the target-specific antibodies to the protein of interest.
    a. For 96 wells, first add 6000 µL of Probe Buffer to a 15 mL tube, followed by 60 µL of Probe A and 60 µL of Probe B.
    b. Change pipette tips after adding Probe A (do not contaminate Probe B with residual Probe A).
    c. For fewer than 96 wells, scale the Probe Buffer volume appropriately.

    ⚠ Important: Always add the Probe Buffer first, followed by Probe A and B. Change pipette tips between Probe A and Probe B.

2. Mix by capping the tube, then gently inverting 5-10 times.
3. Keep the Probe Mix on ice or at 4 °C.

    ⚠ Important: Make the Probe Mix after plating Standards and Samples.

Prepare Binding Reactions

1. Add 50 µL of Probe Mix directly to all plated wells.
    a. Recommended: Use a multichannel pipette and reagent reservoir. Prepare at least 500 µL overage.

    ⚠ Important: Avoid cross-contamination by changing tips between each column (multichannel) or well (single-channel).

Immunocomplex Formation

The target-specific antibodies will now form immunocomplexes with the target proteins.

1. Cover the 96-well plate to prevent contamination or evaporation during the incubation. Adhesive seals can be used to cover the plate. Keep in mind that the seal will be removed after incubation.

2. Incubate the plate to allow for immunocomplex formation.

    a. Option A: Incubate for 2 hours at room temperature. The AmPLAmix and 20x AmPLAmix additive should be removed from the -20 °C freezer at this stage.

    b. Option B: Incubate overnight at 4 °C. For overnight incubation, using an adhesive seal is highly recommended to avoid evaporation or contamination.

Thaw AmPLAmix and 20x AmPLAmix Additive

1. Completely thaw the AmPLAmix and 20x AmPLAmix Additive before proceeding to Step 2.

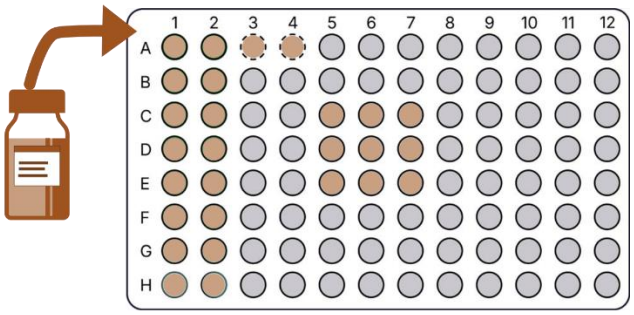
    a. Recommended: Start AmPLAmix thawing process at least 2 hours before initiating the washing step.

2. Check that the AmPLAmix and additive have fully thawed (no residual ice) before proceeding to the next step.

    ⚠ Important: Do not use incompletely thawed AmPLAmix.

Step 2: Washing

Add Magnetic Beads



Add magnetic beads to all plated wells

1. Thoroughly mix Magnetic Beads until homogeneous, either by shaking vigorously for at least 30 seconds or by vortex. The mixture may foam, which is expected and will not negatively affect performance.

2. Gently remove the adhesive seal from the 96-well plate.

Important: Keep plate flat while removing adhesive seal to avoid cross-contamination between wells.

3. Add 50 µL of Magnetic Beads into each plated well. Pipette-mix twice and change tips between wells.
    a. Recommended: Use a multichannel pipette and reagent reservoir. Make at least 500 µL overage when using multichannel pipette and reagent reservoir.
4. After adding magnetic beads, visually confirm that the liquid levels are uniform between all plated wells.

Capture Immunocomplexes

1. Incubate the Magnetic Beads with the immunocomplexes for at least 10 minutes at room temperature from the last bead addition.

Caution: Pinch hazard. The magnetic rack contains strong magnets that can snap together with, or onto, nearby metal objects. Fingers or skin caught between them can be pinched or bruised. Keep fingers away from the magnet surface when placing or removing the plate and keep metal tools and other magnets away from the rack.

Add 20x AmPLAmix Additive to AmPLAmix

1. Ensure that both 20x AmPLAmix Additive and AmPLAmix are fully thawed. Thoroughly mix the 20x AmPLAmix Additive by vigorously shaking for 30 seconds or vortex.
2. For 96 wells, add 300 µL of the 20x AmPLAmix Additive to 5700 µL of AmPLAmix. If using fewer than 96 wells, scale the volumes appropriately.
    a. Extra AmPLAmix with additive may be stored at -20 °C.
3. Cap and invert the tube 5-10 times to mix, or quick vortex.
4. Keep on ice or at 4 °C.

Wash

Wash 1

1. Press the 96-well plate onto the magnetic rack to pull down the beads.
2. Wait at least 1 minute before proceeding to the next step.

Important: Do not remove the plate from the magnet until washing and AmPLAmix addition are complete.

3. Decant the liquid from the plate while the plate is on the magnetic rack.
    a. Option A: Hold plate and magnetic rack together and decant into an appropriate waste container.
        i. Press the plate down on the magnet and keep the assembly horizontal as it is slid or lifted off the laboratory bench. Do not allow the plate and magnet to separate.
        ii. In a smooth, fast motion with a sudden stop, invert the plate over the waste container.
        iii. Shake the inverted plate and magnet assembly 1-2 times to remove the liquid.

    ⚠ Important: Invert the plate and magnet smoothly and quickly. Flipping the plate too slowly may lead to cross-contamination or incomplete wash.

    b. Option B: Keep plate on magnetic rack and aspirate liquid with a pipette, taking care not to remove the magnetic beads.

    ⚠ Important: If aspirating with a pipette, change pipette tips for each well.

4. Add 200 µL of Wash Buffer to each plated well. It is not necessary to change tips.
    a. Recommended: Use a multichannel pipette and reagent reservoir.

Wash 2

1. Follow steps in the previous section:
    a. Decant the first wash buffer from the plate while the plate is on the magnetic rack.
    b. Add 200 µL of Wash Buffer to each plated well.
    c. Decant the second wash buffer from the plate while the plate is on the magnetic rack.
2. While the plate is on the magnetic rack, invert and firmly tap the plate onto a paper towel 3-4 times to remove any residual liquid on the surface of the plate or in the wells.

    ⚠ Important: Tap plate and magnetic rack assembly firmly against paper towels until the residual liquid has been removed.

3. Leave the plate on the magnetic rack and immediately proceed to the next step.

Step 3: qPCR

Add AmPLAmix and Seal

1. While the plate is on the magnetic rack, add 50 µL of AmPLAmix (with 20x AmPLAmix Additive added) to each plated well.
    a. Recommended: Use a multichannel pipette and reagent reservoir.
2. Seal the 96-well plate with an optical adhesive seal appropriate for the plate type.
3. Remove the 96-well plate from the magnetic rack.
4. Visually verify that the liquid levels for each relevant well are even—all wells should contain the same volume. The liquid line should be above the bead pellet.
    a. If pellet is above liquid line, gently tap the plate against the laboratory bench.
5. Load the plate into a qPCR instrument and start the protocol within 15 minutes of adding the AmPLAmix and 20x AmPLAmix Additive.

Readout by qPCR

1. Run the following qPCR protocol:

Step	Temperature	Time	Stage
Ligation	30 °C	15 minutes	Hold
Ligase inactivation	95 °C	3 minutes	Hold
PCR Denature	95 °C	30 seconds	3 cycles
PCR Anneal	60 °C	1 minute	
PCR Denature	95 °C	15 seconds	37 cycles
PCR Anneal[1]	60 °C	30 seconds	

[1]Turn on data collection in FAM during the 37 cycles of 60 °C PCR anneal.

2. Kit-specific templates for some common qPCR instruments are available at https://taudia.com/splash-ultrasensitive-biomarker-kits/ in the Documentation section under each kit.
    a. Contact hello@taudia.com for additional support.

Step 4: Analysis

Quantify Protein Concentration in TallyPro

TallyPro, Taudia's analysis software, converts Cq values from the qPCR instrument into concentration values in pg/mL.

1. Import the Cq output generated by the qPCR analysis software into TallyPro.
2. Load the kit-specific analysis template (available for download from https://taudia.com/splash-ultrasensitive-biomarker-kits/ in the Documentation section under each kit).
    a. If necessary, adjust the well positions for Standard Curve, CheckPoint, and Blank.
    b. If necessary, adjust the sample dilution factor.
3. Export the pg/mL output from TallyPro as an Excel-compatible table.

TallyPro Analysis with Kit-Specific Templates

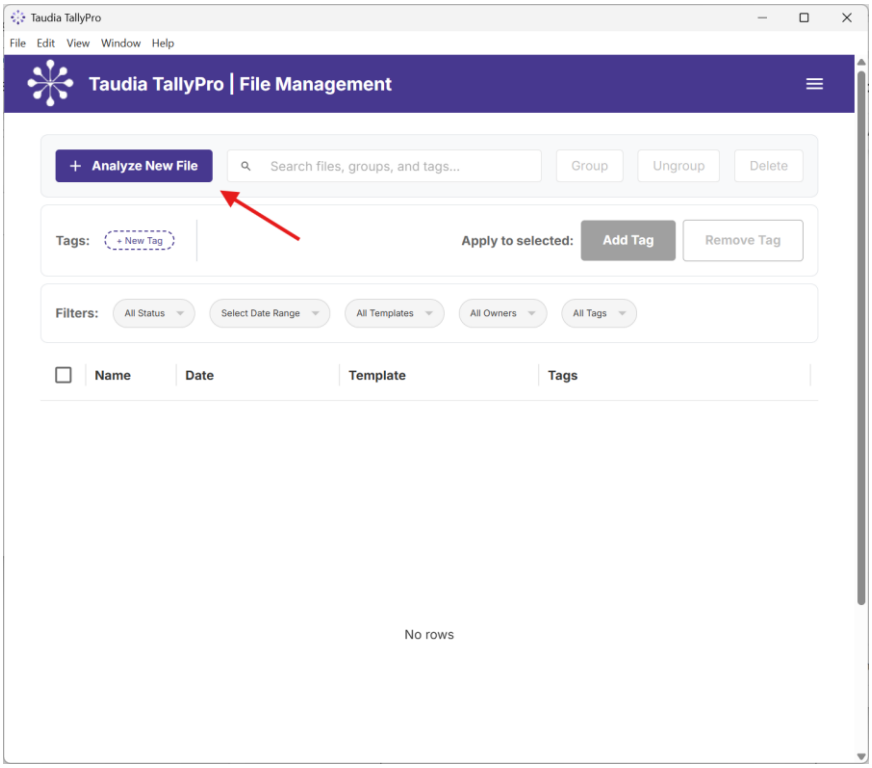
Kit-specific templates assume that the standard curve is plated in columns 1 and 2, with Standard 1 (S1) in A1 & A2, and Blank (B) in H1 & H2. The templates also assume that the CheckPoint (C) is in A3 and A4. Samples may be in any of the other wells. If the layout of the experimental plate differs from the template, go to the

Adjust Well Positions within Plate section.

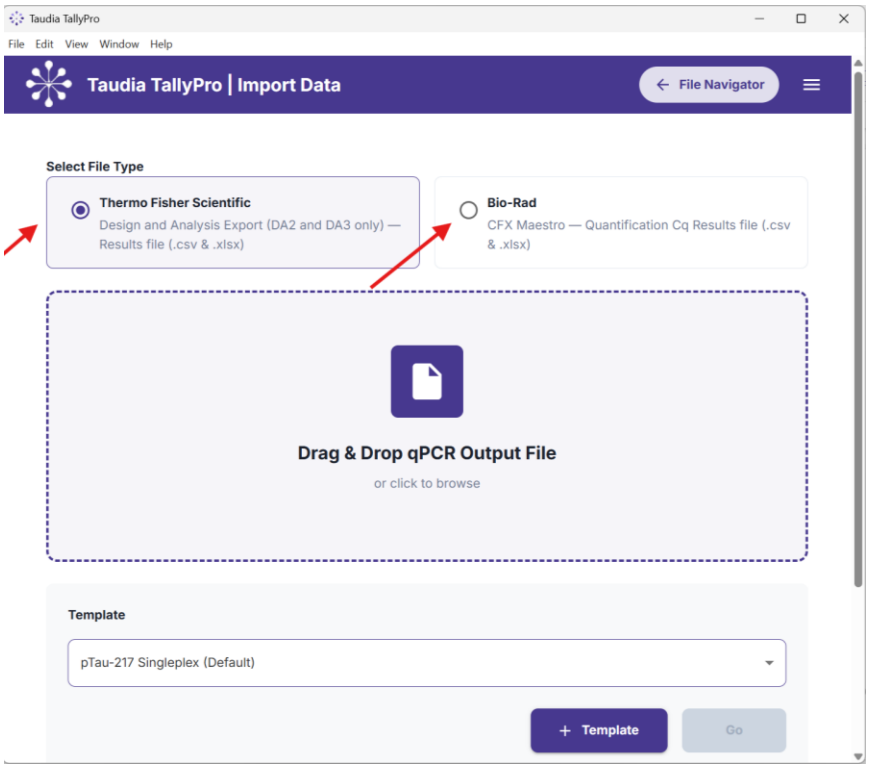
The template assumes a sample dilution factor. To update the sample dilution factor, go to “

Adjust Sample Dilution Factor" after completing steps #1 through 6 below.

1. Click on the “Analyze New File” button in the upper left.



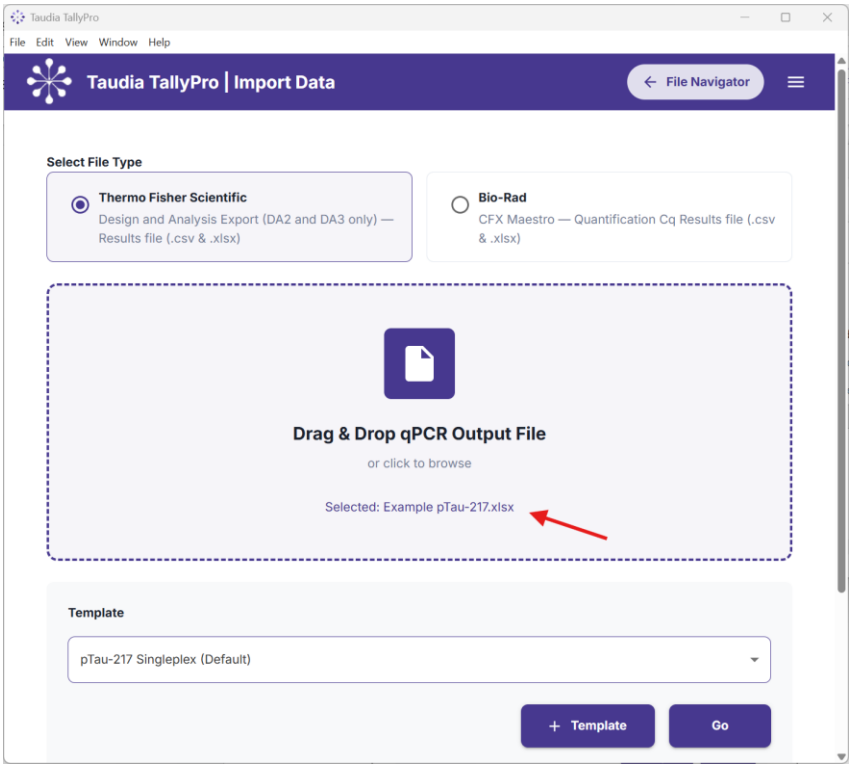
2. Import the qPCR Output File exported from the qPCR instrument software by selecting the qPCR instrument source (radio button at top).



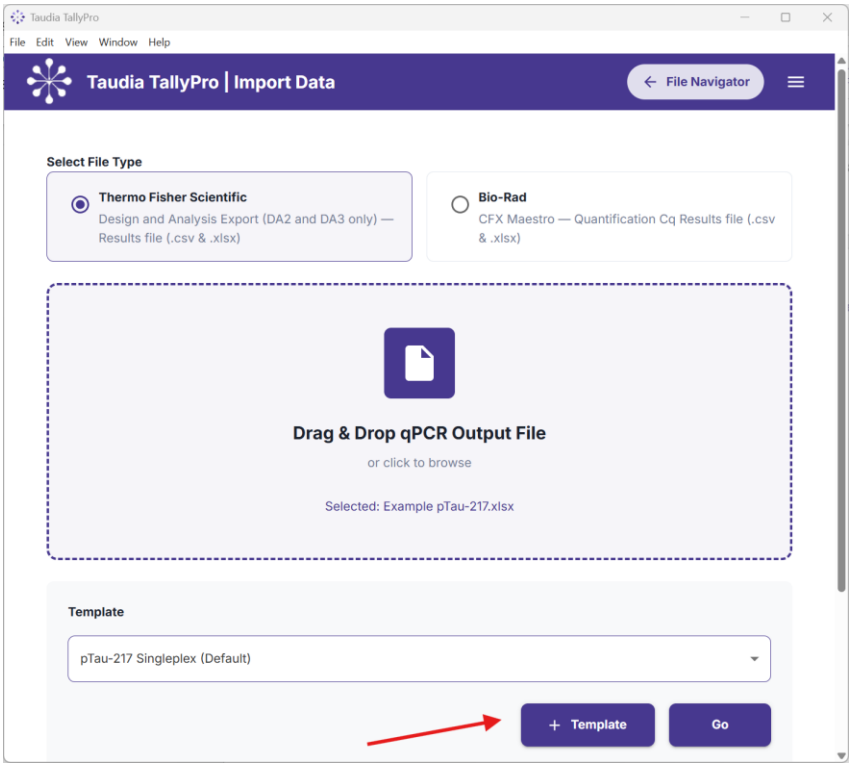
a. For Thermo Fisher Scientific, Design and Analysis Exports, choose the "Results" file (.xlsx format). If the "Combined all export tables in one file" option has been selected when exporting from Design and Analysis, import the single output file into TallyPro.

b. For Bio-Rad Laboratories, CFX Maestro Exports, choose the "Quantification Cq Results" file (.csv format).

3. After importing, the file name will appear in the light purple rectangle.

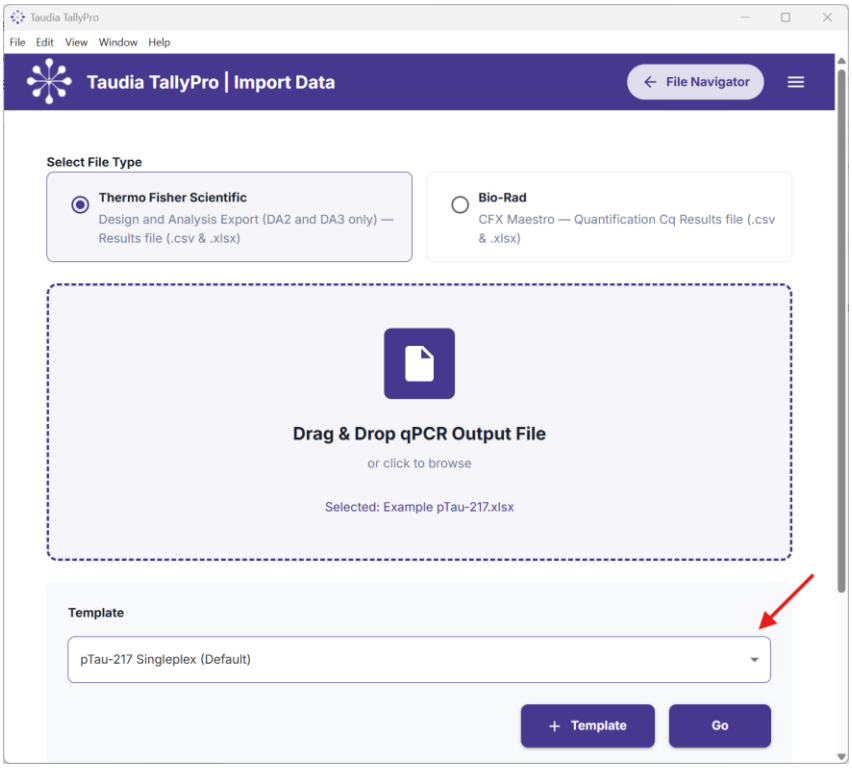


4. If necessary, import the Analysis Template file matching the assay by clicking on the purple "+Template" button and navigating to the desired template. After adding the file, a green message should pop up at the top of the screen indicating that the template was successfully added.

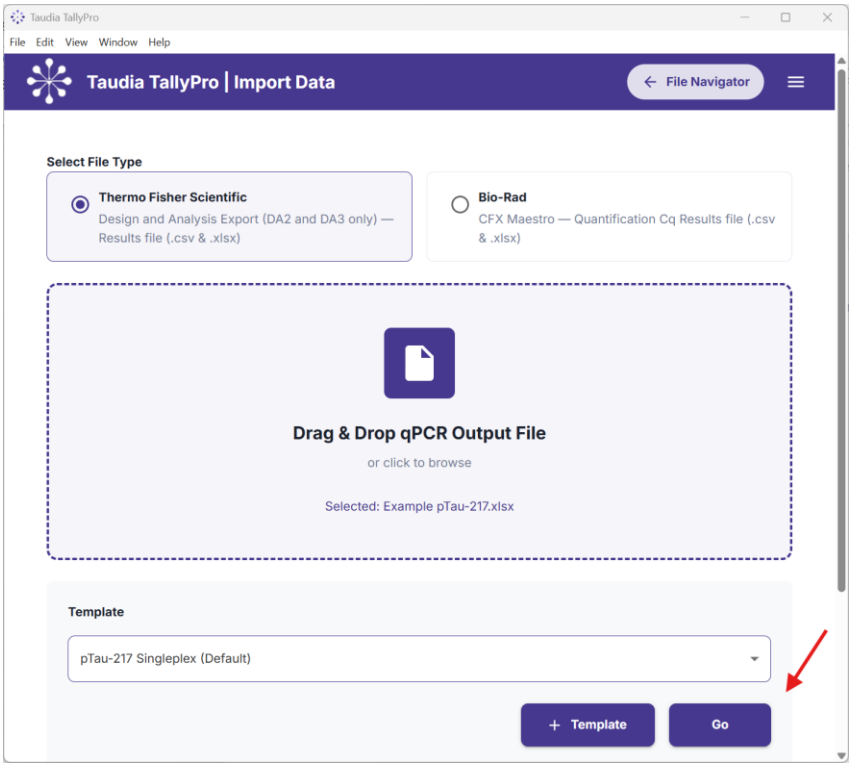


a. Analysis template files specific for each kit can be found at https://taudia.com/splash-ultrasensitive-biomarker-kits/ in the Documentation section under each kit.

5. Select the desired template specific for the kit from the pull-down menu.



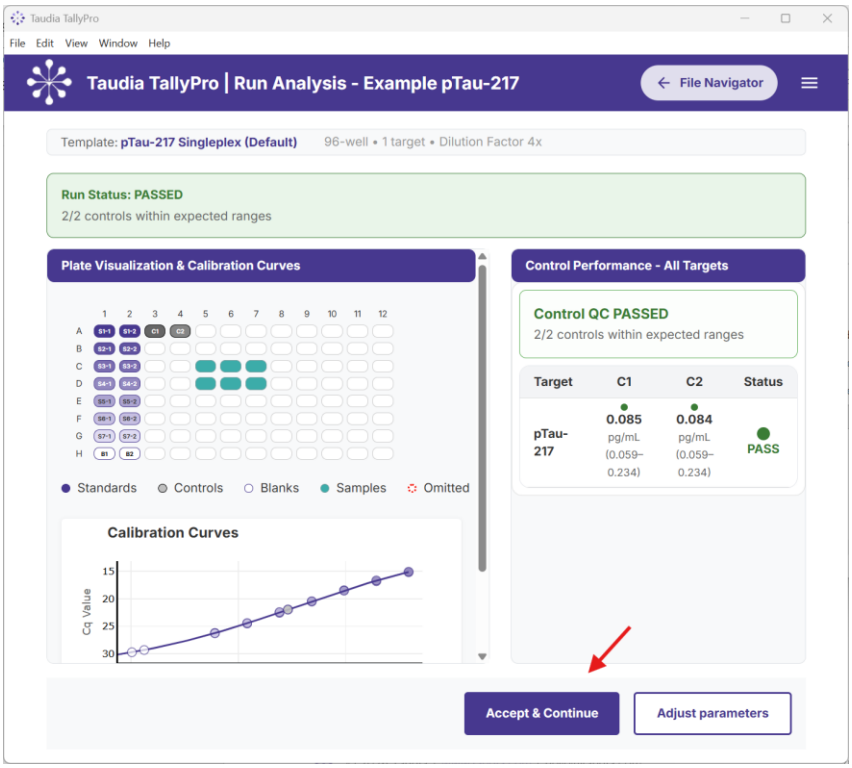
6. With the template selected, click on the "Go" button to proceed to the next step.



7. If the plate layout contains the standard curve in columns 1 and 2 and CheckPoint in wells A3 and A4, the default template positions will not need to be adjusted. The screen will contain the "Plate Visualization and Calibration Curves" on the left, and the "Control Performance" assessment on the right. Click "Accept & Continue" to proceed to the next step.

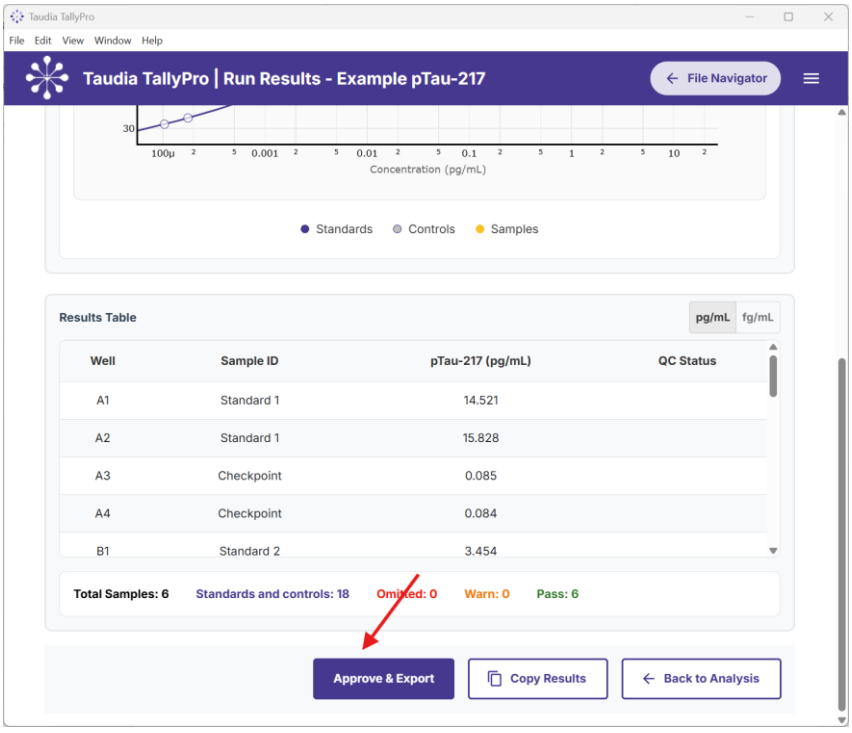
If the layout of the experimental plate is different, please go to the next section of the guide, "

a. Adjust Well Positions within Plate" for a description of how to manually change the well position identities.



8. The next screen shows the "Results Visualization" on the top and the "Results Table" on the bottom. Samples are shown as yellow dots in the "Results Visualization" graph and the blanks are empty dots. Scroll down and click on "Approve & Export" to move to the next step.





9. After clicking on the "Approve & Export" button, the software will ask for a save location. The Results Table output will be saved as a .csv in the desired location. The "Well" column corresponds to the position in the 96-well plate, the "Sample" column is the name of the sample in the qPCR file, the protein quantitation is in the third column, and the QC Status is in the final column. If QC status is "Pass," then the CheckPoint or control values fell within the expected range.

	A	B	C	D
1	Well	Sample	pTau-217 (	QC Status
2	A1	Standard 1	14.52103	
3	A2	Standard 1	15.82786	
4	A3	Checkpoir	0.085014	
5	A4	Checkpoir	0.083809	
6	B1	Standard 2	3.453543	
7	B2	Standard 2	3.879002	
8	C1	Standard 3	0.982667	
9	C2	Standard 3	0.927939	
10	C5	4X Plasma	0.035565	Pass
11	C6	4X Plasma	0.038067	Pass
12	C7	4X Plasma	0.034526	Pass
13	D1	Standard 4	0.228554	
14	D2	Standard 4	0.239022	
15	D5	4X QC Plas	0.024304	Pass
16	D6	4X QC Plas	0.02421	Pass
17	D7	4X QC Plas	0.024372	Pass
18	E1	Standard 5	0.055855	
19	E2	Standard 5	0.06166	
20	F1	Standard 6	0.014088	
21	F2	Standard 6	0.015034	
22	G1	Standard 7	0.003783	
23	G2	Standard 7	0.003571	
24	H1	Backgroun	0.000176	
25	H2	Backgroun	0.000104	

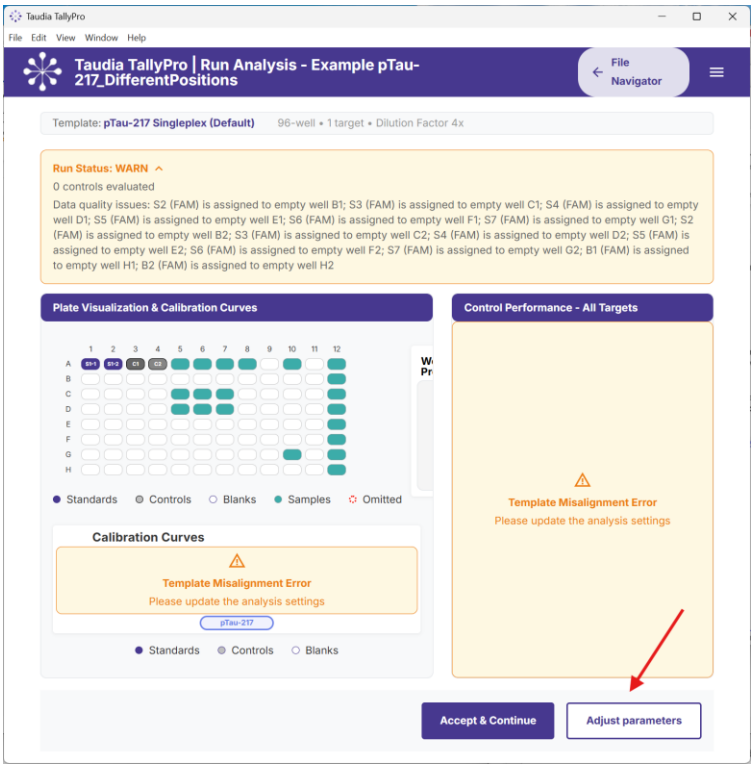
Important: Set sample dilution factor to match experimental value.

Adjust Well Positions within Plate

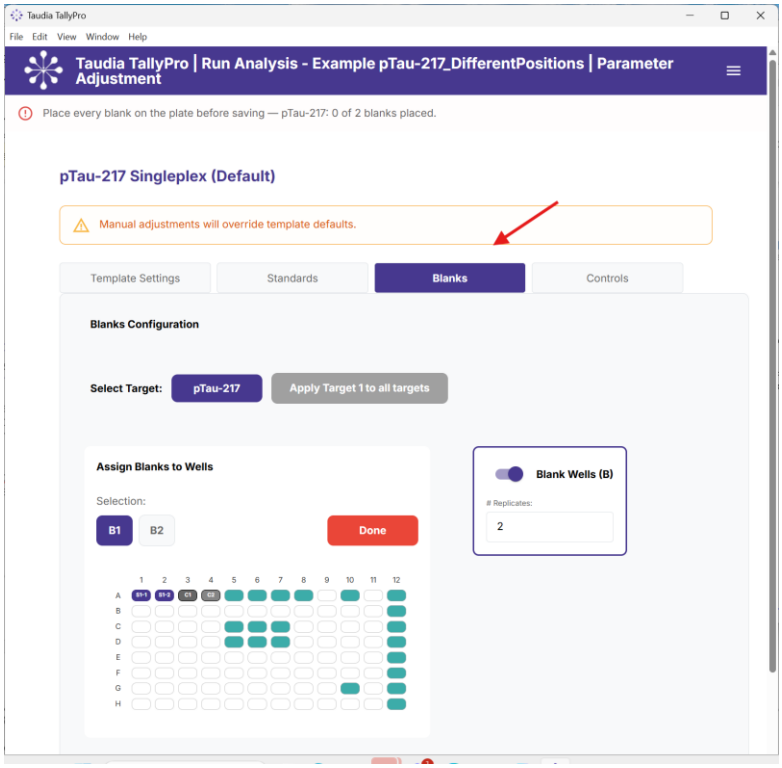
1. Follow steps #1 through #6 in the prior section, "TallyPro Analysis with Kit-Specific Templates," to import qPCR data and load the Analysis Template specific for the kit.

2. In this example, the positions of the Standard Curve, CheckPoint, and Blanks need to be updated. The first replicate of the Standard Curve is in row A, and the second replicate is in column 12. The CheckPoint samples are in A10 and G10, and the samples are in rows C and D.

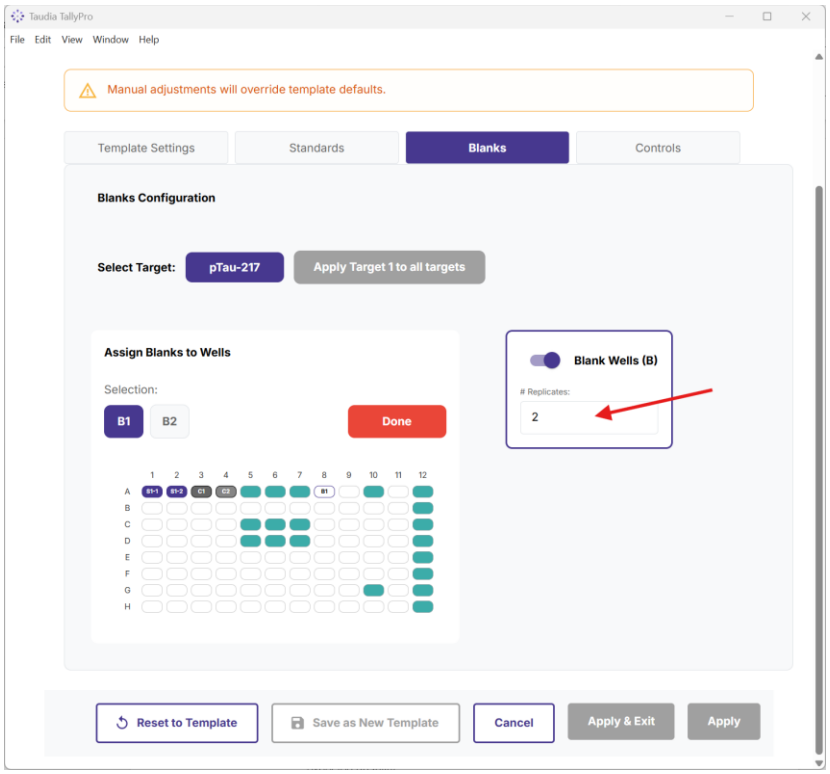
3. TallyPro will display several warning messages because the standard curve is not in the expected position (columns 1 and 2). Click on "Adjust parameters" in the lower right.



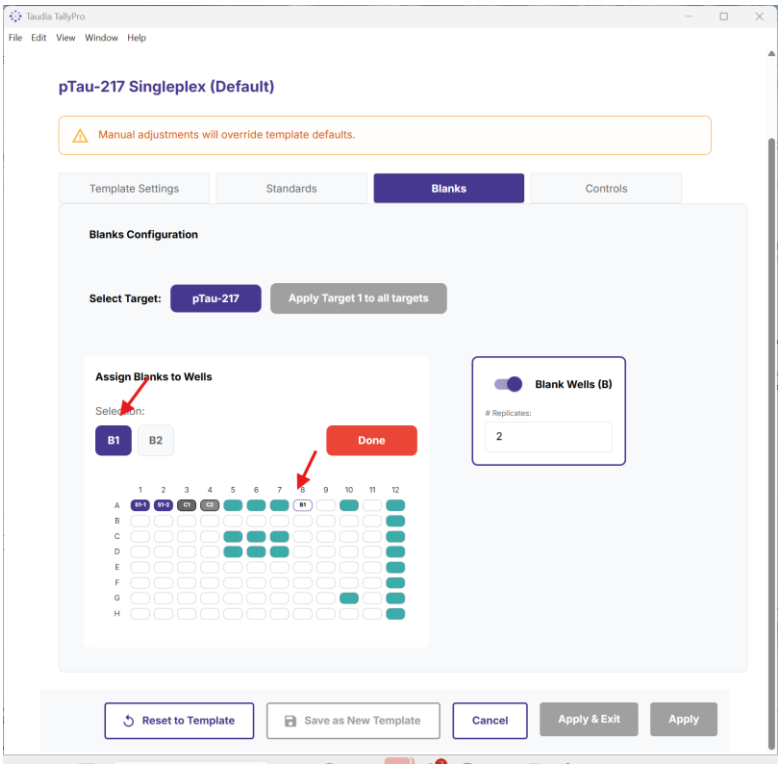
4. Click on the “Blanks” tab to adjust the position of the blanks to match the plate layout.



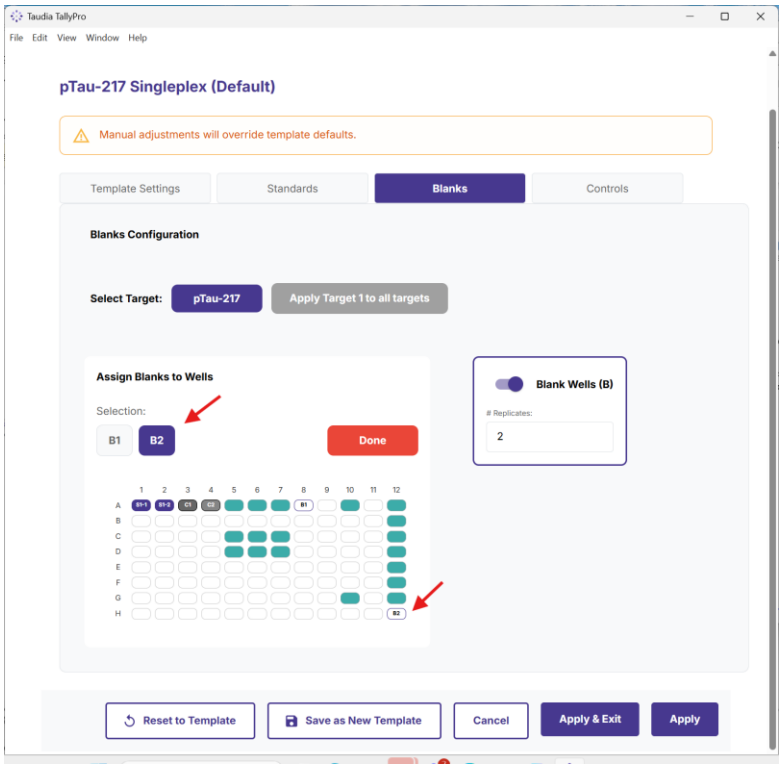
5. If needed, adjust the number of blank wells to match the experiment. Blanks can also be removed by clicking on the purple slider to the left of “Blank Wells (B)”.



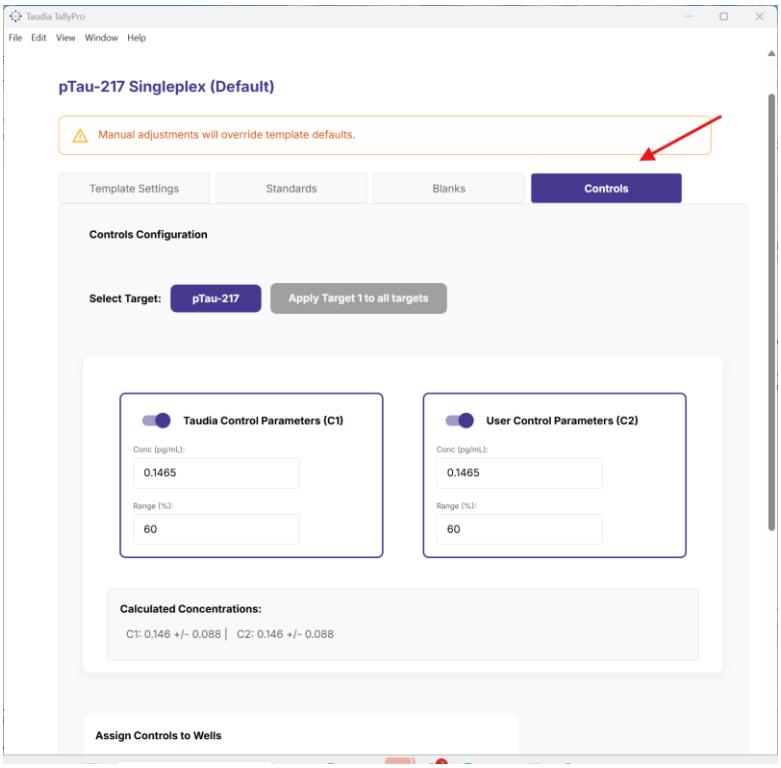
6. Click on “B1” (Blank #1) so that it is highlighted in purple. Then click the well on the plate map where Blank #1 is located. In this example, Blank #1 is in well A8.



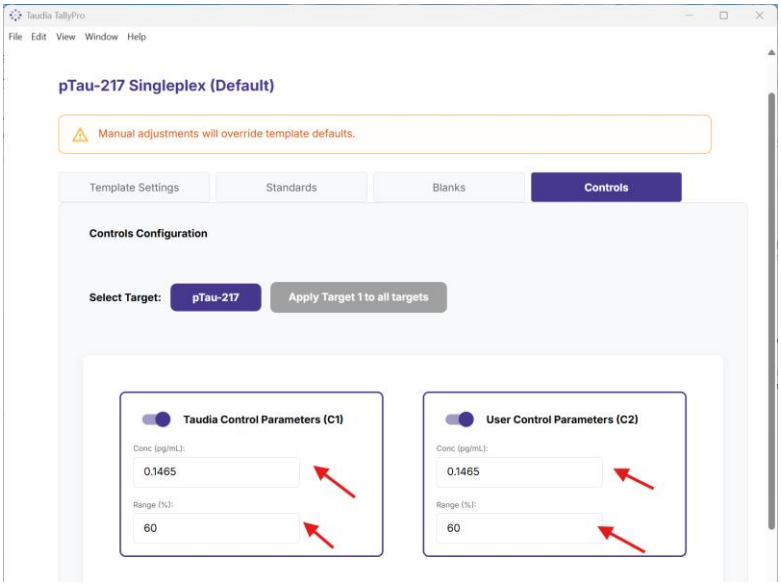
7. Repeat the process for all blank wells in the plate. In this example, there are two blank wells, located in A8 and H12.



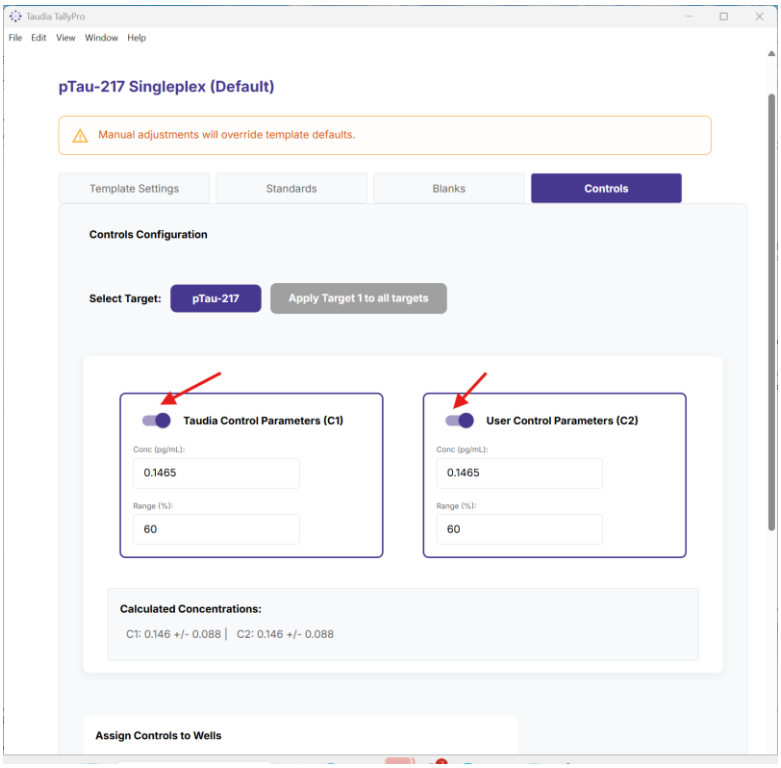
8. Click on the tab "Controls" to adjust the position of the CheckPoint (or user-defined controls).



a. If using user-defined controls, the expected concentration and acceptable range can be edited in the concentration and range fields.

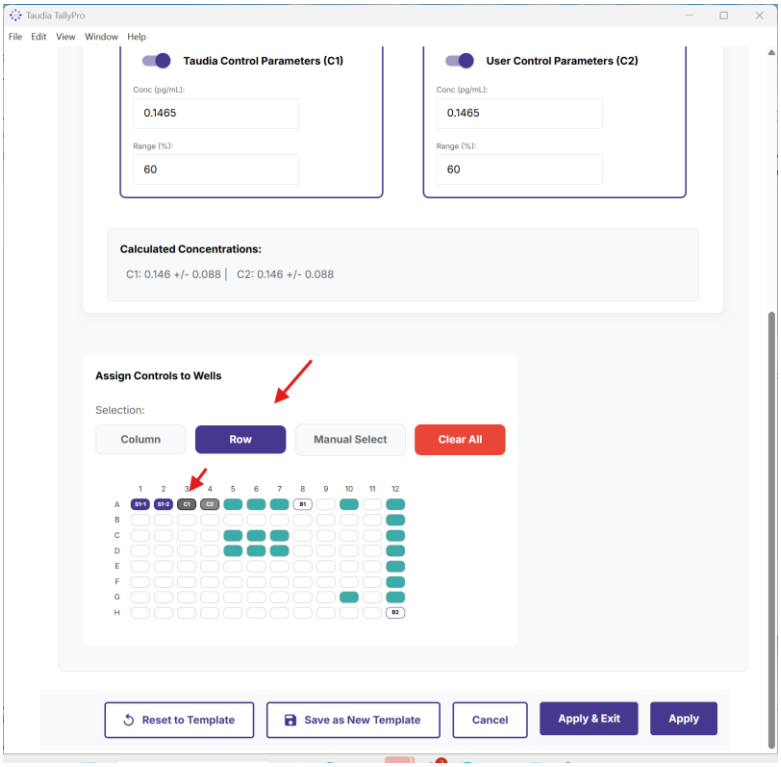


b. Controls (or CheckPoints) can be turned off as desired by sliding the toggle to the left of “Control Parameter.”

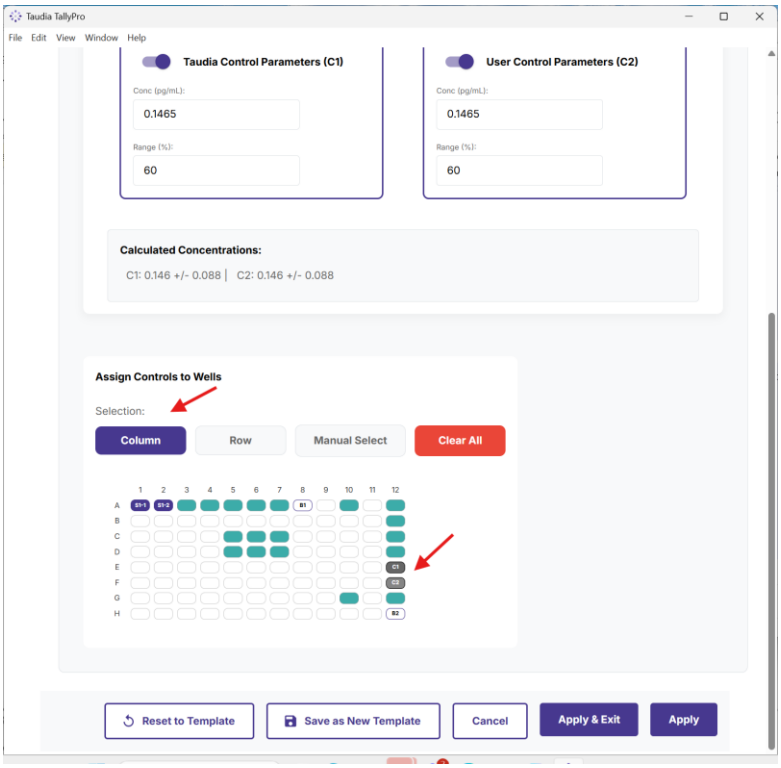


9. Scroll down to the plate map.

    a. If the CheckPoints are in adjacent wells in the same row, select “Row” and then click on the right-most well with CheckPoint.

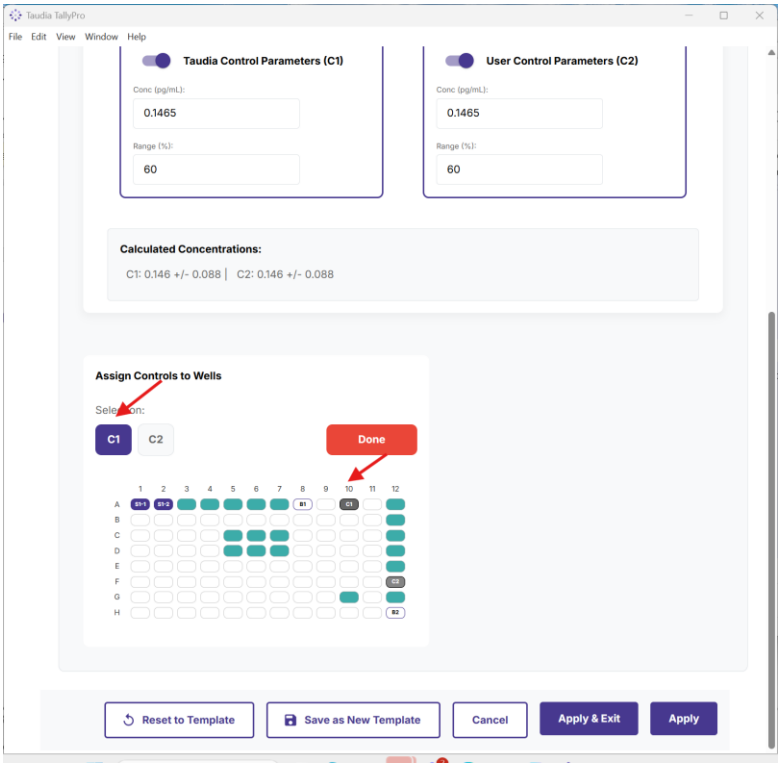


b. If CheckPoints are in adjacent wells in the same column, select “Column” and then click on the top-most well with a CheckPoint.

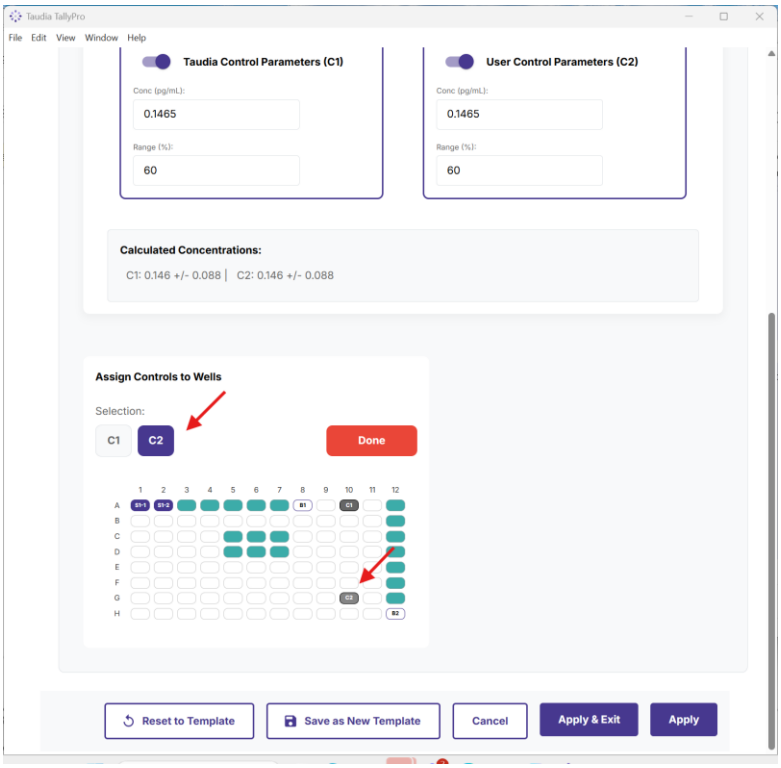


c. If CheckPoints are in non-adjacent wells, select "Manual Select" and then click on the wells containing CheckPoint.

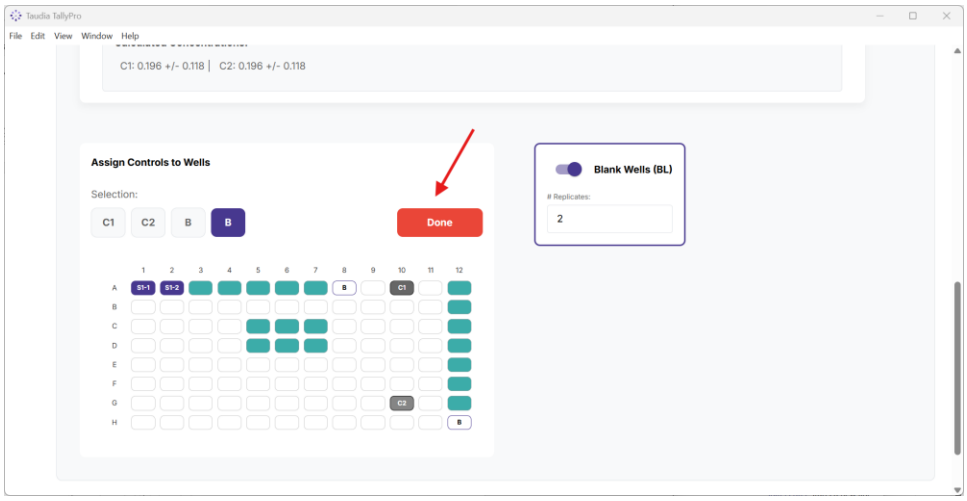
i. First click on "C1", then click the location on the plate map for the first CheckPoint. In this example, the first CheckPoint is in well A10.



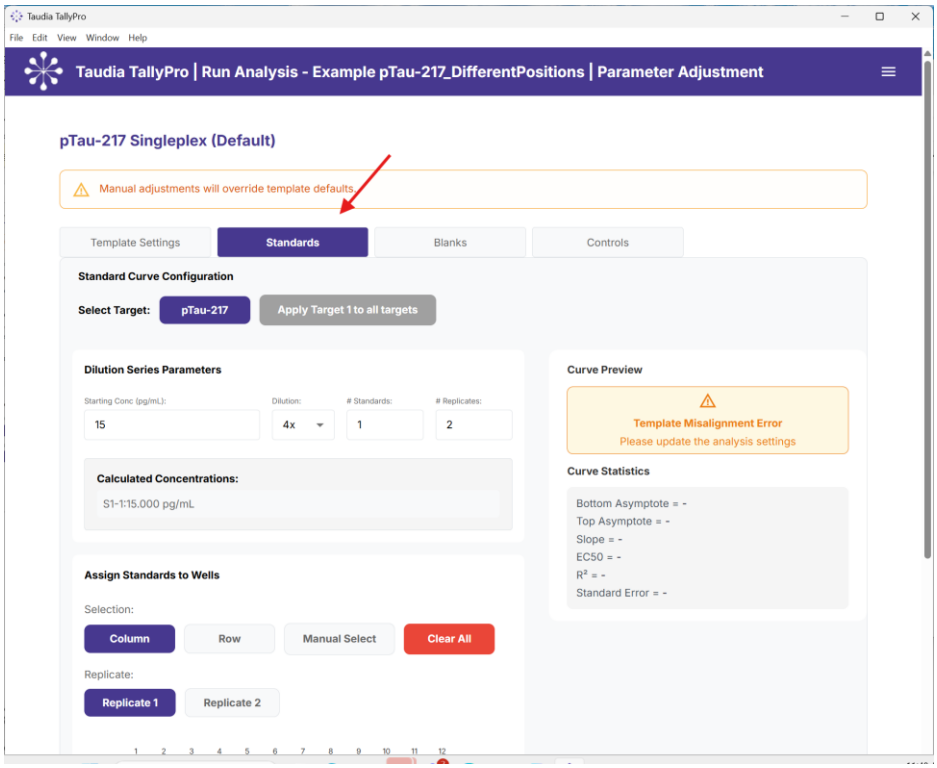
ii. Next click on "C2", then click the location on the plate map for the second CheckPoint. In this example, the second CheckPoint is in well G10.



d. When finished, click "Done".



10. Click on the tab “Standards” to adjust the positions of the Standard Curve. Standards can be located in the same row or column, or in any positions in the plate.



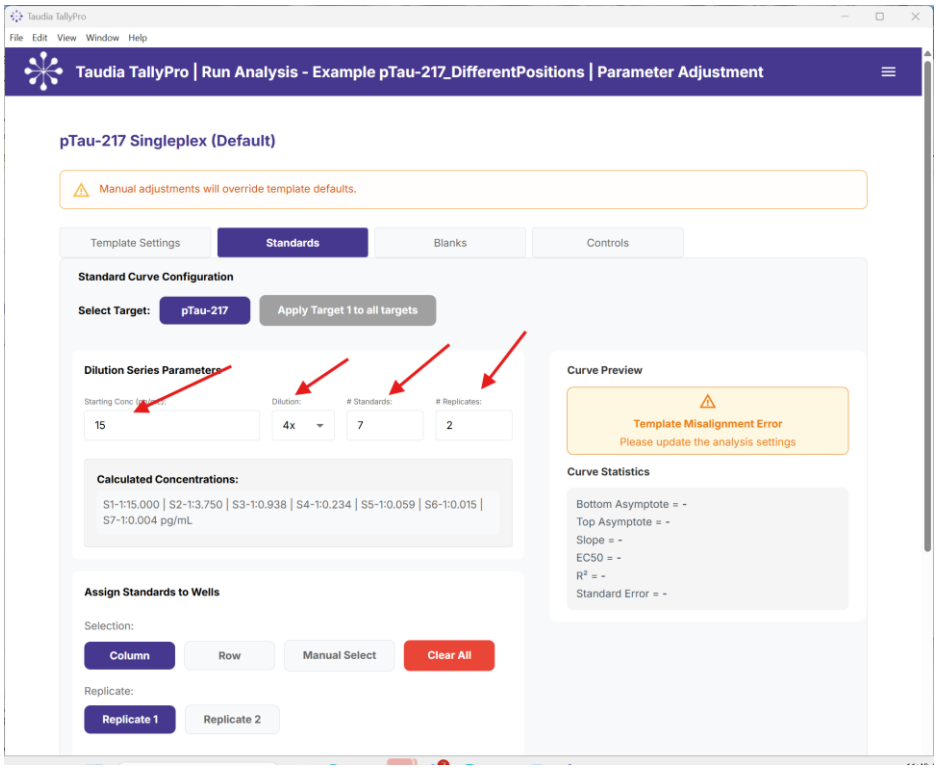
a. Adjust the Standard Curve Dilution Series Parameters as necessary.

   i. Check that starting concentration matches the kit values.

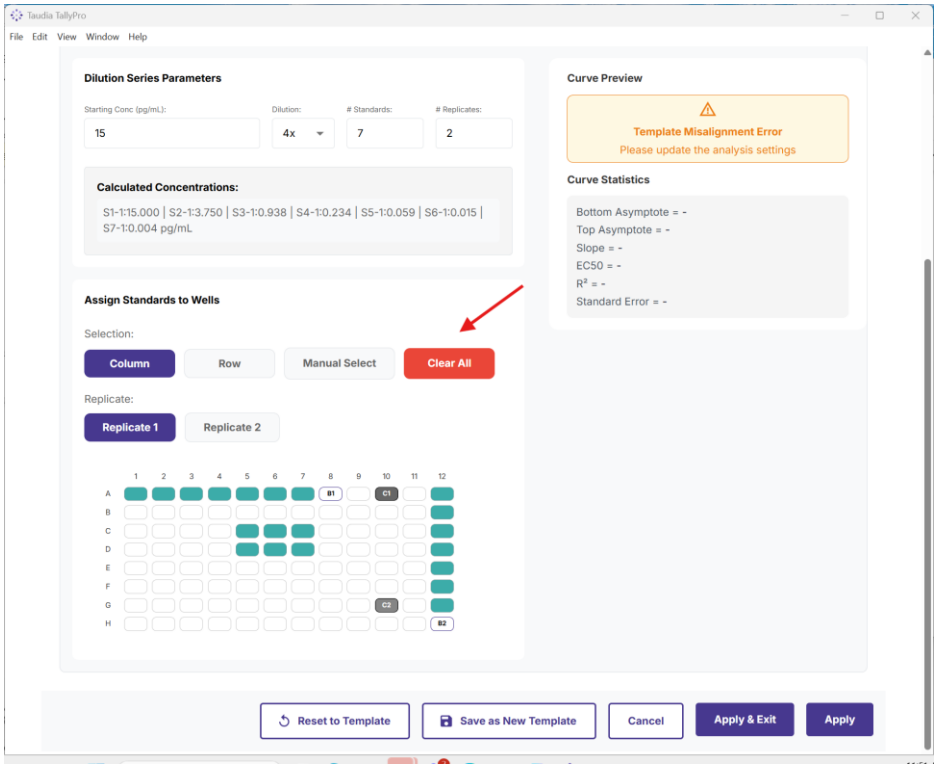
   ii. Ensure that dilution is set to 4x.

   iii. Set the number of standards to be 7.

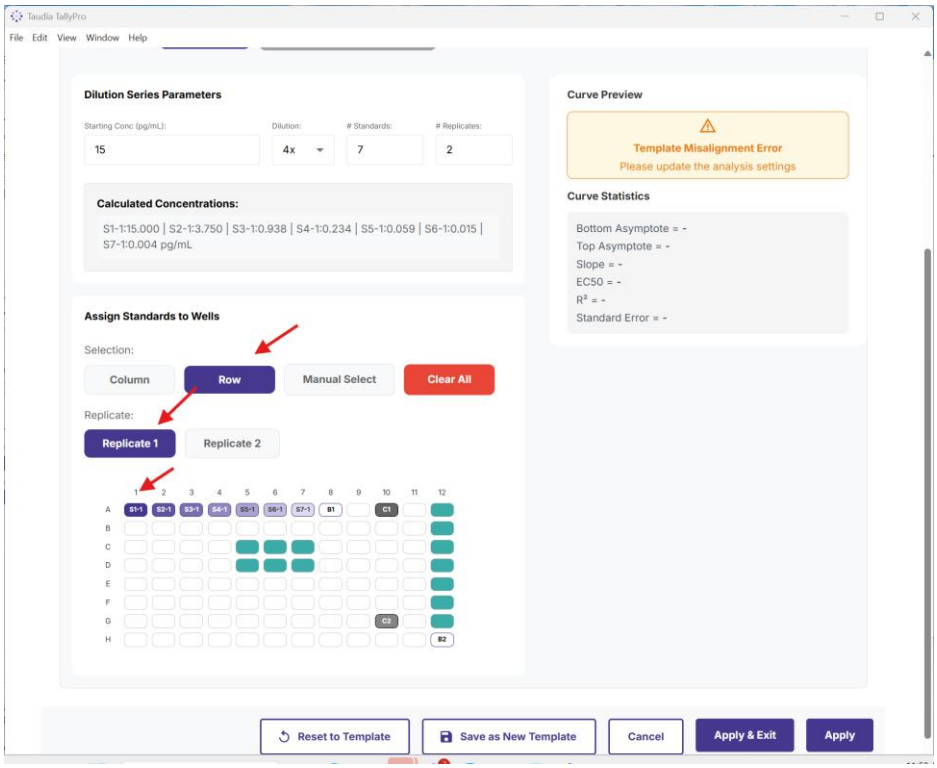
   iv. Set number of replicates to match the experiment. The default is 2.



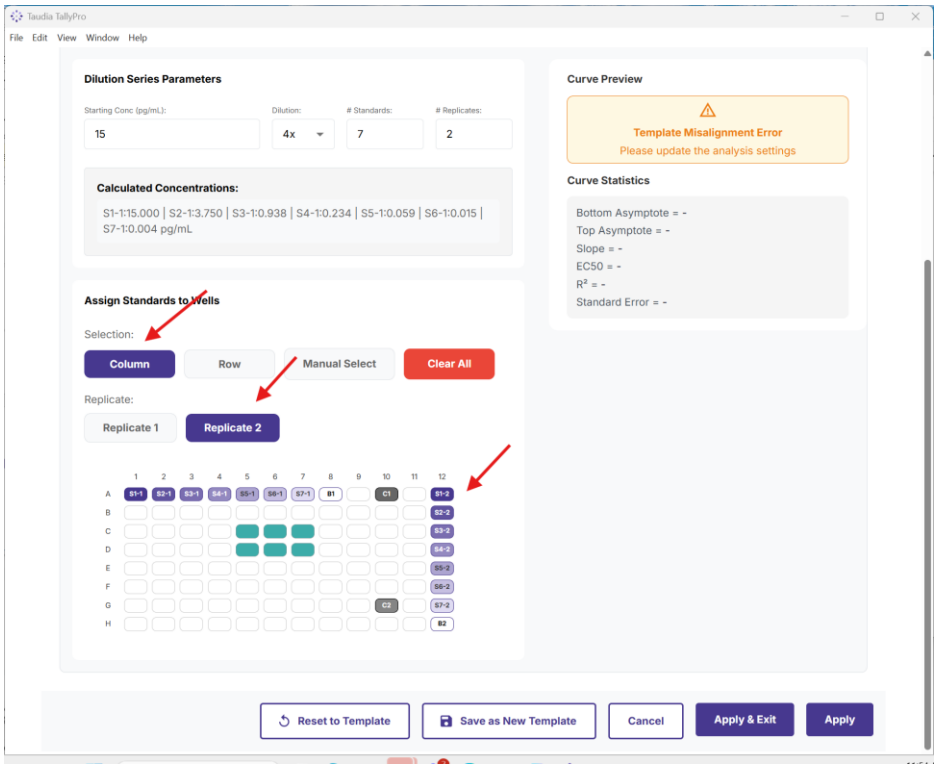
b. Clear the assigned wells by clicking “Clear All”.



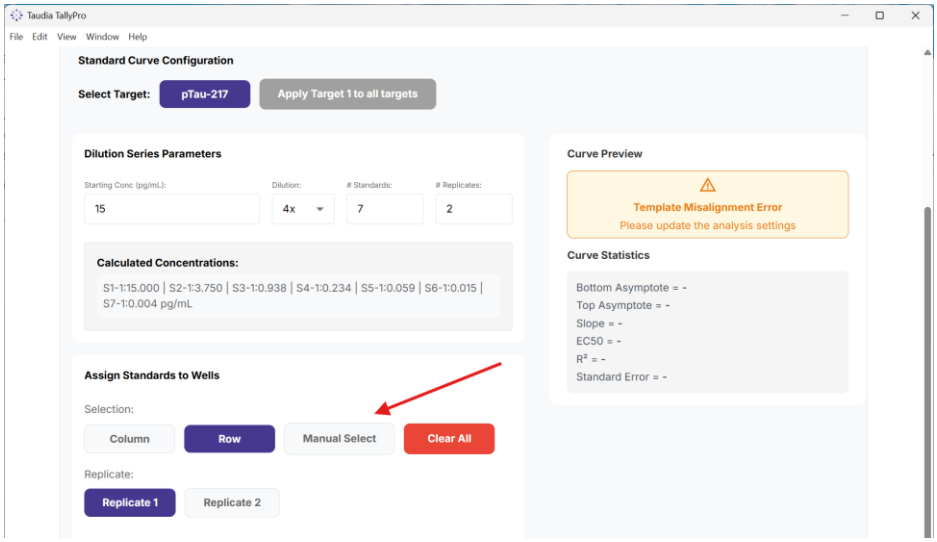
c. If the standard curve is within the same row, click on "Row", then the desired replicate number (in this example, replicate #1), and finally the well containing Standard 1 (in this example, A1).

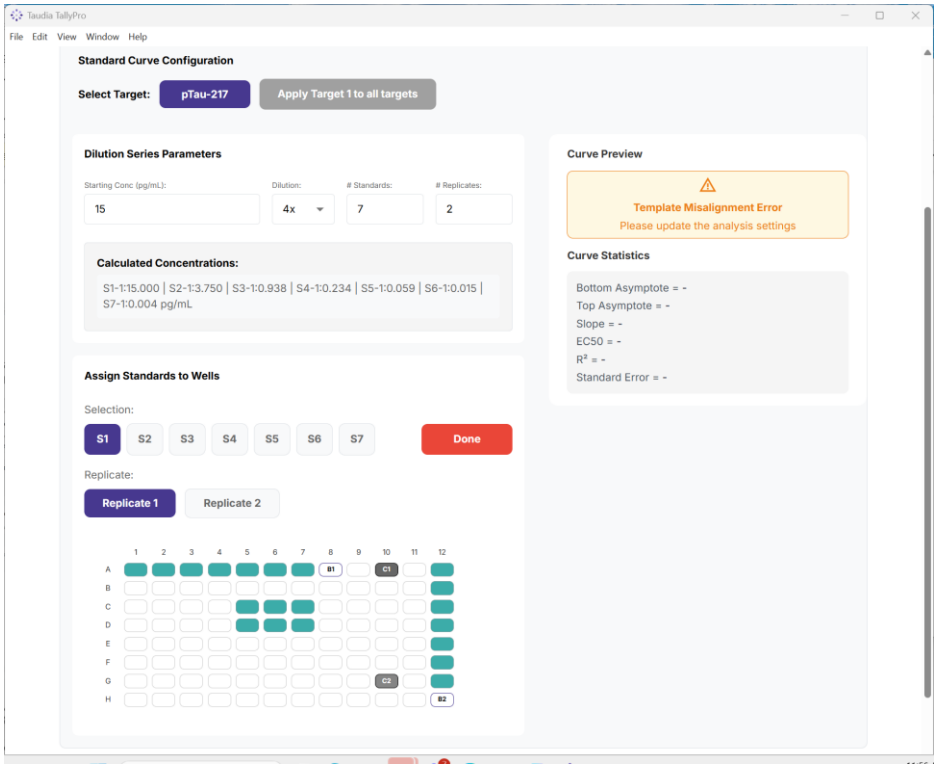


d. If the standard curve is in the same column, click on "Column", then the desired replicate number (in this example, replicate #2), and finally the well containing Standard 1 (in this example, A12).

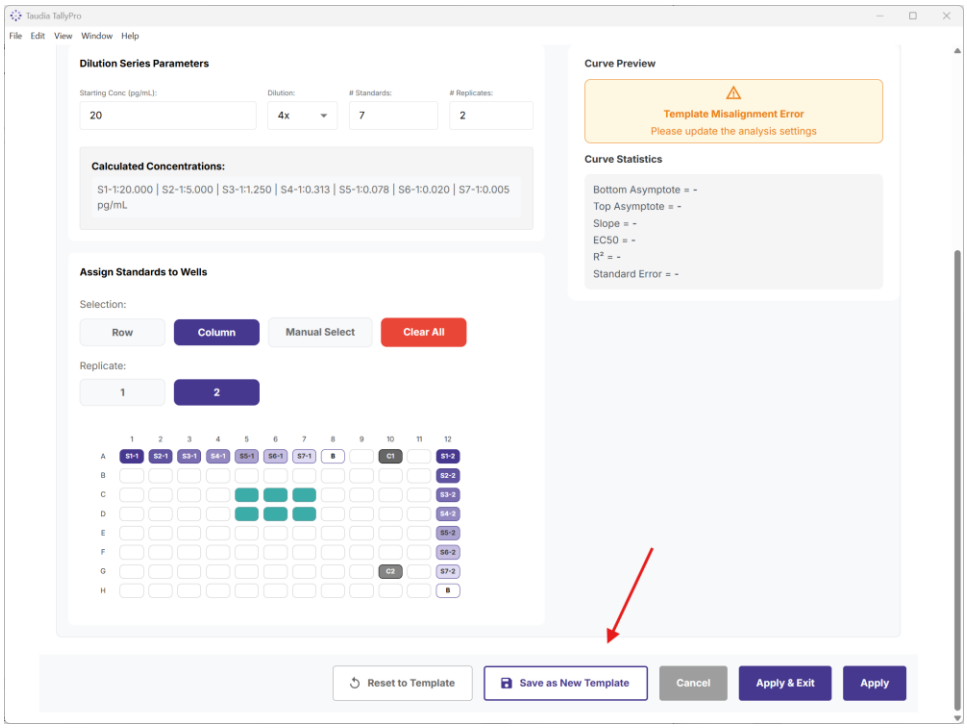


e. If the standard curve is in non-adjacent rows or columns, click on “Manual Select”, then the desired Standard (in this image, Standard 1), then the desired replicate number (in this image, replicate #1), and finally the well containing Standard 1 (in this example, A3). Repeat for all Standard Curve wells and replicates.

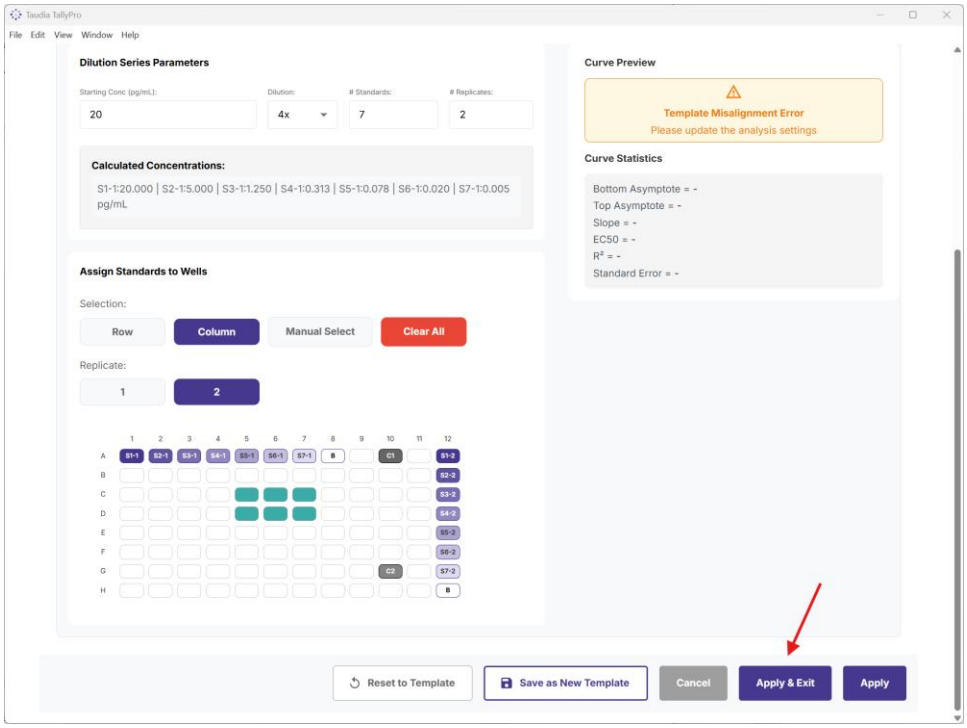




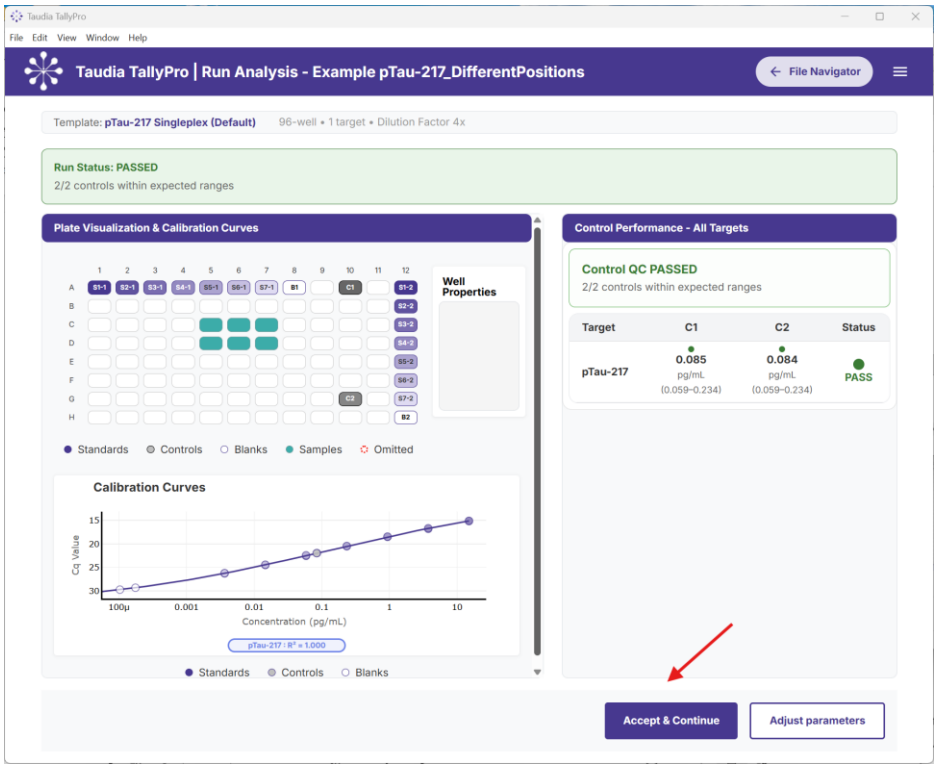
11. After making the changes, the configuration can be saved as a new Analysis Template by clicking on "Save as New Template."



12. When the positions of Standard Curves, CheckPoint, and Blank match the plate, click "Apply & Exit" to return to the Plate Visualization Screen.



13. The plate map in the analysis settings should now match the experiment. If it looks correct, click “Accept & Continue” in the bottom right. If the well positions are not correct, click on “Adjust parameters” to return to the prior screens.

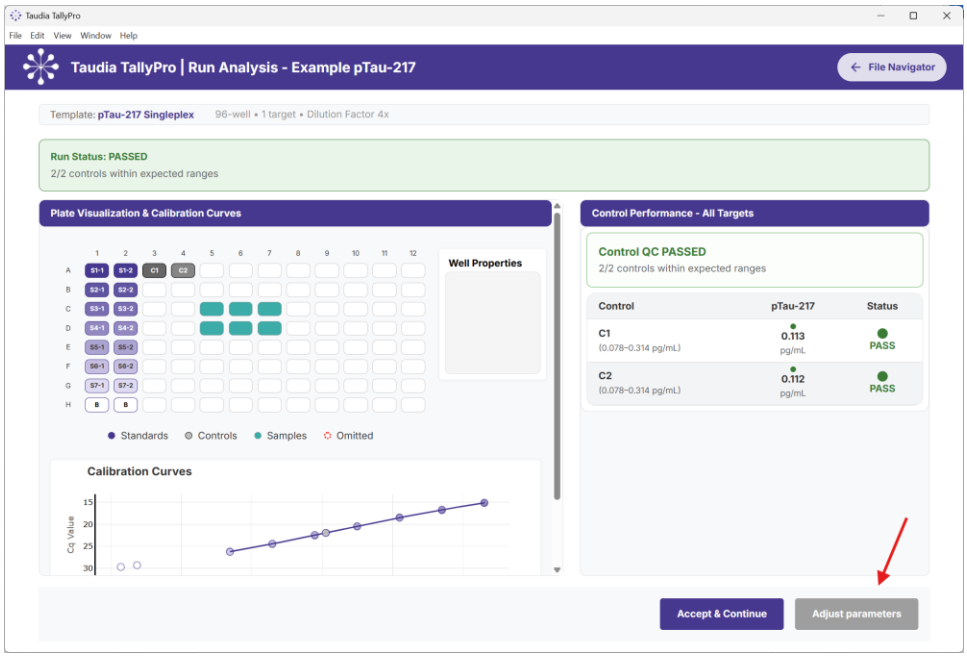


14. The data can now be exported as described in the prior section.

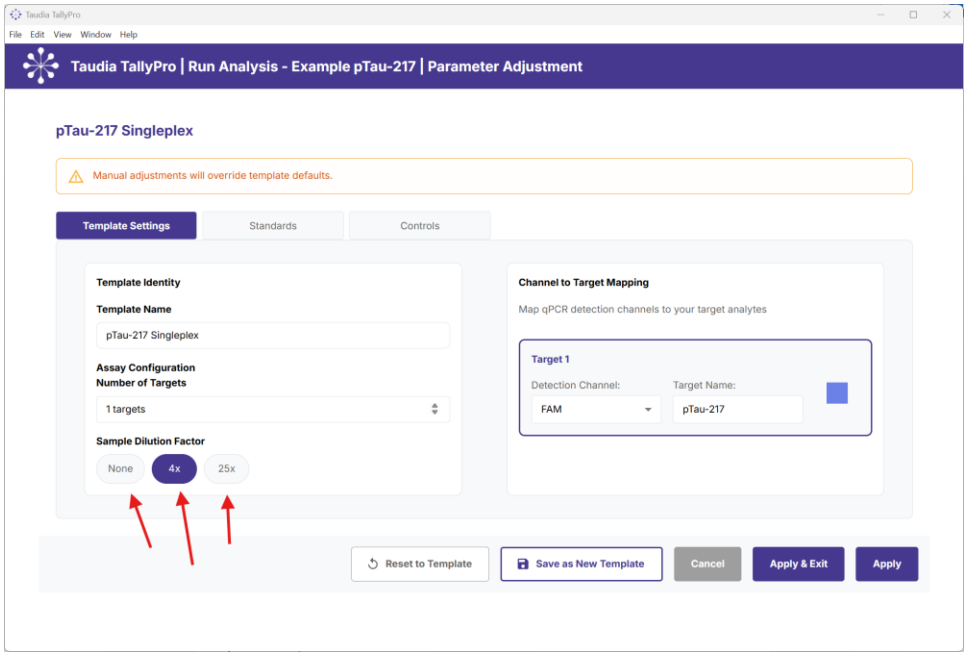
Adjust Sample Dilution Factor

1. Follow steps #1 through #6 in the prior section, "TallyPro Analysis with Kit-Specific Templates," to import qPCR data and load the Analysis Template specific for the kit.

2. Click on "Adjust parameters" in the lower right.



3. Sample dilutions can be set to "None", "4x", or "25x" by clicking on the associated values under "Sample Dilution Factor." For other dilution values (for instance, 50x dilution), select "None" and scale the calculated sample concentrations by the dilution factor after export from the software.



4. After updating the Sample Dilution Factor, click on "Apply & Exit" to continue with the analysis. Note that the changes can be saved as a new template file if desired by clicking "Save as New Template."

