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	March 20, 2026	New user guide for SPLASH singleplex assay kits

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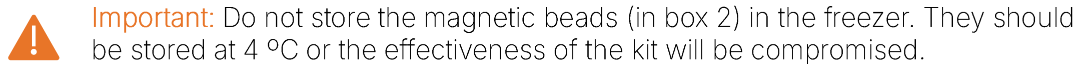
Product Information

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 arrive in two shipments: Box 1 is shipped on dry ice and should be stored at -20 ºC upon receipt. Box 2 is shipped on gel pack and should be stored at 4 ºC 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 ºC or the effectiveness of the kit will be compromised.

Box #	Item	P/N	Quantity	Storage
1	Lyophilized Protein Standard	Target-specific	2 vials	-20 ºC
	Lyophilized CheckPoint	Target-specific	2 vials	
	Target Protein AmPLAmix	Target-specific	5 mL	
2	Sample Buffer	SPL-00013	8 mL	4 ºC
	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	

A variety of singleplex SPLASH kits are available. Each kit has specific components for the Target Protein Standard, the Target Protein CheckPoint, the AmPLAmix, and the Target Protein Probes A and B.

Target Protein	Acronym	Kit	P/N	Target-specific Components	P/N
Phosphorylated Tau 217	pTau-217	pTau-217 Singleplex	TSK-00001	Lyophilized pTau-217 Standard	SPL-00011
				pTau-217 CheckPoint	SPL-00012
				pTau-217 AmPLAmix	SPL-00001
				pTau-217 Probe A	SPL-00009
				pTau-217 Probe B	SPL-00010
Amyloid-beta 40	Aβ40	Aβ40 Singleplex	TSK-00002	Lyophilized Aβ40 Standard	SPL-00034

Target Protein	Acronym	Kit	P/N	Target-specific Components	P/N
				Aβ40 CheckPoint	SPL-00041
				Aβ40 AmPLAmix	SPL-00002
				Aβ40 Probe A	SPL-00020
				Aβ40 Probe B	SPL-00027
Amyloid-beta 42	Aβ42	Aβ42 Singleplex	TSK-00003	Lyophilized Aβ42 Standard	SPL-00035
				Aβ42 CheckPoint	SPL-00042
				Aβ42 AmPLAmix	SPL-00003
				Aβ42 Probe A	SPL-00021
				Aβ42 Probe B	SPL-00028
Neurofilament light chain	NfL	NfL Singleplex	TSK-00004	Lyophilized NfL Standard	SPL-00036
				NfL CheckPoint	SPL-00043
				NfL AmPLAmix	SPL-00004
				NfL Probe A	SPL-00022
				NfL Probe B	SPL-00029
Glial fibrillary acidic protein	GFAP	GFAP Singleplex	TSK-00005	Lyophilized GFAP Standard	SPL-00037
				GFAP CheckPoint	SPL-00044
				GFAP AmPLAmix	SPL-00005
				GFAP Probe A	SPL-00023
				GFAP Probe B	SPL-00030

Resources

Each SPLASH assay kit has an associated Quick Guide, Product Sheet, qPCR template, and a TallyPro™ Analysis Template available for download from the eShop at https://taudia.com/shop.

Methods

Workflow Overview

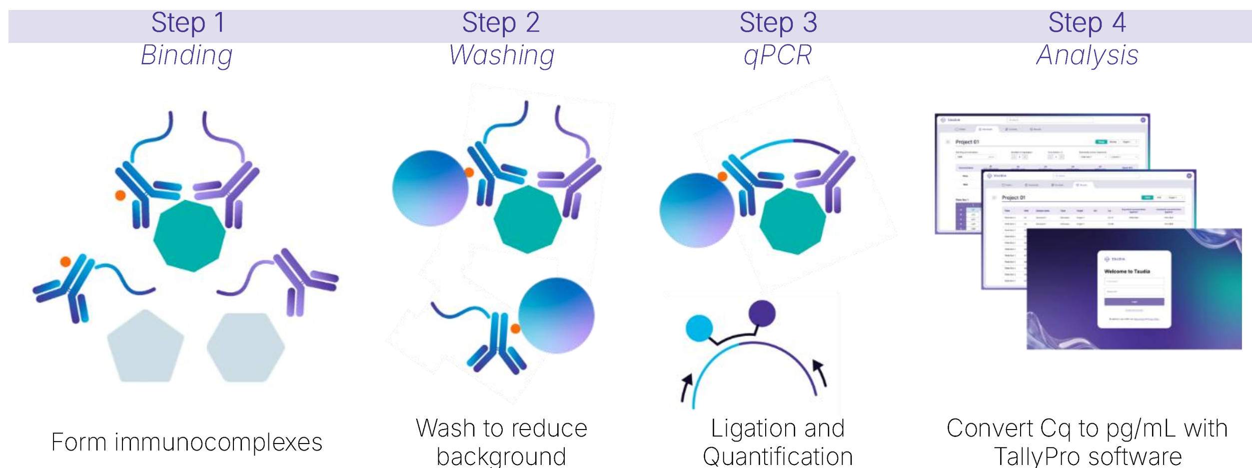
SPLASH assay kits enable ultrasensitive protein detection for specific protein targets. The workflow consists of four distinct steps: (1) Binding, (2) Washing, (3) qPCR Readout, and (4) Analysis. Protein binding, wash, and qPCR readout occur within the same 96-well consumable plate.

During binding, the sample is incubated 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 ligation of oligonucleotides and completed strand amplification 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 standard curve, 80 wells remain on each plate for samples.



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 10-15 minutes before creating the standard curve.
- Ensure complete mixing when creating the standard curve by slowly pipetting up and down at least fifteen 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 4 ºC 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 rpm 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

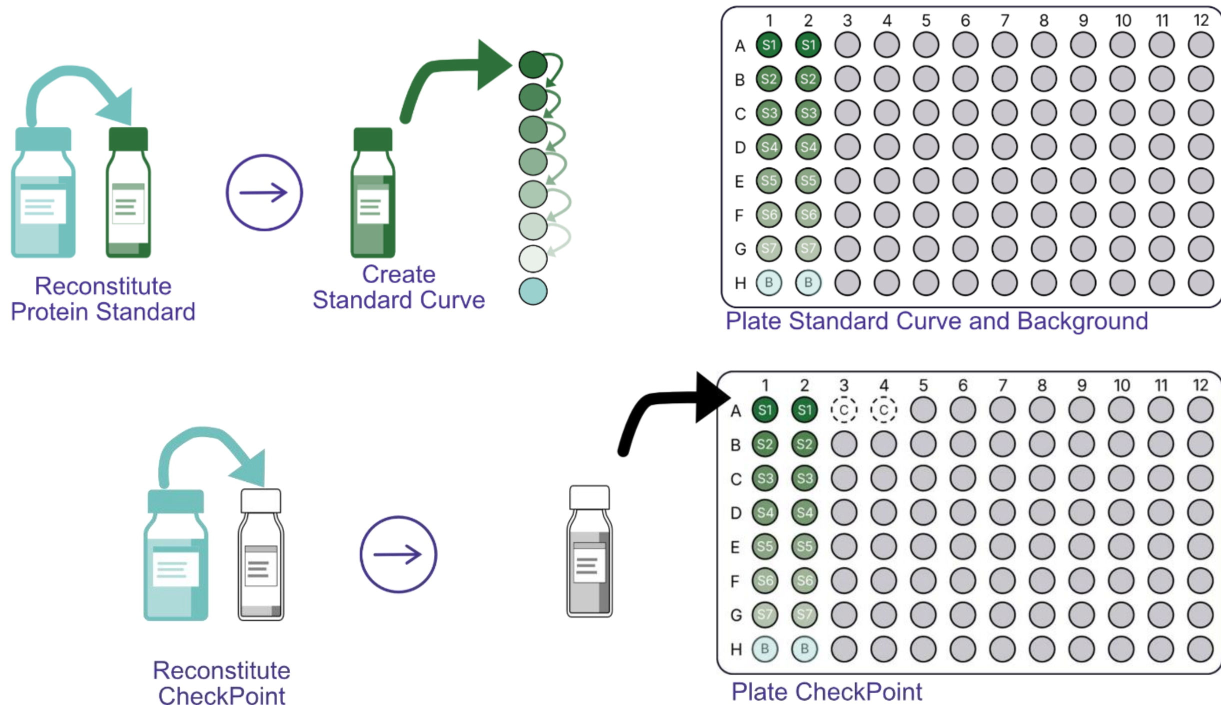
- SPLASH assays leverage qPCR for signal readout, so performance will be compromised in the presence of PCR inhibitors or amplicon contamination.
- Follow best lab practices for DNA and protein assays: wear gloves, use low-bind and DNase/RNase-free polypropylene plastics, clean the laboratory bench before 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, available at https://taudia.com/shop
- 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:* 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.

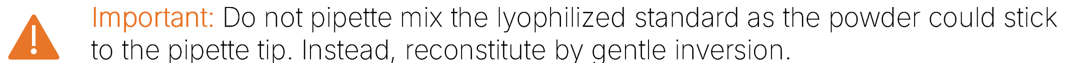
3. *Optional:* Reconstitute the CheckPoint by adding 500 µL of Sample Buffer to 1 vial of CheckPoint.

    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 ºC.


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. 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.

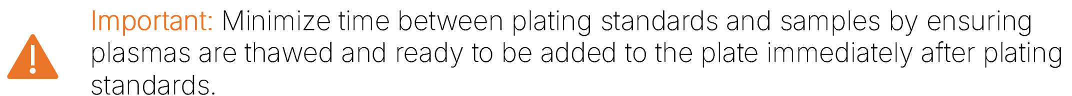
Plate Standard Curve and CheckPoint

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.

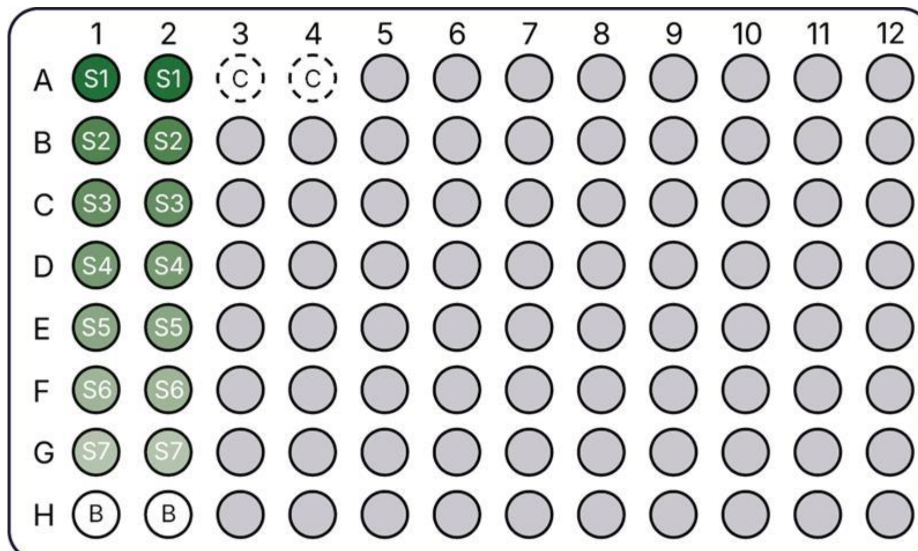
2. *Optional:* Plate 50 µL of the reconstituted CheckPoint into wells A3 and A4.

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 the Taudia Analysis Software as desired when performing the analysis.



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



Prepare and Plate Samples

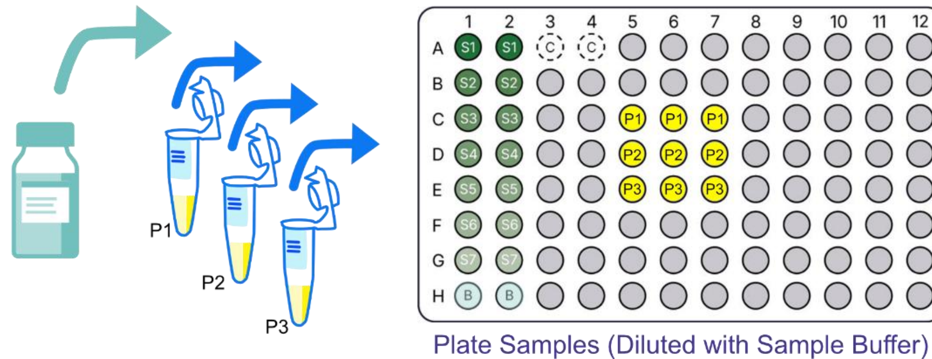


Plate Samples (Diluted with Sample Buffer)

Plasma Samples

1. Dilute plasma samples 4X into Sample Buffer for a total volume of 50 µL.

    a. Optional 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 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 pipetting 10-15 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

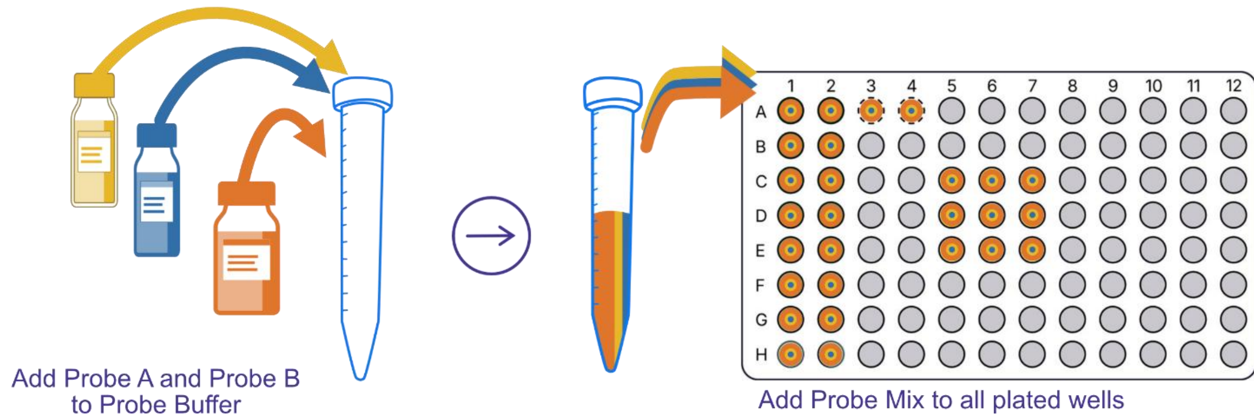
1. Dilute CSF samples 25-50X into Sample Buffer for a total volume of 50 µL

    a. Option A (Recommended for 1-2 replicates): The dilution can be performed directly in the 96-well plate by adding 48-49 µL of Sample Buffer into an empty well, followed by 1-2 µL of CSF sample, for a total volume of 50 µL. Pipette up and down a few times to mix.

    b. Option B (Recommended for >2 replicates): Create bulk 25x-50X dilution in a separate tube with overage, then transfer 50 µL volumes to 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 samples, 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 samples, 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.

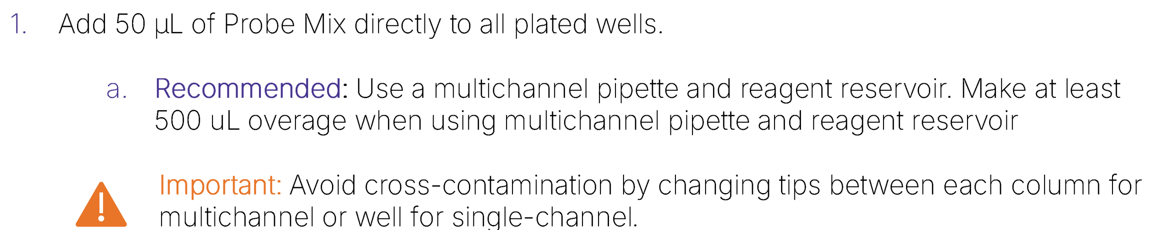
2. Mix by capping the tube, then gently inverting 5-10 times.

3. Keep the Probe Mix on ice or at 4 ºC.

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. Make at least 500 uL overage when using multichannel pipette and reagent reservoir

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

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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.

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

Thaw AmPLAmix

1. Thaw the AmPLAmix on ice before proceeding to Step 2.

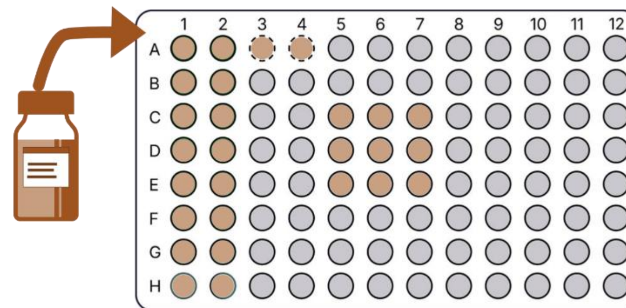
    a. Recommended: Start AmPLAmix thawing process at least 2 hours before initiating the washing step.

2. Check that the AmPLAmix has fully thawed (no residual ice) before initiating the wash.

Important: Do not use incompletely thawed AmPLAmix.

Step 2: Washing

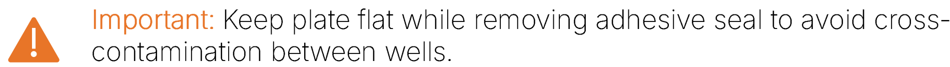
Add Magnetic Beads



Add magnetic beads to all plated wells

1. Thoroughly mix Magnetic Beads until homogenous, either by shaking vigorously for at least 30 seconds or by vortex. The mixture will 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 uL 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.

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 is 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 of 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 decant liquid with a pipette, taking care not to remove the magnetic beads.

Important: If decanting 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 to each plated well.
    a. Recommended: Use a multichannel pipette and reagent reservoir.
2. Seal the 96-well plate with an optical adhesive well 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.

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 are available for download from https://taudia.com/shop for some common qPCR instruments, like the QuantStudio line manufactured by Applied Biosystems, and the Opus96, manufactured by Bio-Rad.

Singleplex Kit	Target Name for PCR software
pTau-217 Singleplex	pTau-217
Aβ40 Singleplex	AB40
Aβ42 Singleplex	AB42
NfL Singleplex	NfL
GFAP Singleplex	GFAP

   a. Contact hello@taudia.com for additional support.

Step 4: Analysis

Quantify Protein Concentration in TallyPro

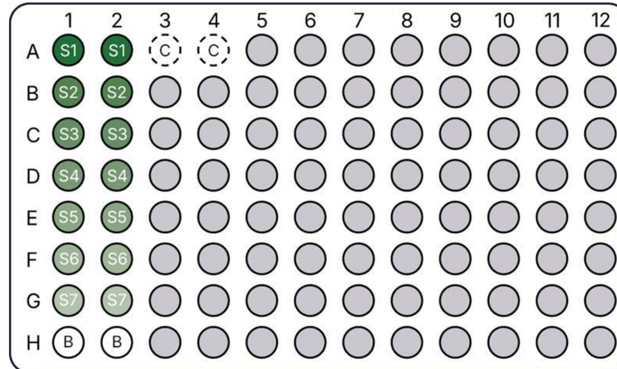
TallyPro, Taudia's analysis software, converts Cq values from the qPCR instrument into concentration values in pg/mL.

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/shop)
   a. If necessary, adjust the well positions for Standard Curve, CheckPoint, 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

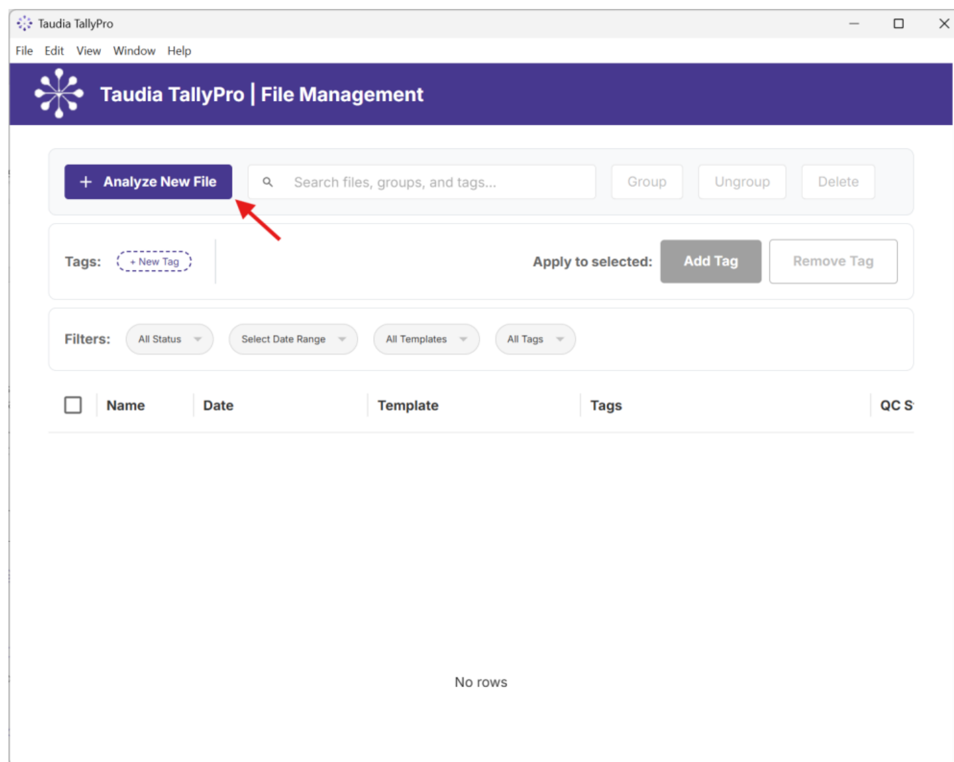
Kit-specific templates assume that the standard curve is plated in columns 1 and 2, with Standard 1 (S1) in A1 & A2, and Blank (B) in H1 & H2. The templates also assume that the CheckPoint (C) is in A3 and

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 that the sample is diluted by 4x. To update the sample dilution factor, go to "Adjust Sample Dilution Factor" after completing steps #1 through 6 below.

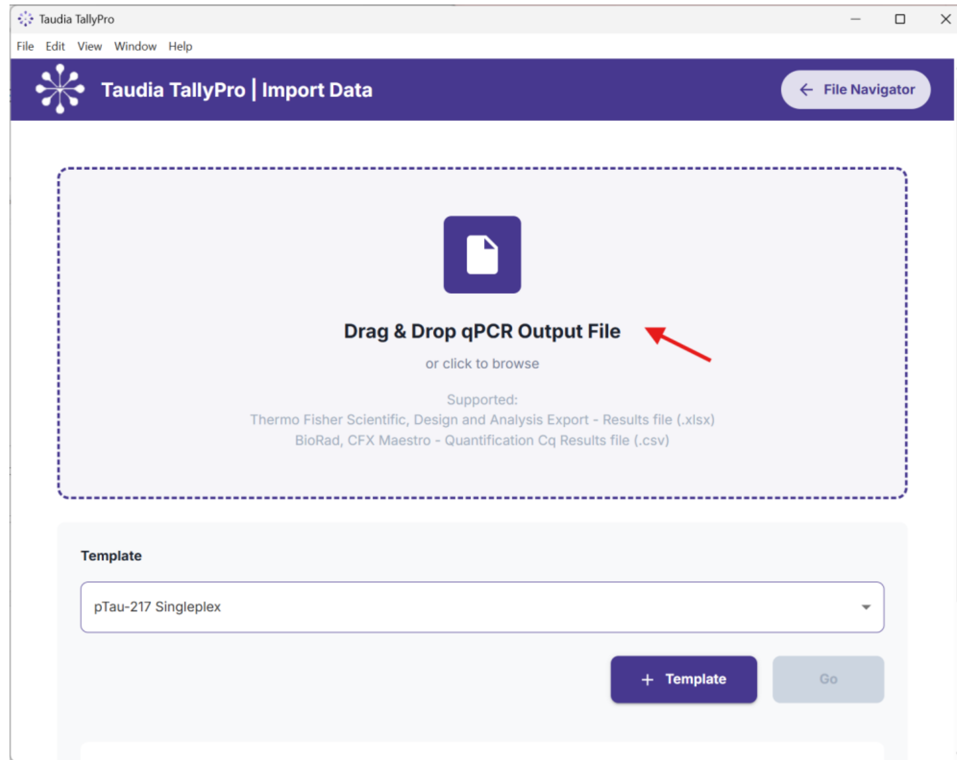
1. Click on the "Analyze New File" button in the upper left.



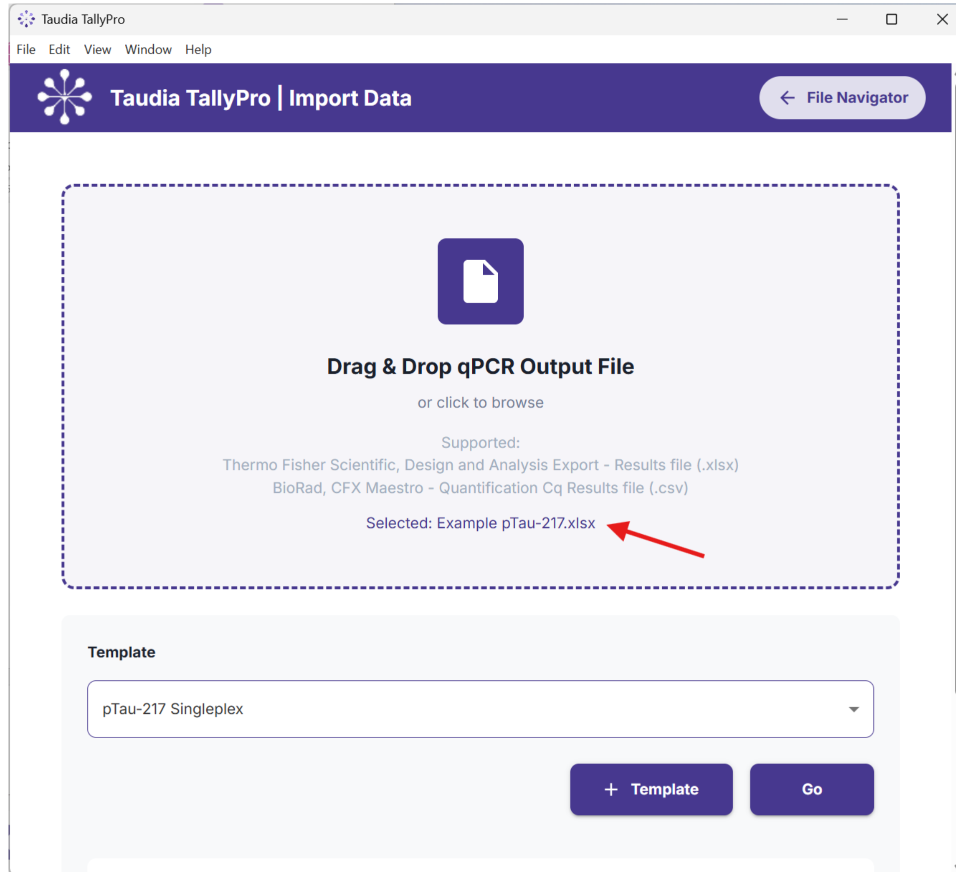
2. Import the qPCR Output File exported from the qPCR instrument software.

    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 Results file (.csv format). Import the "Quantification Cq Results" file into TallyPro.

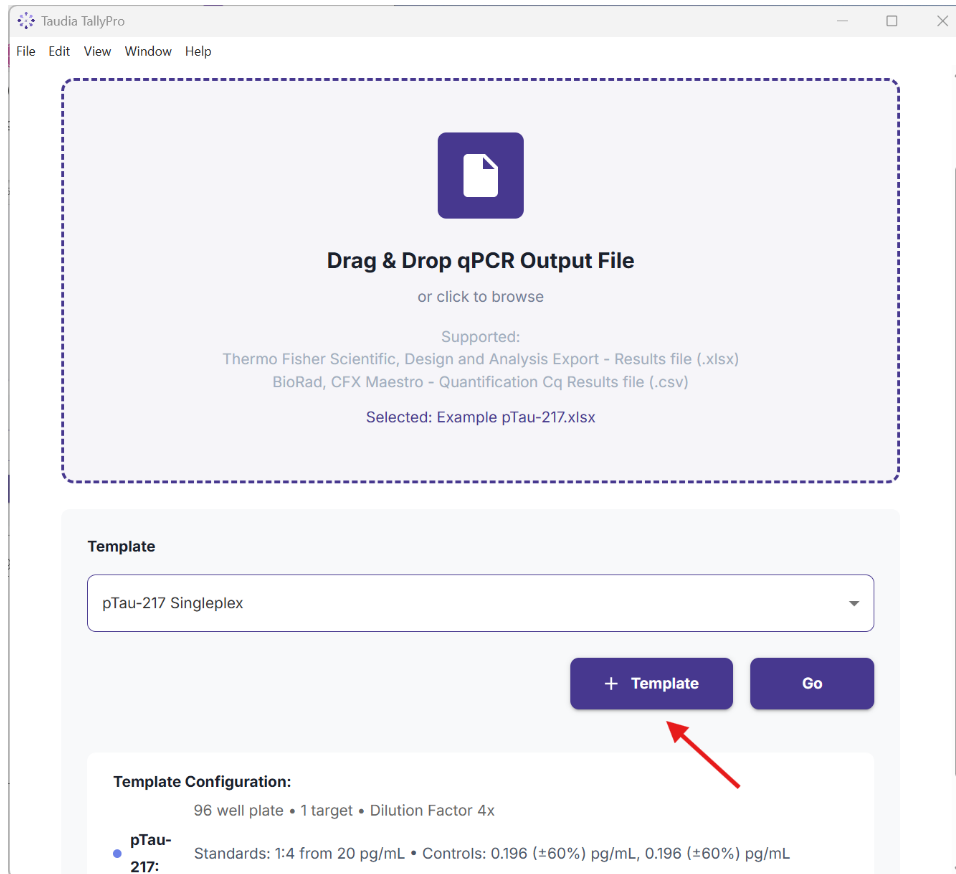


3. After importing, the file name will appear in the light purple rectangle.

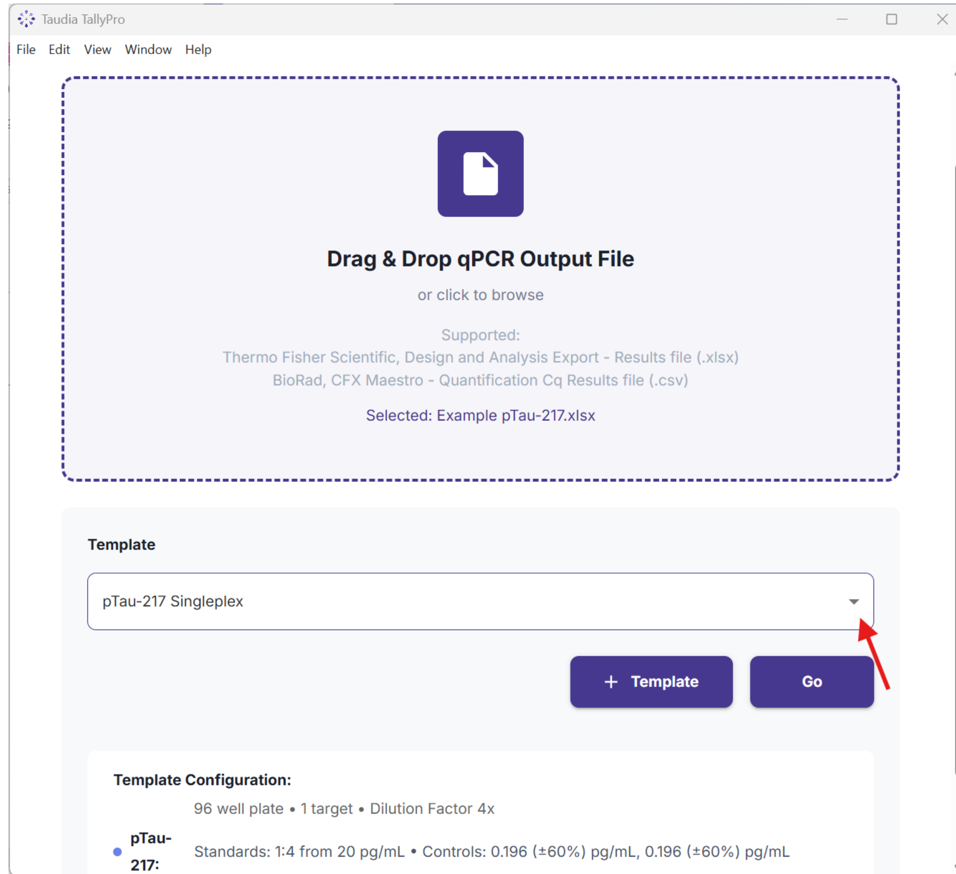


4. If necessary, import the Analysis Template file matching the assay by clicking on the purple "+Template" button and navigating to the desired template. After adding the file, a green message should pop up at the top of the screen indicating that the template was successfully added.

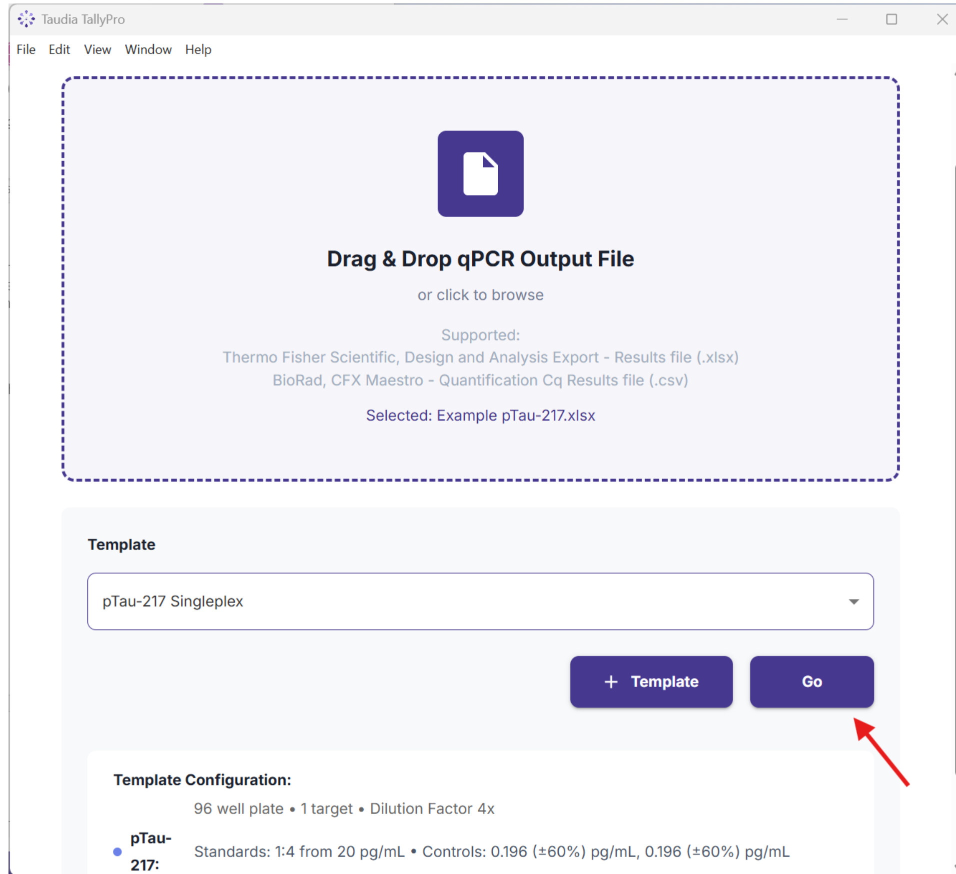
    a. Analysis template files specific for each kit can be found at https://taudia.com/shop



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

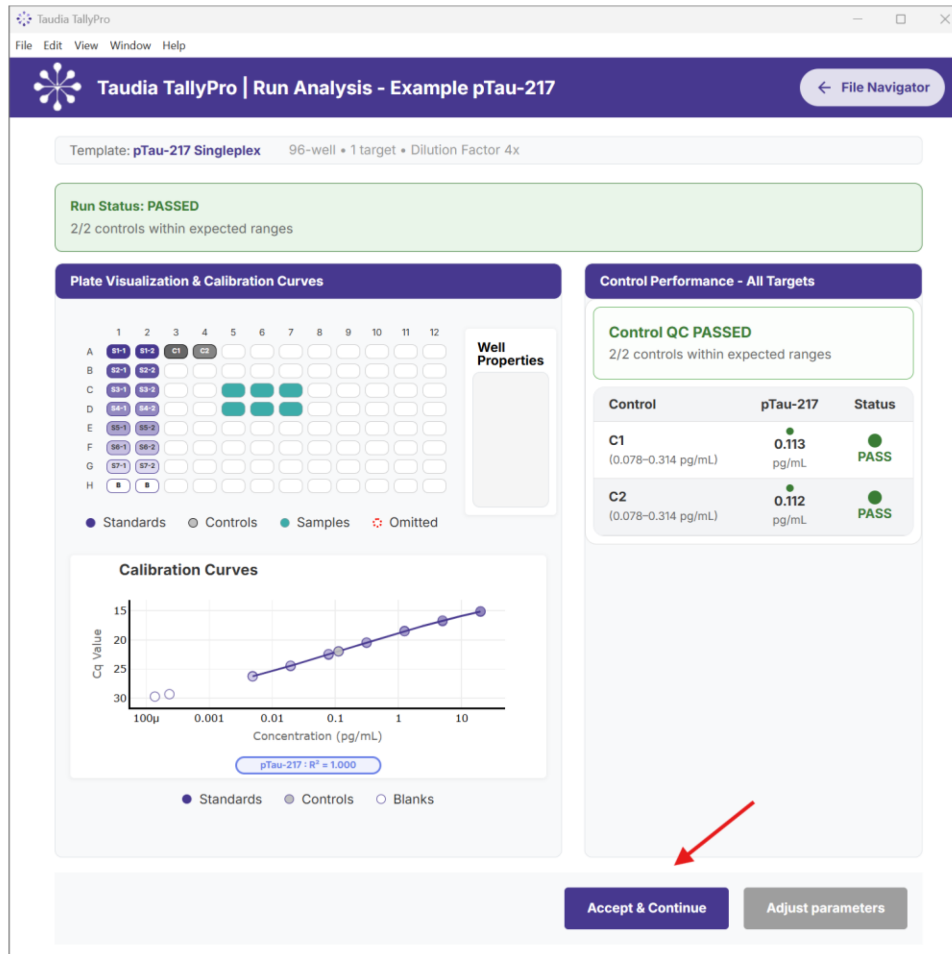


6. With the template selected, click on the “Go” button to proceed to the next step.

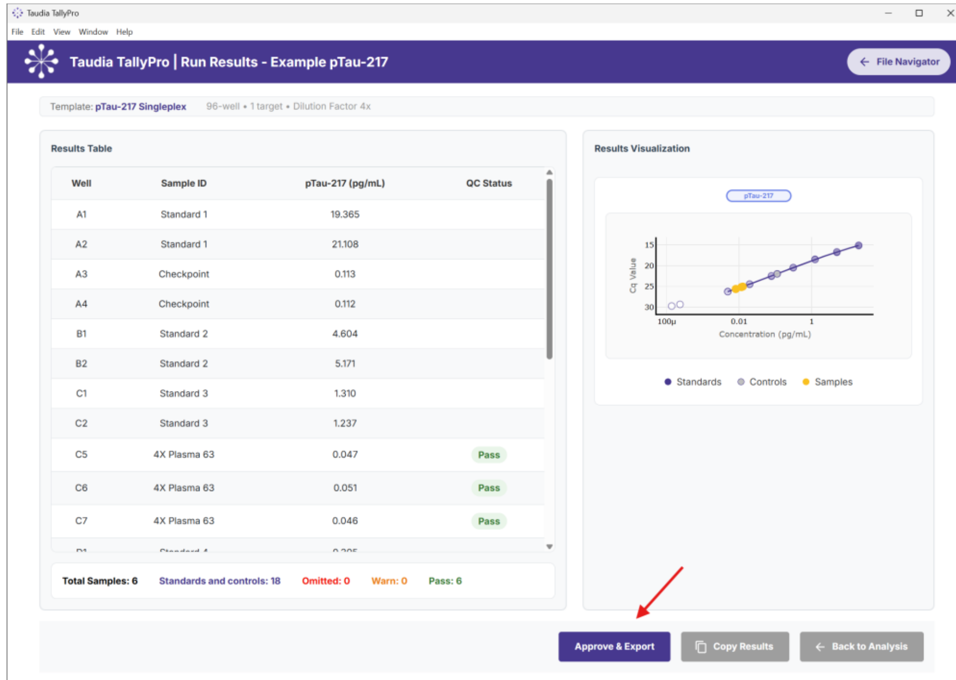


7. If the plate layout contains the standard curve in columns 1 and 2 and CheckPoint in wells A3 and A4, the default template positions will not need to be adjusted. The screen will contain the "Plate Visualization and Calibration Curves" on the left, and the "Control Performance" assessment on the right.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

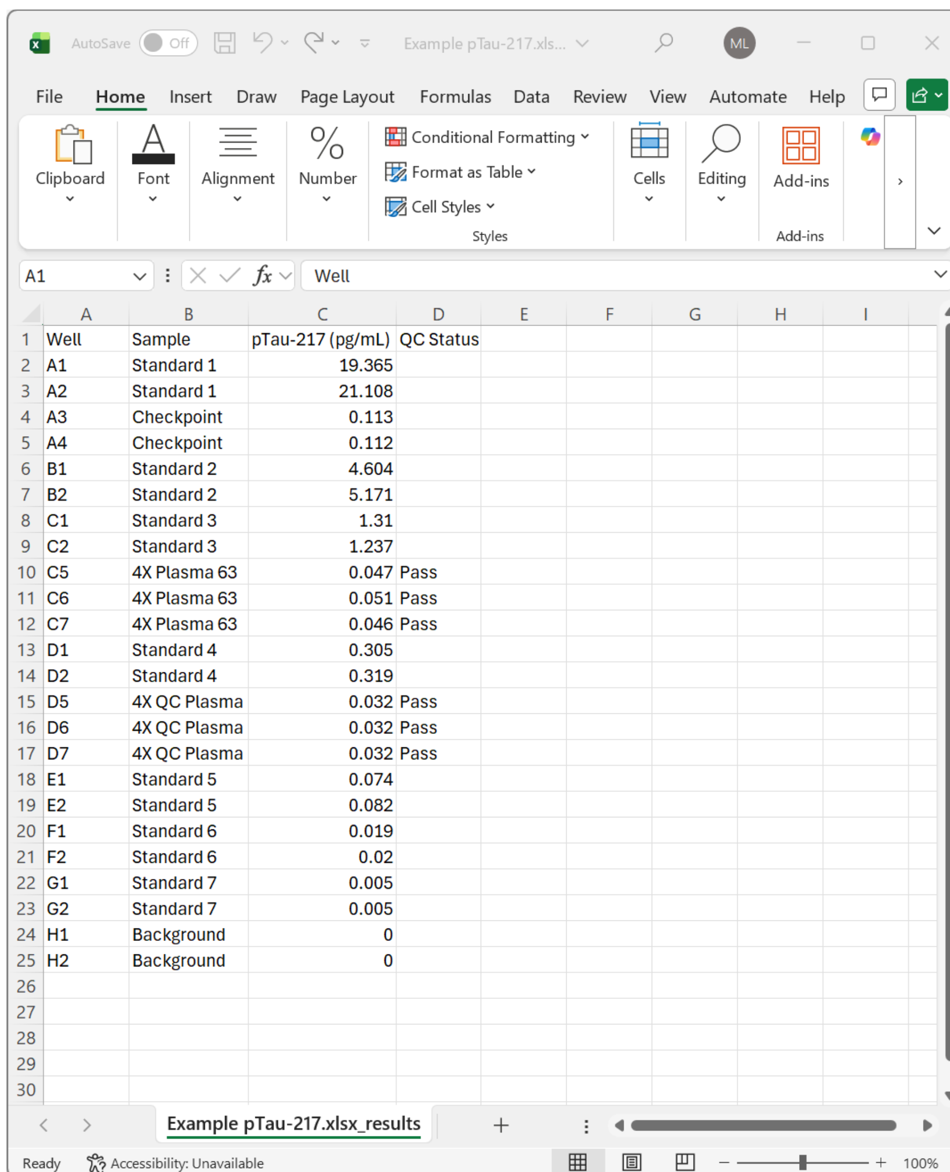
    a. If the layout of the experimental plate is different, please go to the next section of the guide, "Adjust Well Positions within Plate" for a description of how to manually change the well position identities.



8. The next screen shows the “Results Table” on the left and “Results Visualization” on the right. Samples are shown in as yellow dots in the “Results Visualization” graph and the blanks are empty dots. 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.



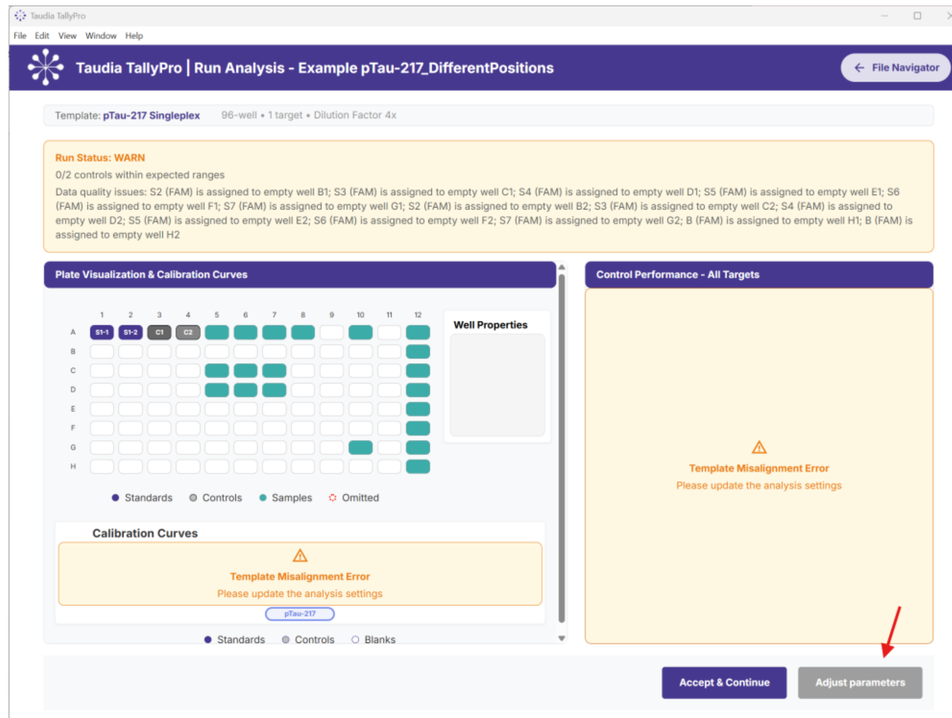
Well	Sample	pTau-217 (pg/mL)	QC Status
A1	Standard 1	19.365	
A2	Standard 1	21.108	
A3	Checkpoint	0.113	
A4	Checkpoint	0.112	
B1	Standard 2	4.604	
B2	Standard 2	5.171	
C1	Standard 3	1.31	
C2	Standard 3	1.237	
C5	4X Plasma 63	0.047	Pass
C6	4X Plasma 63	0.051	Pass
C7	4X Plasma 63	0.046	Pass
D1	Standard 4	0.305	
D2	Standard 4	0.319	
D5	4X QC Plasma	0.032	Pass
D6	4X QC Plasma	0.032	Pass
D7	4X QC Plasma	0.032	Pass
E1	Standard 5	0.074	
E2	Standard 5	0.082	
F1	Standard 6	0.019	
F2	Standard 6	0.02	
G1	Standard 7	0.005	
G2	Standard 7	0.005	
H1	Background	0	
H2	Background	0	

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.

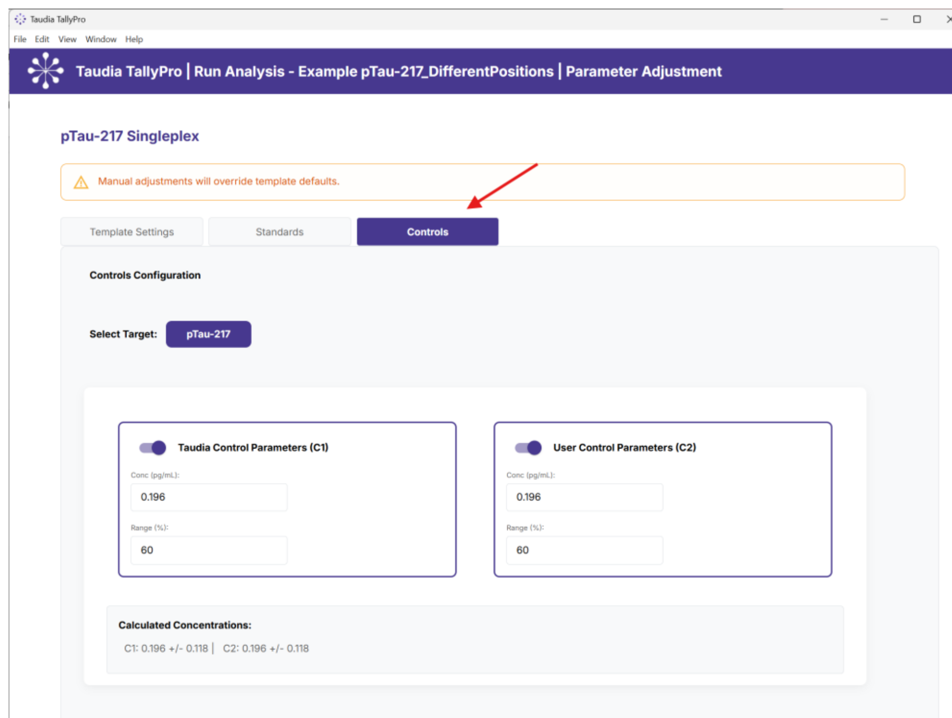
2. In this example, the positions of the Standard Curve, CheckPoint, and Blanks need to be updated. The first replicate of the Standard Curve is in row A, and the second replicate is in column 12. The CheckPoint samples are in A10 and G10, and the samples are in rows C and D.

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.



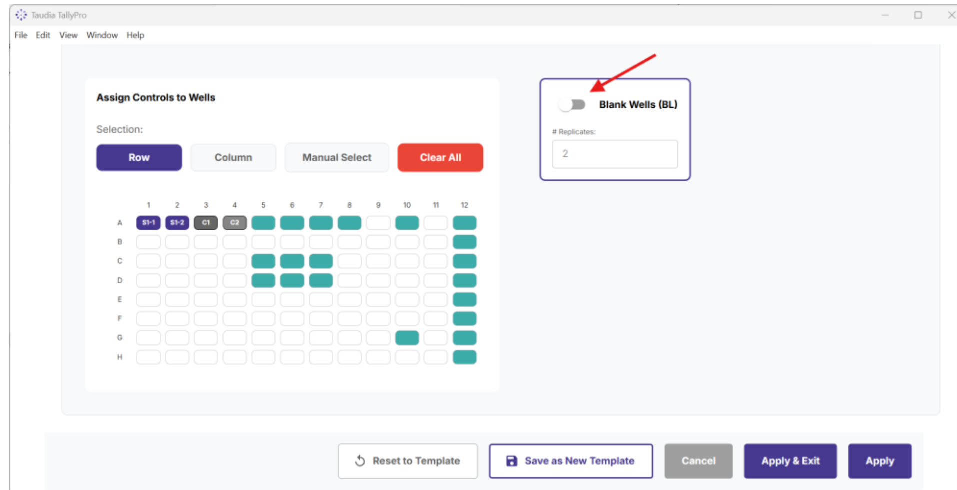
4. Click on the tab "Controls" to adjust the position of the CheckPoint.

    a. If there is a separate control, the default value can be updated to match the expected quantity.

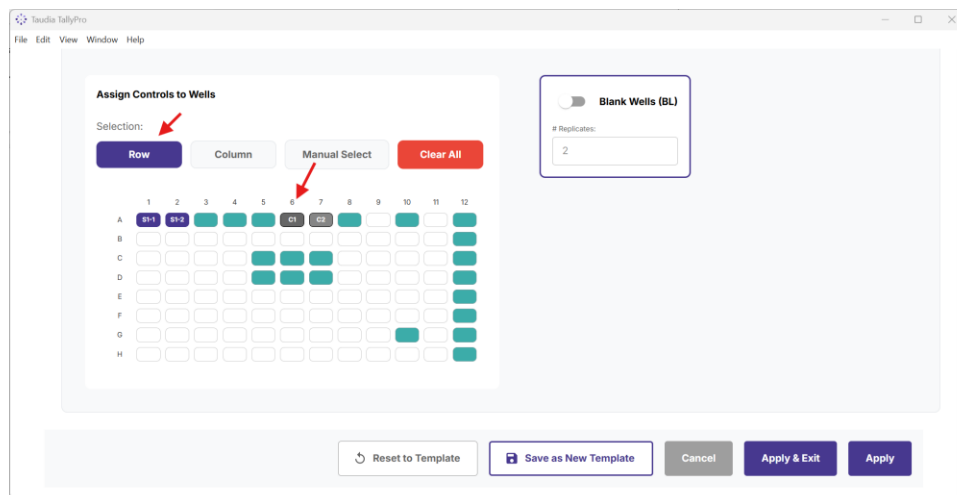


5. Scroll down to the plate map.

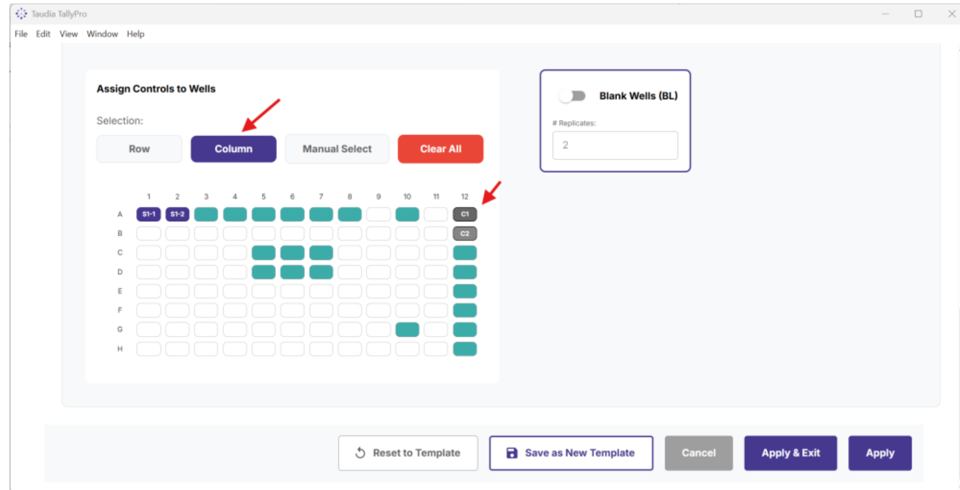
a. Toggle off "Blank Wells" on the right side of the screen.



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

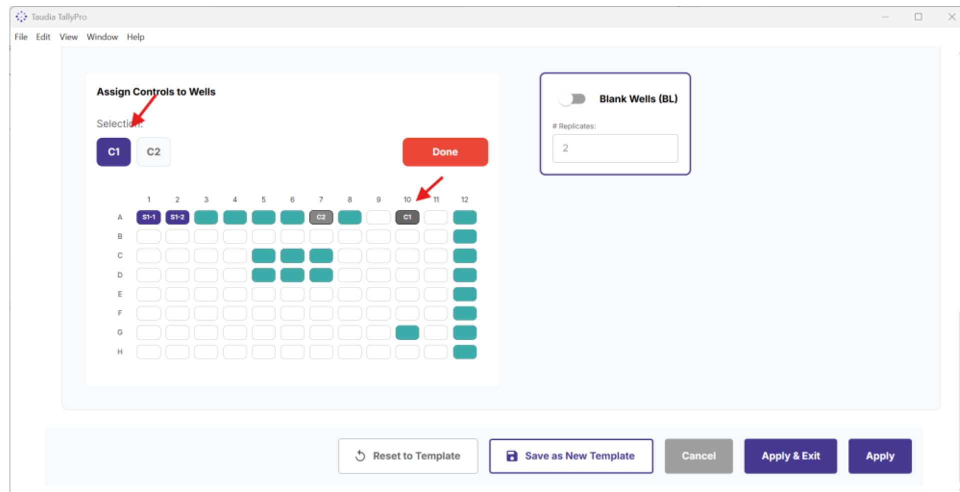


c. If CheckPoints are in adjacent wells in the same column, select "Column" and then click on the top-most well with a CheckPoint.

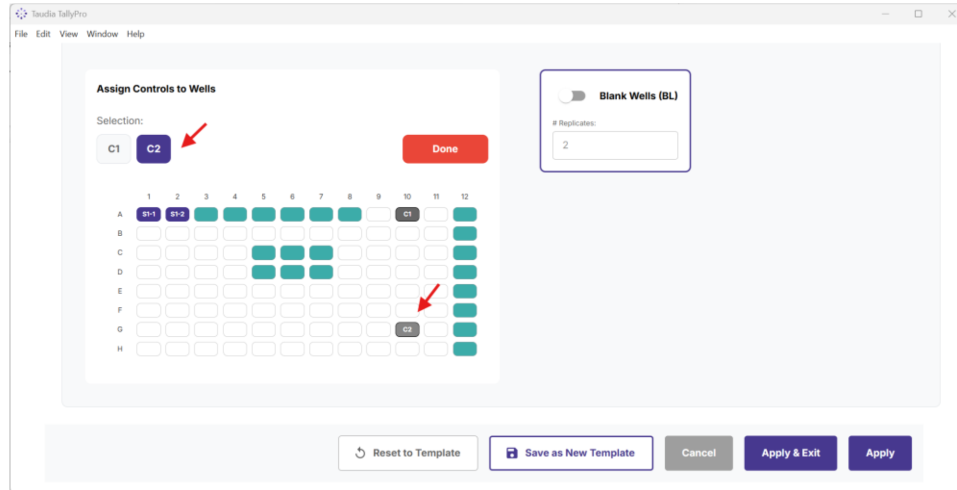


d. If CheckPoints are in non-adjacent wells, select “Manual” and then click on the wells containing CheckPoint.

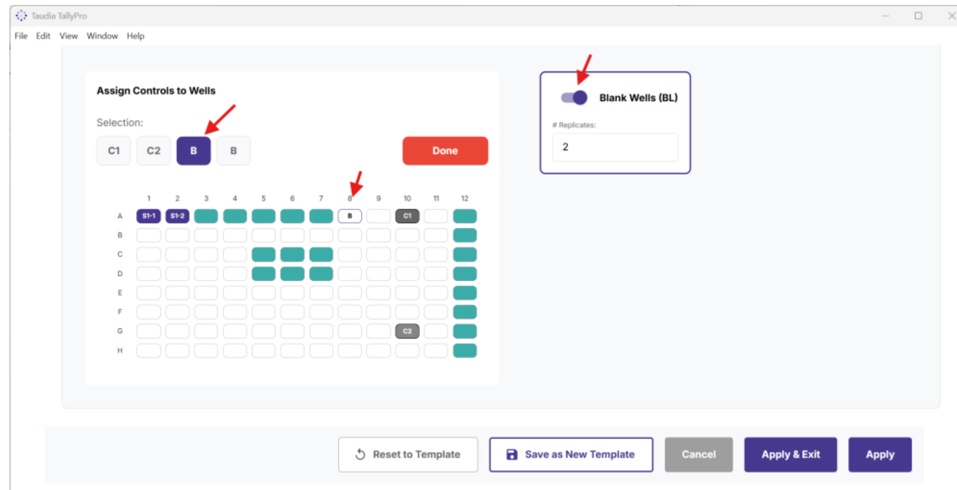
i. First click on “C1”, then click the location on the plate map for the first CheckPoint

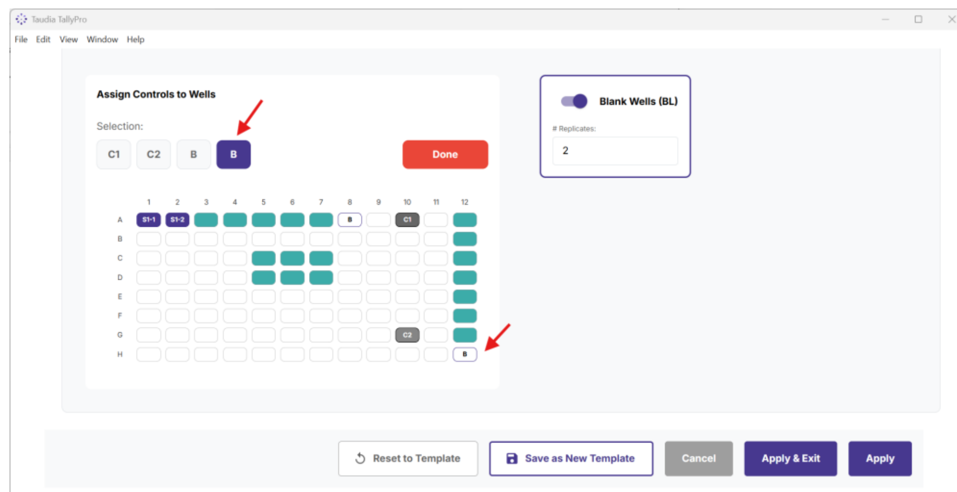


ii. Next click on “C2”, then click the location on the plate map for the second CheckPoint



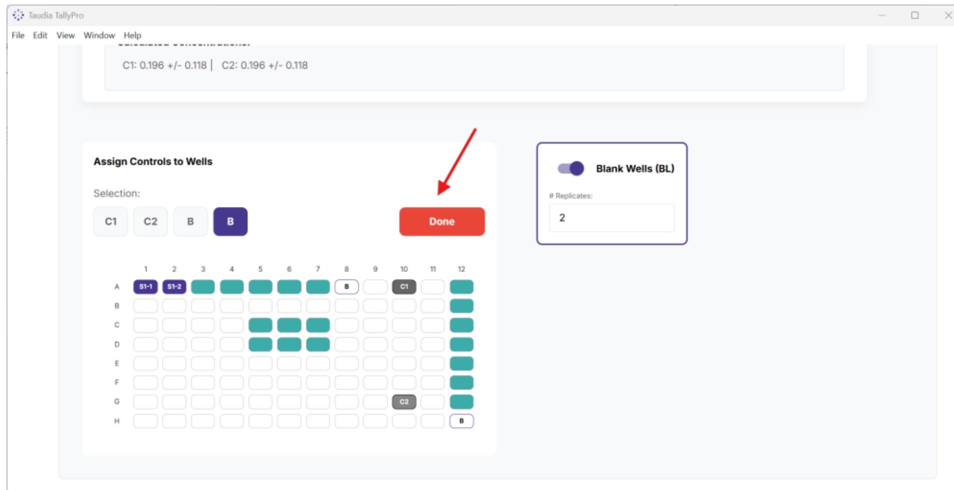
e. The "Blank Wells" can be toggled back on, and blank wells can be selected by first clicking on "B", then the location on the plate for the blank well.



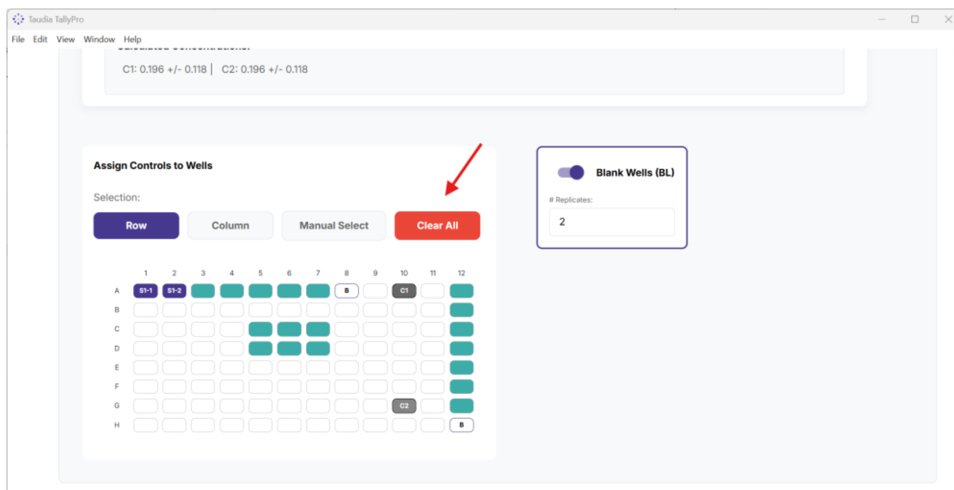


f. The number of blank wells can be increased as desired.

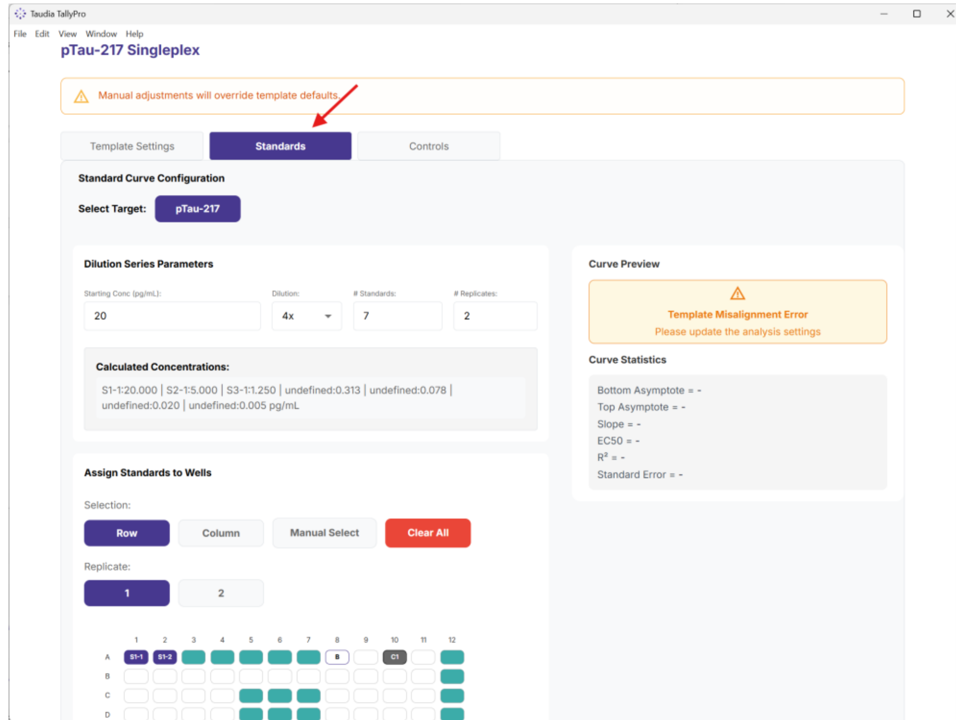
g. When finished, click “Done”



6. If there are no CheckPoint wells, click on the “Clear All” button to remove CheckPoints and Blanks from the well map.



7. Click on the tab “Standards” to adjust the positions of the Standard Curve. Standards can be sequentially in rows or columns, or in any positions in the plate.



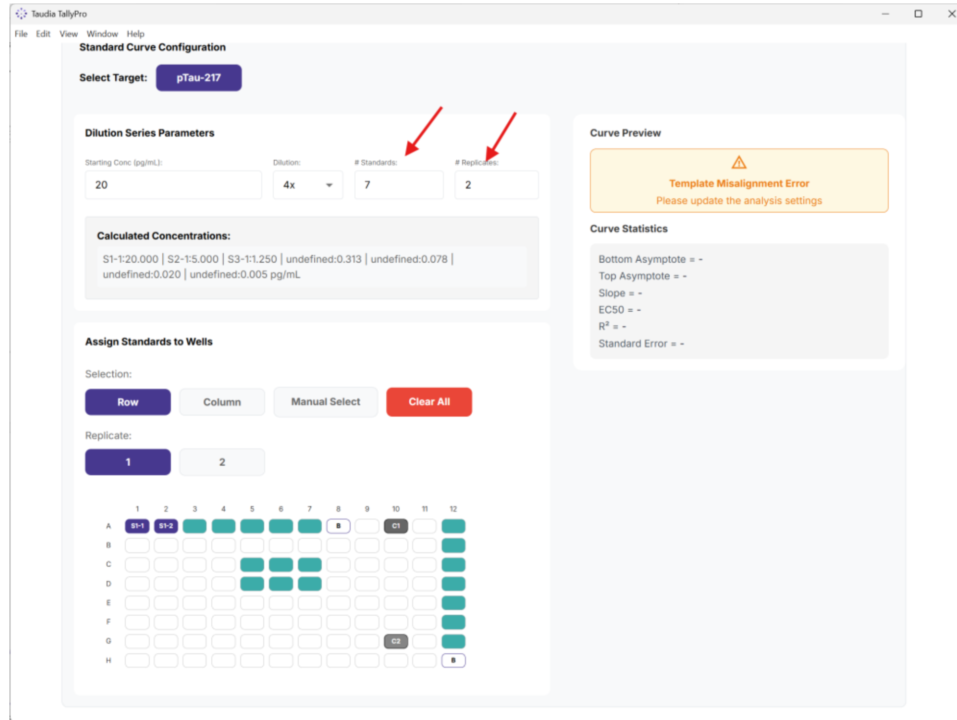
a. Adjust the Standard Curve Dilution Series Parameters as necessary

    i. Check that starting concentration matches the kit values

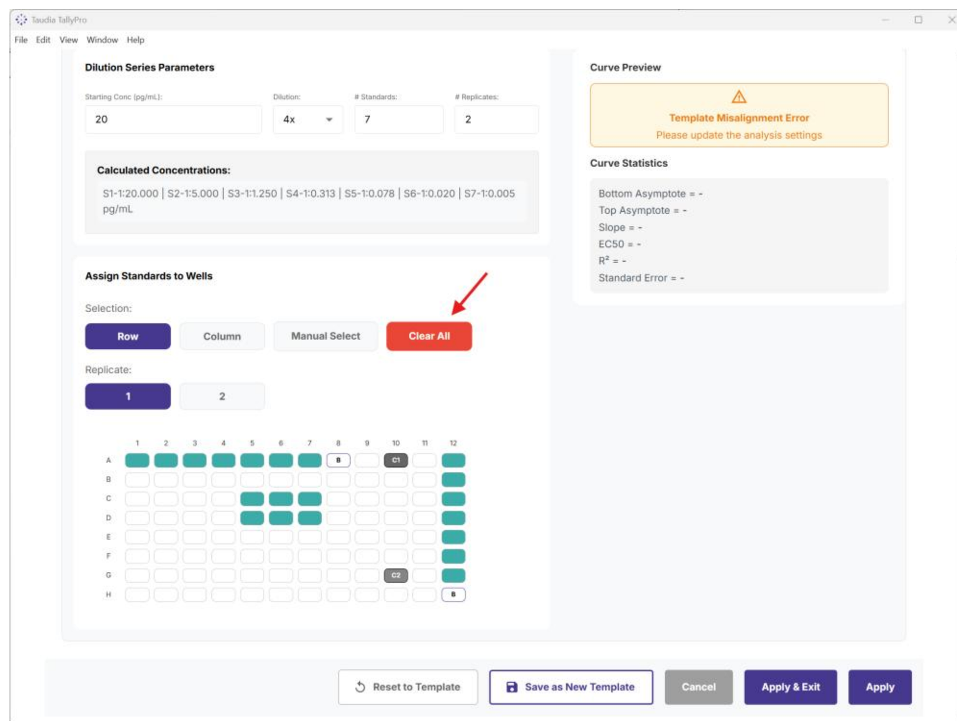
    ii. Ensure that dilution is set to 4x

    iii. Set number of standards to be 7

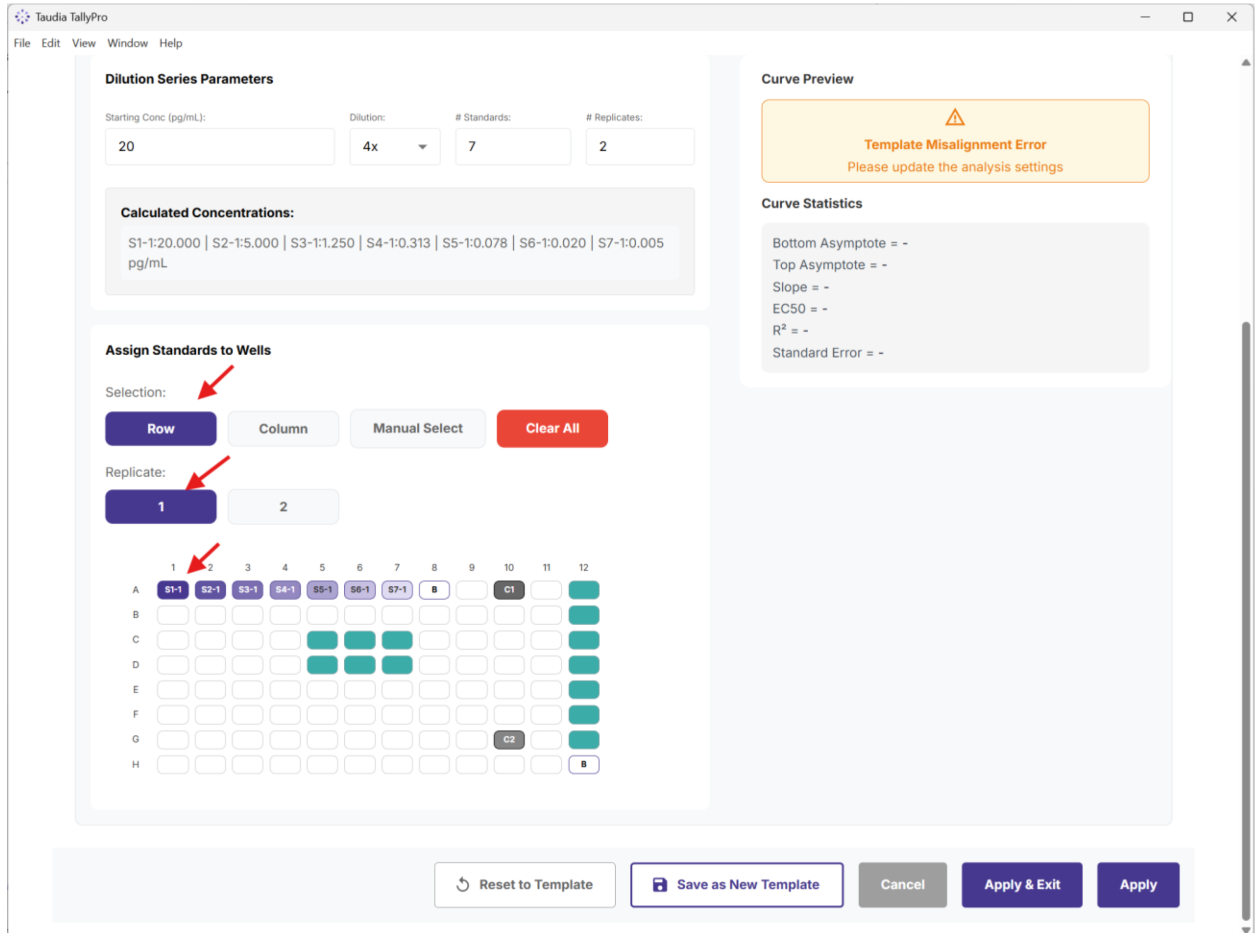
    iv. Set number of replicates to match the experiment



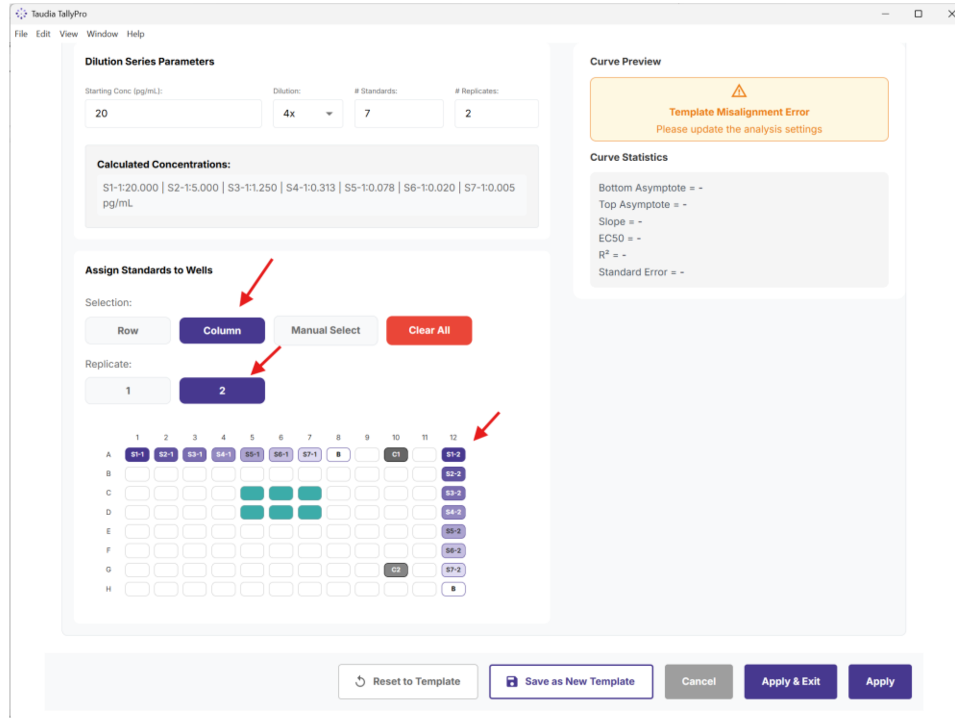
b. Clear the assigned wells by clicking "Clear All"



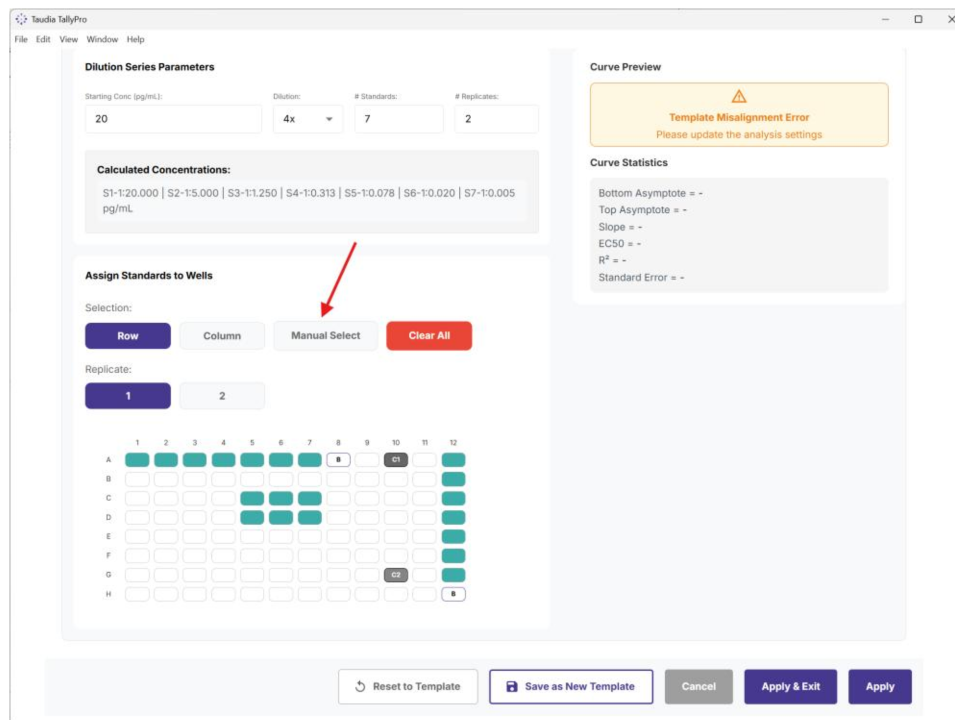
c. Standard Curve in Sequential Rows: Click on "Row", then the desired replicate number (in this image, replicate #1), and finally the well containing Standard 1 (in this example, A1).

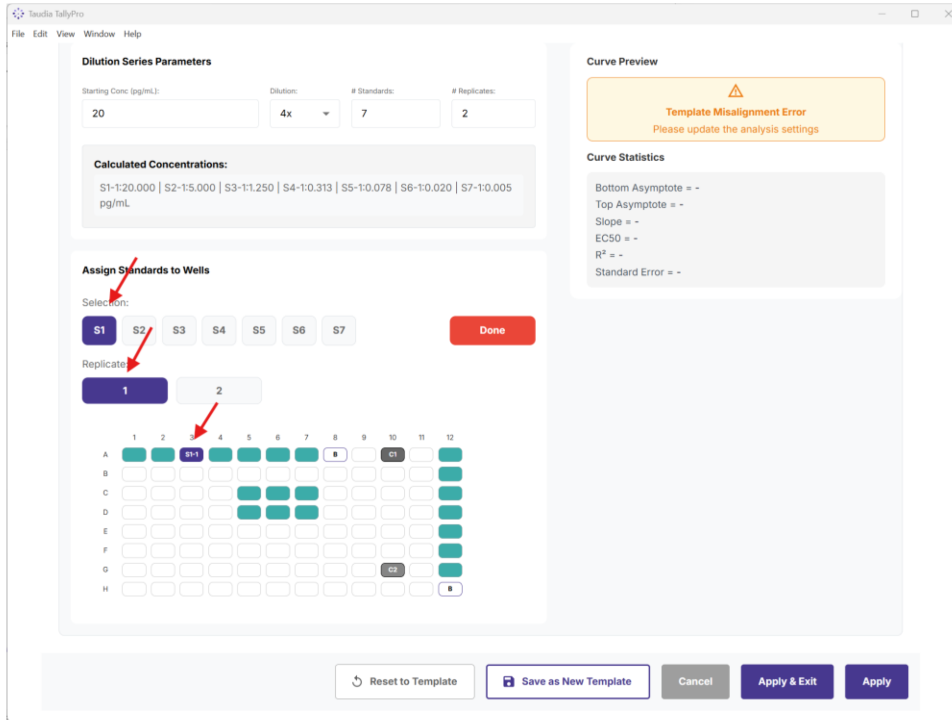


d. Standard Curve in Sequential Column: Click on “Column”, then the desired replicate number (in this image, replicate #2), and finally the well containing Standard 1 (in this example, A12).

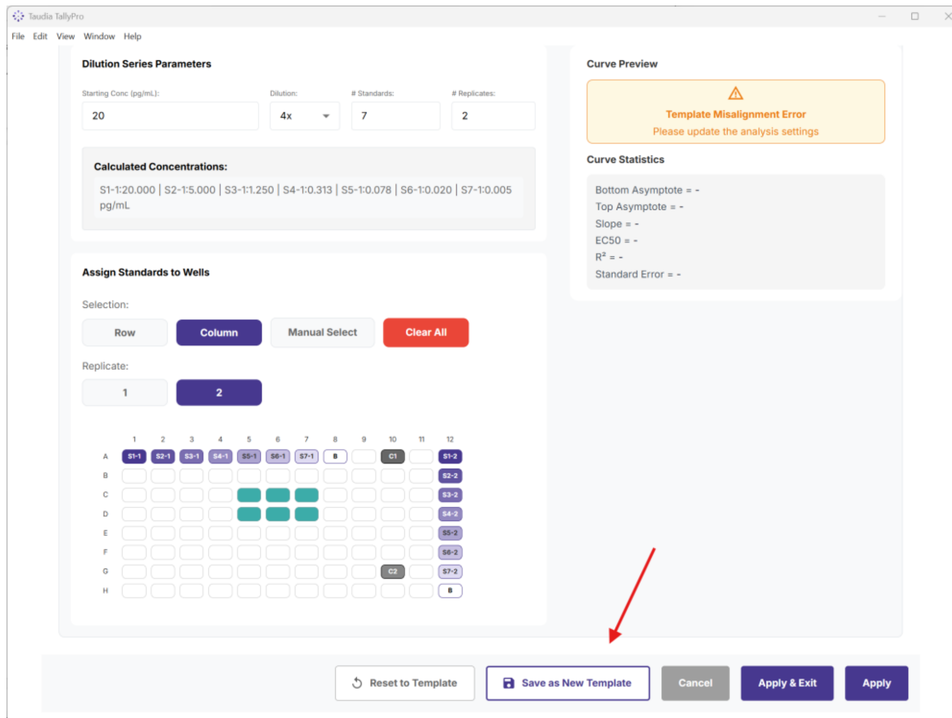


e. Standard Curve in Arbitrary Wells: Click on “Manual Select”, then the desired Standard (in this image, Standard 1), then the desired replicate number (in this image, replicate #1), and finally the well containing Standard 1 (in this example, A3). Repeat for all Standard Curve wells and replicates.

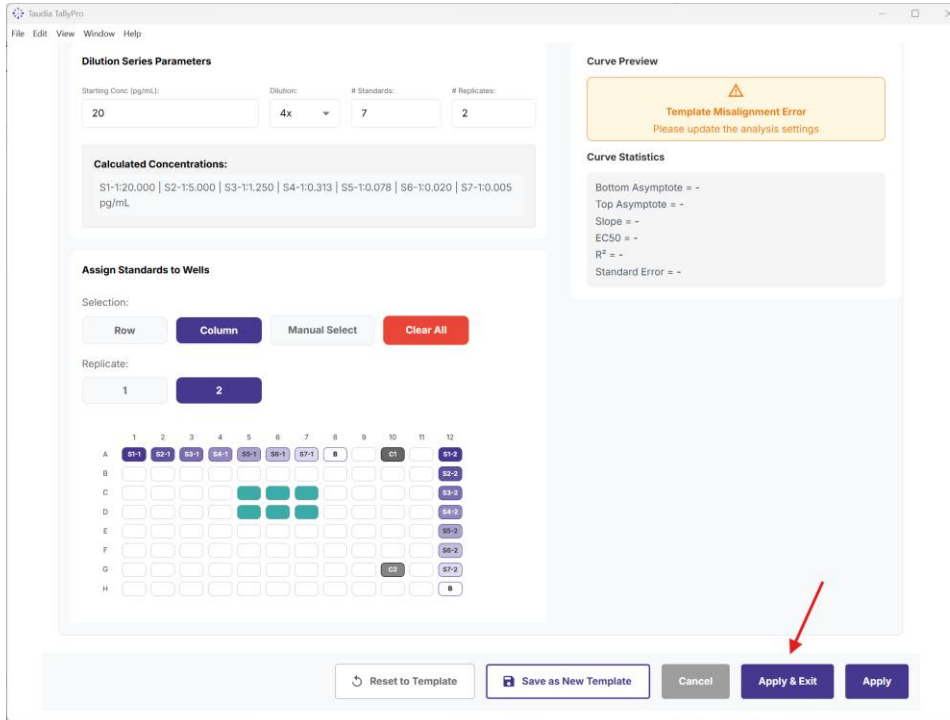




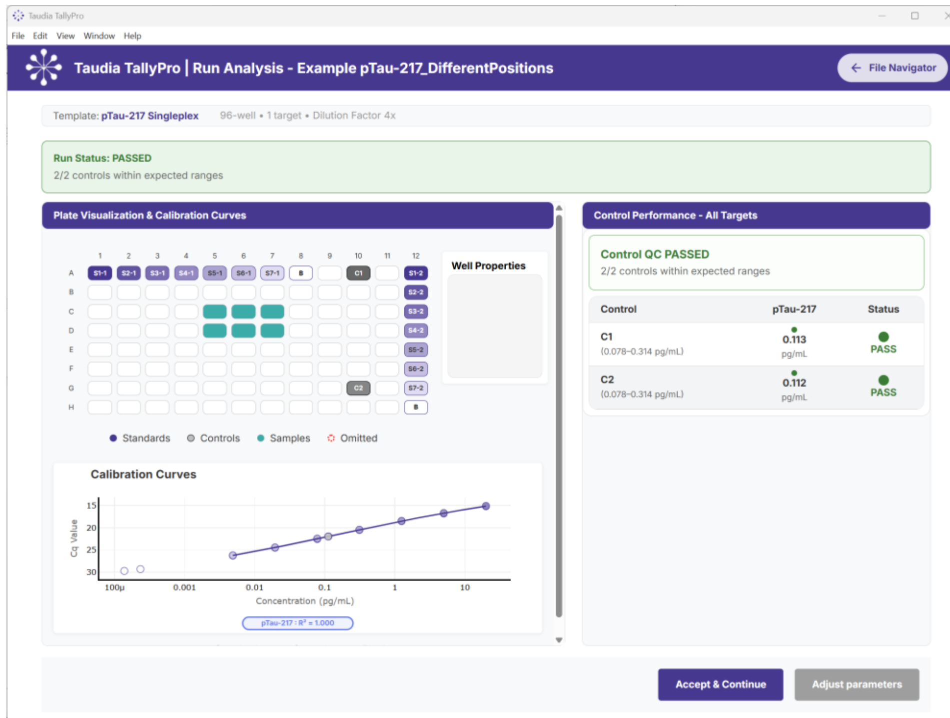
8. After making the changes, the configuration can be saved as a new Analysis Template by clicking on “Save as a New Template.”



9. When the positions of Standard Curves, CheckPoint, and Blank match the plate, click “Apply and Exit” to return to the Plate Visualization Screen.



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

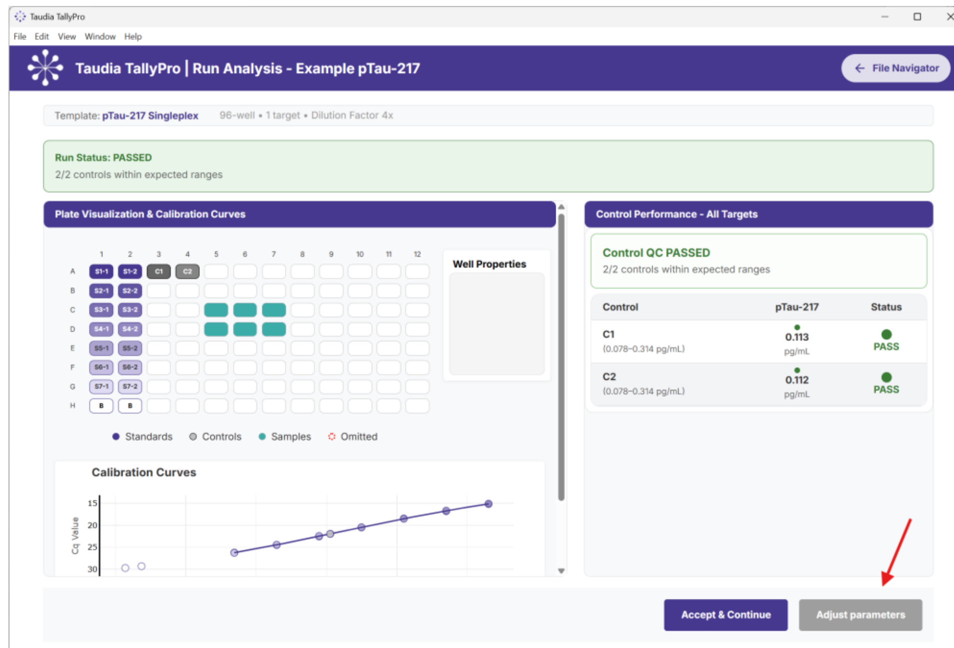


11. The data can now be exported as described in the prior section.

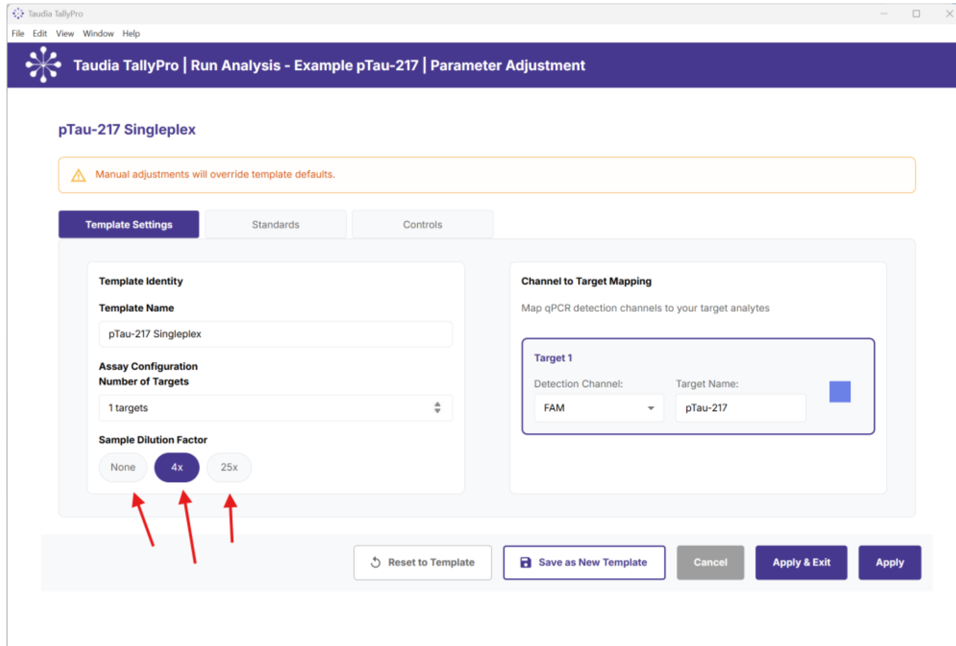
Adjust Sample Dilution Factor

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 dilutions values, 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.”