MARBIONC Enzyme-linked Immunosorbent Assay (ELISA) for the determination of Neurotoxic Shellfish Poisoning (NSP) toxins in molluscan shellfish

Principle of Analysis

In this indirect competitive ELISA based on Naar et al. (2002), a 96-well ELISA plate is coated with protein-linked brevetoxin, and any remaining binding sites in the wells are blocked. Polyclonal goat anti-brevetoxin antibodies are then incubated with samples or standards in the plate wells. The antibodies will react with the brevetoxins in the samples or standards or will be immobilized on the plate. Antibodies that are not attached to the plate after incubation are washed out during subsequent rinses. Antibodies immobilized on the plate are detected through steps linking the antibodies to horseradish peroxidase (HRP)-linked secondary antibodies and addition of an HRP substrate (3,3'5,5'-Tetramethylbenzidine [TMB]), which yields a blue color (Amax = 370 nm and 652 nm) that changes to yellow (Amax = 450 nm) upon addition of a sulfuric acid stop solution. The intensity of this color is inversely proportional to the amount of brevetoxin that was present in the well during incubation. Using this method, one ELISA plate can be used to quantitatively assay five shellfish samples. For qualitative (+/-) screening, more samples can be run on one plate (up to 40).

Included in MARBIONC ELISA Kit (store in freezer):

- Reagent A BSA-linked PbTx-3
- Reagent C Goat anti-brevetoxin Ab
- Reagent D HRP-linked anti-goat secondary Ab
- Brevetoxin standard (PbTx-3, 10 μg)

Reagents required but not included (Brands and product numbers are for convenience. Unless otherwise noted, equivalents are acceptable)**:**

- Methanol (ACS grade or better)
- Reagent B: Superblock Blocking Buffer (Thermo Scientific 37545)
- Phosphate Buffered Saline, pH 7.4 (PBS, Sigma P-3813)
- Phosphate Buffered Saline, 0.05% Tween 20, pH 7.4 (PBS-Tween, Sigma P-3563)
- Gelatin (Sigma G-6144)
- 3,3'5,5'-Tetramethylbenzidine (TMB, Sigma T0440)
- Sulfuric acid stop solution (H_2SO_4, 0.5M)
- Nanopure water (or equivalent quality water)

Consumables needed:

- Disposable glass test tubes
- Disposable plastic dilution tubes (96-well cluster format)
- 15-ml and 50-ml graduated polypropylene centrifuge tubes
- Nunc flat-bottom polystyrene 96-well Maxisorp Immunoplates (**substitution NOT recommended**)
- Microplate sealing film
- Assorted pipet tips
- Solution basins
- Aluminum foil

Equipment needed:

Basic laboratory glassware (beakers, 1-L graduated cylinders, bottles, 10-ml volumetric flask)
Balance capable of measuring to 0.1g
Number 10 sieve
Laboratory blender
Vortex mixer
Centrifuge capable of 3,000*xg*, with rotor for 15 ~~ml~~ml or 50 ml centrifuge tubes
Microplate reader with filter for measurement at 450 nm
Multichannel pipettor (100-300 µl), individual pipettors (10-1000 µl)
Orbital microplate shaker
Refrigerator (4°C)/freezer (-20°C)

Pre-Assay Preparation

In advance:

PbTx-3 for positive control. Each set of kit reagents (15-plate supply) comes with 10 µg of PbTx-3 for use as a positive control.
Stock solution (1 µg/ml): Dissolve in 10 ml of 100% methanol. Store at -20°C. (May be stored for up to 1 year.)
Working solution (100 ng/ml): From this stock, dilute 1 ml to 10 ml with 100% methanol. Store at -20°C. (May be used for several months.)

80% aqueous methanol. Add 800 ml of methanol to a 1L graduated cylinder and bring to 1L with Nanopure water (or equivalent quality water). Good for up to 1 year.

5% gelatin stock solution. Dissolve 5 g gelatin in 100 ml Nanopure water - stir on heated stir plate until clear. Portion into 15-ml centrifuge tubes and refrigerate. Good for several weeks at 4°C.

SuperBlock - Dissolve 1 pouch in 200 ml Nanopure water. Portion 50-ml aliquots into 50-ml centrifuge tubes and refrigerate. Good for several weeks at 4°C.

PBS, pH 7.4 1 L - Dissolve 1 pouch of PBS powder in 1 L of Nanopure water. (Unused buffer may be stored for no more than one week at 4°C.)

PBS-Tween (0.05% Tween), pH 7.4 1L - Dissolve 1 pouch of PBS-Tween powder in 1 L of Nanopure water. (Unused buffer may be stored for no more than one week at 4°C.)

Make fresh daily:
PGT (PBS, 0.05% Tween, 0.5% gelatin) - Immerse a tube of stock gelatin in warm water for a few minutes to liquefy. Pour 5 ml gelatin into a 50-ml centrifuge tube and fill to 50 ml with PBS-Tween. Make one tube per plate.

Shellfish Sample Preparation (follows requirements for the NSP mouse bioassay)

At least 12 animals and a total mass of 100-120 grams of meat should be collected per sample. Immediately after collection, shellfish should be placed in dry storage between 0 and 10°C. Shellfish not shucked on the day of collection should be refrigerated. Refrigeration must not exceed 48 hours. If shellfish are refrigerated, only live animals are used in the analysis.

The outside of shellfish are cleaned with fresh water. Adductor muscles are cut and the shell is opened. The inside of the shellfish is rinsed with fresh water to remove sand and other foreign material. Meats are shucked from shell being careful not to cut or damage the body of the mollusk. Approximately 100-120 grams of meat are collected, in a single layer, on a number 10 sieve, and the sample is drained for 5 minutes. Any pieces of shell are discarded. Drained meats are blended at high speed until homogenous (60-120 seconds) and extracted for brevetoxins. Samples must be processed within 24 hours of shucking.

Rapid Extraction of Shellfish for Brevetoxins

1. Weigh 1.0 g of homogenized shellfish into a 15-~~mL~~ ml or 50-ml polypropylene centrifuge tube.
2. Add 9 ~~mL~~ ml of 80% aqueous methanol, and cap tightly.
3. Vortex for 2 minutes at highest speed.
4. Centrifuge at a minimum of 3000xg for 10 minutes.
5. Pour off supernatant into clean, labeled graduated 15-~~mL~~ ml centrifuge tube.
6. Bring the volume of the supernatant to 10mL with 80% methanol.
7. Vortex for 15 seconds to mix.
8. Transfer to a clean labeled glass vial and store at -20°C until assayed.

ELISA Protocol

****IMPORTANT NOTE**** Kit Reagents A, C, and D are diluted in a glycerol solution to prevent freezing. To avoid pipetting error due to viscosity, only place the very tip of the pipet into the vial to withdraw the desired amount. DO NOT PRE-RINSE THE TIP. Submerge the tip into the buffer when dispensing, and rinse the tip several times with buffer to ensure complete transfer.

Step 1 - Reagent A
Shake vial of Reagent A gently by hand. Dilute Reagent A. 1:300 (or as specified in kit instructions) in **PBS**. (For 1 plate, add 40 µl of A to 12 ml **PBS**; for 2 plates, add 80 µl A to 24 ml **PBS**).

Fill each well of a 96-well Maxisorp Immunoplates with 100 µl of diluted Reagent A. Cover with microplate sealing film, and incubate on a plate shaker for 1 hour at room temperature. After 1 hour, pour liquid from plate and rinse each well 3 times with 300 µl **PBS**. (**No Tween for this step**.)

Step 2 - Reagent B
Fill each well with 250 µl of Reagent B-Blocking Buffer. Cover with microplate sealing film, and incubate on plate shaker for 30 minutes at room temperature. Pour the liquid from the plate and rinse each well 3 times with 300 µl PBS-Tween.

Step 3 - Sample and positive control dilutions *(This step can be done while Step 1 and 2 are incubating.)*

Note: Sample extracts and PbTx-3 working solution should be brought to room temperature before diluting.

Arrange dilution tubes in a rack according to plate layout - see below. Eight (8) tubes are needed for each sample or positive control.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Pos Ctrl (PbTx-3)
A	tube A	tube A	tube A	tube A	tube A	tube A
B	tube B	tube B	tube B	tube B	tube B	tube B
C	tube C	tube C	tube C	tube C	tube C	tube C
D	tube D	tube D	tube D	tube D	tube D	tube D
E	tube E	tube E	tube E	tube E	tube E	tube E
F	tube F	tube F	tube F	tube F	tube F	tube F
G	tube G	tube G	tube G	tube G	tube G	tube G
H	tube H	tube H	tube H	tube H	tube H	tube H

Leave dilution tubes in row **A** empty. To all other tubes in rows **B-H** (for both samples and Pos Ctrl) add 250 µl of PGT. For each sample, add 975µl of PGT to a small glass test tube. Add 25 µl of sample extract to the tube, and vortex briefly to mix. Transfer 250 µl of this diluted extract into dilution tube **A**. Withdraw another 250 µl from the glass tube, place into tube **B**, and vortex to mix. Then withdraw 250 µl from tube **B**, place into tube **C**, and vortex to mix. Continue this **serial dilution** for tubes **D** through **G**. **DO NOT DILUTE INTO TUBE H.** Do this for each sample.

Positive Control (PbTx-3)
To make the positive control, add 950µl of PGT to a small glass test tube. Add 50 µl of brevetoxin working solution (at 100 ng PbTx-3/ml) to the tube (50 µl PbTx-3 + 950 ul PGT= 5 ng PbTx-3/ml). (This is sufficient for up to two plates.) For each plate, transfer 250 µl of diluted PbTx-3 into dilution tube **A**. Withdraw another 250 µl from the glass tube and place into tube **B**, and vortex to mix. Then withdraw 250 µl from tube **B**, place into tube **C**, and vortex to mix. Continue this **serial dilution** for tubes **D** through **G**. **DO NOT DILUTE INTO TUBE H.**
(Tube H are PGT only and will serve as Reference Wells for maximum absorbance in the absence of brevetoxin.)

Step 4 - Transfer Samples On to Plate

After the plate has been blocked and washed (after Step 2 is complete), use a multichannel pipette to transfer the diluted samples and standards to the plate.

Fill wells of the microplate with 100 µl of each tube **in duplicate** (side by side wells), according to the figure below.

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Pos. Ctrl.	
	1	2	3	4	5	6	7	8	9	10	11	12
A	1:400	1:400	1:400	1:400	1:400	1:400	1:400	1:400	1:400	1:400	PbTx-3	5 ng/ml
B	1:800	1:800	1:800	1:800	1:800	1:800	1:800	1:800	1:800	1:800	PbTx-3	2.5 ng/nl
C	1:1600	1:1600	1:1600	1:1600	1:1600	1:1600	1:1600	1:1600	1:1600	1:1600	PbTx-3	1.25 ng/ml
D	1:3200	1:3200	1:3200	1:3200	1:3200	1:3200	1:3200	1:3200	1:3200	1:3200	PbTx-3	0.625 ng/ml
E	1:6400	1:6400	1:6400	1:6400	1:6400	1:6400	1:6400	1:6400	1:6400	1:6400	PbTx-3	0.31 mg/ml
F	1:12800	1:12800	1:12800	1:12800	1:12800	1:12800	1:12800	1:12800	1:12800	1:12800	PbTx-3	0.156 ng/ml
G	1:25600	1:25600	1:25600	1:25600	1:25600	1:25600	1:25600	1:25600	1:25600	1:25600	PbTx-3	0.078 ng/ml
H	PGT	PGT	PGT	PGT	PGT	PGT	PGT	PGT	PGT	PGT	PGT	PGT

Step 5 - Reagent C
Dilute Reagent **C** 1:300 (or as specified in kit instructions)
(For 1 plate, add 40 µl of ~~A~~ C to 12 ml PGT; for 2 plates, add 80 µl ~~A~~ C to 24 ml PGT)
To each well add 100 µl of diluted Reagent C. Cover with microplate sealing film, and shake the plate on the plate shaker for 90 minutes at room temperature. Pour the liquid from the plate and rinse each well 3 times with 300 µl PBS-Tween.

Step 6 - Reagent D
Dilute Reagent **D** 1:800 (or as specified in kit instructions)
(For 1 plate, add 15 µl of D to 12 ml PGT; for 2 plates, add 30 µl D to 24 ml PGT.)
Fill each well with 100 µl of diluted Reagent D. Cover with microplate sealing film, and incubate on a plate shaker for 1 hour at room temperature.
(When you get to this step – aliquot 12 ml of TMB per plate into a 15 or 50-ml centrifuge tube and warm to room temperature. Keep the tube in the dark (do not expose to light).
After 1 hour, pour liquid from plate and rinse each well 3 times with 300 µl PBS-Tween. **Then rinse each well one time with 300 µl PBS to ensure no Tween remains on the plate.**

Step 7 - TMB
Fill each well with 100 µl of TMB. Cover the plate with a piece of aluminum foil and incubate for 5-7 minutes (or until a blue color develops in the reference wells). Stop the reaction by adding 100 µl of 0.5M H_2SO_4 to each well. The blue color in the wells should turn yellow. Read the plate at 450 nm.

Note: The stop time may vary with kit reagent lots and bottles of TMB. The timing of the final step should be standardized with each new lot of kit reagents and each new lot of TMB to achieve maximum optical densities (at 450 nm) of 1.0 ± 30%.

Calculations

Presence of brevetoxin in the sample will prevent color development in the well. Toxin can be quantified by converting absorbance values to % color inhibition and comparing to the positive control.

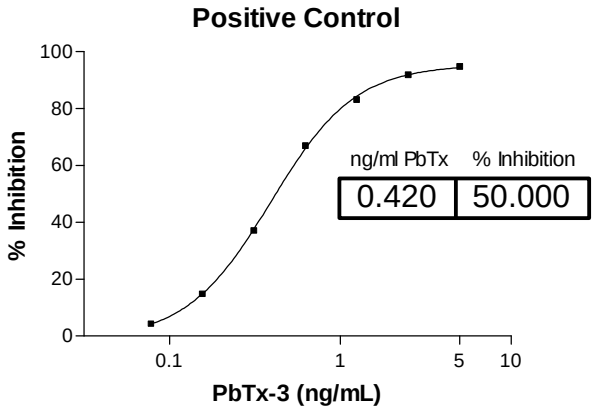
1. Average the values of the duplicate wells for each dilution, and determine the % color inhibition using the following equation:

   % inhibition = [1 - (Avg of dups/Amax)] x 100%

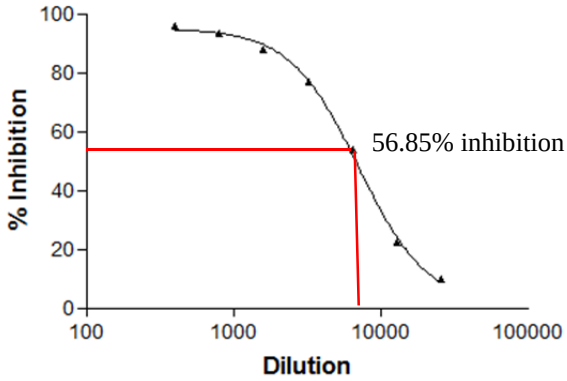
   where Amax is the average absorbance of the reference wells (PGT only) oriented below the sample or standard dilutions.

2. Using the 4-parameter logistic (4PL) curve in a curve-fitting program like Prism or SigmaPlot, fit a curve to the positive control with ng toxin/ml on the x-axis (log scale), and % inhibition on the y-axis (linear scale).
3. Determine the concentration for sample dilutions falling within the linear portion of the standard curve.
4. Multiply the concentration by the sample dilution and divide by 1000 to obtain PbTx-3 eq. results in ppm.

Example Standard Curve (50% inhibition = 0.42 ng PbTx-3/ml)
The control curve should be steep. On the linear part of the curve, the space between the dilutions (on the y-axis) is large. There should be clear plateaus at the top and bottom of the curve.



Example Sample Serial Dilution
Sample curves plotted with dilution on the x-axis (log scale), and % inhibition on the y-axis (linear scale) should have the same features. There should be a clear plateau either at the top or the bottom (or both). Shallow curves with no plateaus or linear curves with little space between points indicate interference in the assay, and results should be discarded.



For a sample with % inhibition of 56.85% at dilution of 1:6,400, the interpolated concentration = 0.495 ng/mL

[PbTx-3 eq] = 0.495 ng/ml x 6400 = 3168 ng/ml or 3.17 ppm

Quality Control Criteria

Acceptance of **assay results** is dependent on meeting the following criteria:

- Absorbance of reference wells must be (Amax) ≥ 0.6. (Optimal absorbance is 1.0 ± 30%.)
- %CV of raw absorbance of duplicate wells for standard curve within the linear range of the assay (~~20~~30-70% inhibition) must be < 20%.

If either ~~criteria~~ criterion is~~are~~ not met, re-run the ELISA plate.

Acceptance of **sample results** is dependent on meeting the following criteria:

- %CV of raw absorbance of duplicate wells for sample dilutions used for quantitation (within the linear range of the assay; ~~20~~30-70% inhibition) must be < 20%.
- %CV of calculated concentrations of different sample dilutions within the linear range of the assay must be < 20%. (A 20% or greater disparity between the calculated concentrations of two different dilutions of the same sample indicates assay interference or dilution error.)

If either criterion~~a~~ is~~are~~ not met, re-run the sample.

In 2017, the ISSC approved the MARBIONC Brevetoxin ELISA as a Limited Use Method under the NSSP (Proposal 17-107). The Standard Operating Procedure (SOP) for the MARBIONC Brevetoxin ELISA submitted as a part of the supporting documents for Proposal 17-107 specifies that quantification of sample dilutions is restricted to those dilutions falling within the linear portion of the standard curve (defined as 20%-70% inhibition). Sample dilutions with signals falling within this portion of the standard curve are used to quantify the brevetoxin concentration in the sample. One of the specified QA/QC criterion requires that the %CV of sample dilutions within this range must be <20%. Since its acceptance as a Limited Use Method, we have conducted numerous assays. Based on our results, we are proposing to narrow the specified range for quantifying sample dilutions to 30%-70% and to modify the QA/QC criteria to reflect this change. Additionally, we have made some minor corrections and additions to the SOP.

Basis for Proposed Modifications

We propose to narrow the specified range for quantifying sample dilutions to 30%-70% and to modify the QA/QC criteria to reflect this change.

Competitive ELISAs yield sigmoidally-shaped standard curves (Figure 1), and the rate of change of signal vs. concentration varies across the range of standard concentrations. The steep vertical portion of the curve exhibits large signal changes with small concentration changes, and the shallow horizontal portions of the curve exhibit small signal changes with large concentration changes. Because of this concentration dependence, accurate quantification in a competitive ELISA requires that sample dilutions fall within the relatively narrow, steep portion of the curve, which has the most reliable concentration dependence. Quantification is most accurate closer to the center of the curve.

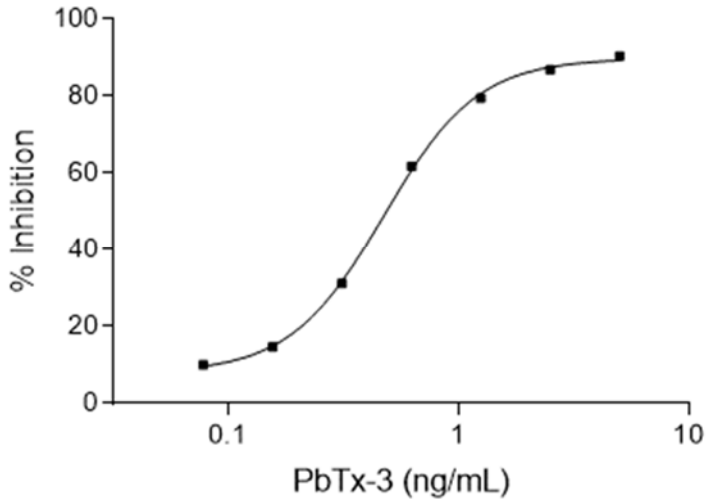


Figure 1. Example of a typical standard curve using the MARBIONC Brevetoxin ELISA

Signals (well absorbance measurements) are first normalized to zero controls, which provide maximum absorbance values, and are expressed as percent of the maximum (A/Amax) or, inversely, as percent inhibition (1-A/Amax). In general, concentration estimates can be obtained from signals that fall within 20% to 80%; however, to achieve variability between dilutions (calculated as %CV) of less than 20%, it is often necessary to use values obtained for a narrower portion of the curve (Sasaki and Mitchell 2002).

As a part of the SLV for the MARBIONC Brevetoxin ELISA, bend points from 60 different standard curves were calculated according to Sebaugh and McCray (2003), and the average of these bend points were used to help define the linear portion of the assay standard curve (20%-70% inhibition). In practice, the linear portion of the standard curve can vary slightly from assay to assay (Figure 2).

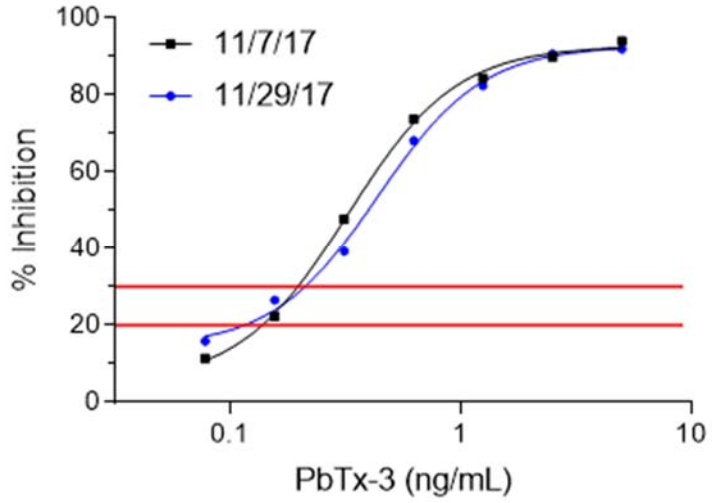


Figure 2. Calibration curves from 2 ELISA assays on different days. Red lines at 20% and 30% illustrate loss of linearity below 30% inhibition on the 11/29/17 standard curve.

Data from 326 shellfish samples tested since the method was approved in October 2017 were examined to determine the effect on variation and outcome of sample results (pass or fail sample QA/QC requirements) when the range for acceptable sample dilution signal was modified from 20%-70% to 30%-70% (Table 1). In most cases (n=281), assay results yielded two dilutions per sample that fell between 20% and 70%. Separating samples with at least one dilution between 20% and 30% from samples where all dilutions were ≥ 30% created two separate sample sets with no overlap. For 45 samples, assay results yielded three dilutions between 20% and 80% (with one dilution between 20% and 30% in 44 of the 45 samples). For these samples, %CV of dilutions was calculated for each pair of adjacent dilutions, and if either comparison yielded %CV ≥ 20% , the sampled failed QC. (For some samples, only a single sample dilution fell within the 20%-70% range. Those samples are not included the data presented.) Full data on sample dilutions included here are provided in Table 2 at the end of Appendix B.

While the range specified in currently accepted QA/QC criteria (20%-70%) yield acceptable variation between sample dilutions most of the time, narrowing this range does make a difference. Unacceptably high %CVs occurred in 17% of samples for which one of two dilutions fell between 20%-30% compared to only 8% unacceptably high %CVs in samples with dilutions between 30%-70%. Samples that had three dilutions within the 20%-70% range were rarer, and 27% of these samples had unacceptably high %CVs.

Table 1. Summary of shellfish samples (hard clams and oysters) included in this proposal.

ELISA dilution results	Number of samples	%CV of dilutions <20% pass QC	%CV of dilutions ≥ 20% fail QC
One of two dilutions between 20%-30%	179	148 (83%)	31 (17%)
Two dilutions between 30%-70%	193	177 (92%)	16 (8%)
Three dilutions between 20%-70%	45	33 (73%)	12 (27%)

An unpaired t-test also demonstrates a clear statistical difference ($p = 0.0024$) between the %CVs calculated for each of the two data sets for samples with two dilutions in the linear range.

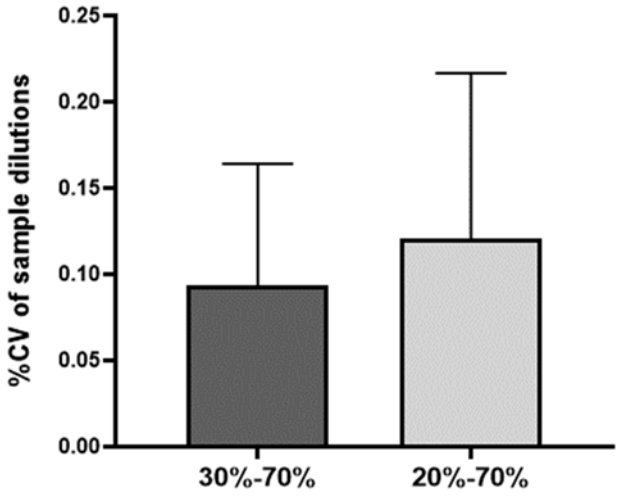


Figure 3. Mean %CV calculated for samples that had two dilutions between 30%-70% (left bar, n=193) and those with one of two dilutions between 20%-30% (right bar, n=179). Means are statistically different (unpaired t-test, $p = 0.0024$). Error bars indicate standard deviation (in the positive direction only for clarity).

The sigmoidal curves generated in a competitive ELISA are similar to those generated in receptor binding assays. In the Approved PSP RBA, the quantitative range specified is 0.2-0.7 B/B_0 (percent of maximum binding). If expressed in terms of binding inhibition ($1-B/B_0$), this

would be 0.3-0.8. VanDolah stated in that submission (Proposal 13-114) that they selected a more conservative cutoff (at the lower end of the curve) than the 0.8 B/B_0 (or 0.2 binding inhibition) frequently used in receptor assays because quantification was unacceptably variable at that cutoff. Our reasoning in this proposal is similar.

High sample variation between dilutions are most often due to analyst error, matrix effects generated by the sample, or poor quantification at the extreme ends of the linear portion of the standards curve. These are the reasons for imposing QA/QC criteria that specify the acceptable range for quantification and the acceptable variation (%CV) between dilutions that fall within that range. Matrix effects were not seen in any of the species studied in the SLV at the starting sample dilutions of 400, which is the minimum dilution specified in the protocol. Therefore, we feel that any unacceptable variation between dilutions is more likely due to interpolation error or analyst error. Revising the acceptable range of quantification to a more conservative one as proposed will minimize interpolation error and avoidable QC failures while still controlling for assay quality.

Minor corrections and additions to the SOP

- Corrections to the protocol are indicated in Appendix A and include grammatical corrections, minor changes for consistency in how units are expressed (e.g. ml vs mL), and correction of typos.
- Glassware was added to "Equipment needed" on Page 2.
- For the first step of the extraction on Page 3, the option of using 50 ml centrifuge tubes was added. The size of the centrifuge tubes used for this step of the extraction is not critical. This change adds flexibility. (In "Equipment needed" on Page 2 the rotor required was therefore also made optional.)
- At "Step 7" on Page 5, additional guidance was provided on when to stop the color development. This reinforces the note at the bottom of the page that the timing can change with reagents.
- Quality control criteria were modified as described above.

References cited:

Sasaki D. and R.A. Mitchell (2002). How to obtain reproducible quantitative ELISA results. Oxford Biomedical Research, Inc. https://www.oxfordbiomed.com/sites/default/files/2017-02/How%20to%20Obtain%20Reproducible%20Quantitative%20ELISA%20results.pdf

Sebaugh, J. L. and P. D. McCray (2003). Defining the linear portion of a sigmoid-shaped curve: bend points. Pharmaceutical Statistics 2: 167-174.

Table 2. Samples included in the analyses for this proposal with the dilutions used, calculated sample concentrations, and %CVs of paired dilutions. Dilutions <30% inhibition are highlighted in blue, and unacceptably high %CVs are highlighted in red.

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T17-1239	1600	64.3%	1.33	2.13		
T17-1239	3200	35.4%	0.54	1.73	1.93	14.5%
T17-1240	800	53.6%	0.97	0.78		
T17-1240	1600	29.8%	0.45	0.73	0.75	4.9%
T17-1241	1600	64.7%	1.37	2.20		
T17-1241	3200	27.7%	0.42	1.34	1.77	34.1%
T17-1333	800	64.1%	1.51	1.21		
T17-1333	1600	36.5%	0.55	0.87	1.04	22.9%
T17-1383	800	65.6%	1.63	1.30		
T17-1383	1600	45.3%	0.75	1.19	1.25	6.1%
T17-1383	3200	22.4%	0.34	1.08	1.14	7.3%
T17-1384	800	69.8%	2.02	1.61		
T17-1384	1600	52.9%	0.97	1.55	1.58	2.6%
T17-1384	3200	21.5%	0.32	1.04	1.30	28.2%
T17-1389	3200	48.3%	0.32	1.02		
T17-1389	6400	29.5%	0.19	1.24	1.13	13.7%
T17-1390	3200	51.1%	0.34	1.09		
T17-1390	6400	28.8%	0.19	1.21	1.15	7.4%
T17-1391	6400	46.2%	0.30	1.93		
T17-1391	12800	21.6%	0.15	1.89	1.91	1.8%
T17-1526a	1600	60.5%	0.86	1.38		
T17-1526a	3200	24.5%	0.35	1.13	1.26	14.2%
T17-1526b	1600	61.7%	0.99	1.58		
T17-1526b	3200	30.0%	0.46	1.47	1.52	5.2%
T17-1527a	1600	59.0%	0.83	1.33		
T17-1527a	3200	27.6%	0.39	1.24	1.29	4.9%
T17-1527b	1600	61.1%	0.97	1.56		
T17-1527b	3200	31.6%	0.48	1.53	1.54	1.0%
T17-1528a	1600	63.7%	0.94	1.50		
T17-1528a	3200	31.1%	0.43	1.38	1.44	5.9%
T17-1528b	1600	56.6%	0.87	1.39		
T17-1528b	3200	26.8%	0.42	1.34	1.36	2.8%
T17-1529a	1600	56.8%	0.79	1.26		
T17-1529a	3200	25.3%	0.36	1.16	1.21	6.0%
T17-1529b	1600	54.4%	0.82	1.32		
T17-1529b	3200	25.8%	0.41	1.30	1.31	1.2%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T17-1530	3200	67.3%	0.64	2.05		
T17-1530	6400	47.0%	0.36	2.33	2.19	10.4%
T17-1530	12800	23.2%	0.15	1.91	2.12	14.2%
T17-1531	12800	63.4%	0.57	7.29		
T17-1531	25600	32.1%	0.23	5.88	6.58	15.2%
T17-1532	6400	63.5%	0.57	3.65		
T17-1532	12800	36.4%	0.27	3.41	3.53	4.8%
T17-1535	12467.2	49.8%	0.39	4.91		
T17-1535	24934.4	25.5%	0.17	4.26	4.59	10.0%
T17-1535b	12467.2	59.0%	0.51	6.42		
T17-1535b	24934.4	32.0%	0.22	5.49	5.96	11.0%
T17-1536	6233.6	52.4%	0.42	2.63		
T17-1536	12467.2	30.9%	0.22	2.73	2.85	10.2%
T17-1536	24934.4	21.1%	0.13	3.18	2.95	10.7%
T17-1536b	6233.6	51.0%	0.40	2.52		
T17-1536b	12467.2	33.5%	0.24	2.95	2.92	13.0%
T17-1536b	24934.4	20.4%	0.13	3.28	3.11	7.6%
T17-1538	97.4	69.3%	0.61	0.06		
T17-1538	194.8	40.8%	0.27	0.05	0.06	7.8%
T17-1538	389.6	24.2%	0.16	0.06	0.06	10.8%
T17-1549	800	64.1%	0.51	0.41		
T17-1549	1600	41.1%	0.27	0.44	0.42	4.7%
T17-1549	3200	21.2%	0.15	0.47	0.45	5.1%
T17-1555	12800	58.5%	0.44	5.69		
T17-1555	25600	28.6%	0.21	5.28	5.48	5.3%
T17-1558	40000	68.8%	0.59	23.70		
T17-1558	80000	43.5%	0.28	22.36	25.14	4.1%
T17-1558	160000	28.7%	0.18	29.36		19.2%
T17-1559	80000	65.3%	0.52	41.94		
T17-1559	160000	37.2%	0.23	37.59	39.76	7.7%
T17-1566	6400	64.9%	0.77	4.90		
T17-1566	12800	37.8%	0.31	4.01	4.45	14.2%
T17-1570	3200	63.4%	0.72	2.31		
T17-1570	6400	37.3%	0.31	1.97	2.14	11.3%
T17-1574	12800	59.5%	0.63	8.04		
T17-1574	25600	33.7%	0.28	7.06	7.55	9.2%
T17-1576	3200	57.2%	0.41	1.31		
T17-1576	6400	28.7%	0.18	1.17	1.24	7.8%
T17-1578	6400	51.8%	0.35	2.24		

T17-1578	12800	23.6%	0.16	1.99	2.12	8.5%
Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T17-1583	6400	66.1%	0.66	4.20		
T17-1583	12800	41.2%	0.32	4.12	4.16	1.3%
T17-1585	12800	62.2%	0.58	7.40		
T17-1585	25600	30.2%	0.23	6.00	6.70	14.7%
T17-1586	80000	40.8%	0.36	28.41		
T17-1586	160000	22.2%	0.22	34.45	31.43	13.6%
T17-1587	6400	68.2%	0.75	4.81		
T17-1587	12800	39.9%	0.35	4.45	4.63	5.3%
T17-1587	25600	22.4%	0.20	5.22	4.84	11.1%
T17-1588	1600	61.8%	0.62	0.98		
T17-1588	3200	41.0%	0.36	1.14	1.06	10.6%
T17-1591	80000	38.6%	0.34	26.93		
T17-1591	160000	20.3%	0.20	32.39	29.66	13.0%
T17-1592	80000	49.6%	0.44	35.00		
T17-1592	160000	25.6%	0.24	38.28	36.64	6.3%
T18-0001	12800	64.3%	1.52	19.51		
T18-0001	25600	36.4%	0.62	15.87	17.69	14.6%
T18-0002	12800	63.0%	1.26	16.07		
T18-0002	25600	30.1%	0.51	12.99	14.53	15.0%
T18-0006	12800	66.3%	1.40	17.91		
T18-0006	25600	34.0%	0.57	14.64	16.27	14.2%
T18-0007	12800	63.3%	1.47	18.87		
T18-0007	25600	30.3%	0.50	12.83	15.85	27.0%
T18-0008	12800	65.7%	1.30	16.64		
T18-0008	25600	44.0%	0.74	18.90	17.77	9.0%
T18-0014	80000	54.5%	0.44	34.99		
T18-0014	160000	24.7%	0.17	26.62	30.80	19.2%
T18-0058	12800	49.7%	0.65	8.33		
T18-0058	25600	21.1%	0.28	7.26	7.79	9.6%
T18-0059	12800	52.4%	0.68	8.73		
T18-0059	25600	23.7%	0.24	6.14	7.44	24.6%
T18-0060	12800	63.1%	0.98	12.56		
T18-0060	25600	33.5%	0.42	10.63	11.59	11.8%
T18-0061	12800	64.0%	1.02	13.00		
T18-0061	25600	27.2%	0.28	7.24	10.12	40.2%
T18-0070a	6400	59.3%	0.86	5.48		
T18-0070a	12800	25.7%	0.26	3.38	4.43	33.5%
T18-0070b	8000	51.2%	0.83	6.66		
T18-0070b	16000	21.6%	0.49	7.88	7.27	11.9%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-0071	6400	64.3%	1.03	6.60		
T18-0071	12800	28.8%	0.36	4.59	5.60	25.5%
T18-0071b	4000	63.9%	0.90	3.61		
T18-0071b	8000	41.1%	0.41	3.27	3.44	6.9%
T18-0088	6400	61.0%	1.06	6.77		
T18-0088	12800	21.1%	0.36	4.63	5.70	26.7%
T18-0089	6400	62.2%	0.97	6.18		
T18-0089	12800	25.6%	0.44	5.68	5.93	6.0%
T18-0089b	6400	59.8%	0.77	4.95		
T18-0089b	12800	21.2%	0.17	2.19	3.57	54.7%
T18-0091	12800	56.3%	0.68	8.74		
T18-0091	25600	32.2%	0.30	7.56	8.15	10.3%
T18-0135	3200	68.1%	1.22	3.90		
T18-0135	6400	38.4%	0.56	3.61	3.76	5.4%
T18-0135	12800	23.0%	0.37	4.70	4.16	18.5%
T18-0136	3200	66.3%	1.05	3.35		
T18-0136	6400	34.7%	0.49	3.13	3.24	4.9%
T18-0139	12800	53.9%	0.82	10.50		
T18-0139	25600	24.8%	0.39	9.96	10.23	3.8%
T18-0143	3200	55.8%	0.86	2.76		
T18-0143	6400	31.7%	0.48	3.04	2.90	6.9%
T18-0145	3200	65.0%	1.10	3.52		
T18-0145	6400	35.9%	0.49	3.17	3.34	7.4%
T18-0146	3200	64.5%	1.05	3.35		
T18-0146	6400	30.7%	0.49	3.13	3.24	4.8%
T18-0147	6400	47.7%	0.67	4.30		
T18-0147	12800	22.7%	0.33	4.22	4.26	1.3%
T18-0148	6400	55.0%	0.83	5.29		
T18-0148	12800	22.1%	0.40	5.10	5.20	2.5%
T18-0149	12800	66.2%	1.15	14.68		
T18-0149	25600	29.7%	0.41	10.62	12.65	22.7%
T18-0150	12800	66.0%	1.09	13.99		
T18-0150	25600	35.9%	0.55	14.04	14.02	0.3%
T18-0151	400	52.8%	0.75	0.30		
T18-0151	800	25.2%	0.38	0.30	0.30	0.1%
T18-0151b	200	64.7%	1.20	0.24		
T18-0151b	400	47.5%	0.73	0.29	0.27	14.1%
T18-0151b	800	21.0%	0.35	0.28	0.29	2.5%
T18-0155	3200	64.5%	1.11	3.57		

T18-0155	6400	28.2%	0.47	2.99	3.28	12.4%
Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-0156	3200	67.8%	1.10	3.51		
T18-0156	6400	30.0%	0.43	2.72	3.12	17.9%
T18-0157	3200	54.7%	0.86	2.76		
T18-0157	6400	21.0%	0.39	2.48	2.62	7.6%
T18-0164	6400	43.6%	0.66	4.21		
T18-0164	12800	23.8%	0.38	4.83	4.52	9.8%
T18-0165	1600	66.0%	1.21	1.93		
T18-0165	3200	39.9%	0.62	1.98	1.96	2.0%
T18-0166	1600	65.6%	1.25	2.01		
T18-0166	3200	40.1%	0.60	1.92	1.96	3.2%
T18-0167	1600	67.5%	1.26	2.02		
T18-0167	3200	41.1%	0.64	2.04	2.03	0.5%
T18-0168	1600	64.0%	1.18	1.89		
T18-0168	3200	34.9%	0.52	1.67	1.78	8.9%
T18-0186	3200	60.4%	1.02	3.25		
T18-0186	6400	27.0%	0.45	2.88	3.07	8.6%
T18-0187	3200	50.4%	0.79	2.52		
T18-0187	6400	21.1%	0.34	2.21	2.36	9.5%
T18-0188	6400	57.4%	0.94	6.00		
T18-0188	12800	25.8%	0.44	5.58	5.79	5.1%
T18-0189	6400	56.8%	0.95	6.05		
T18-0189	12800	20.5%	0.34	4.32	5.19	23.7%
T18-0226	3200	49.4%	0.77	2.46		
T18-0226	6400	20.7%	0.35	2.24	2.35	6.5%
T18-0227	6400	47.4%	0.73	4.68		
T18-0227	12800	23.7%	0.39	4.98	4.83	4.5%
T18-0229	6400	49.8%	0.79	5.03		
T18-0229	12800	20.7%	0.42	5.37	5.20	4.7%
T18-0230	3200	62.4%	1.11	3.55		
T18-0230	6400	30.8%	0.48	3.08	3.31	10.1%
T18-0231	3200	63.3%	1.11	3.56		
T18-0231	6400	29.3%	0.51	3.27	3.41	6.2%
T18-0234	1600	61.6%	1.14	1.82		
T18-0234	3200	34.3%	0.49	1.57	1.70	10.3%
T18-0235	3200	45.3%	0.69	2.21		
T18-0235	6400	21.9%	0.39	2.52	2.36	9.3%
T18-0238	6400	71.5%	0.92	5.86		
T18-0238	12800	35.3%	0.42	5.38	5.62	6.1%
T18-0239	6400	71.3%	0.91	5.84		

T18-0239	12800	39.2%	0.45	5.82	5.83	0.3%
Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-0296	3200	69.0%	1.39	4.43		
T18-0296	6400	40.8%	0.56	3.58	4.01	15.0%
T18-0298	3200	62.5%	1.07	3.44		
T18-0298	6400	26.4%	0.37	2.37	2.90	25.9%
T18-0300	12800	66.7%	1.72	22.00		
T18-0300	25600	39.3%	0.70	17.98	19.99	14.2%
T18-0301	6400	69.0%	1.90	12.13		
T18-0301	12800	38.1%	0.68	8.68	10.41	23.4%
T18-0302	6400	54.3%	1.11	7.11		
T18-0302	12800	23.7%	0.42	5.40	6.25	19.3%
T18-0309	6400	68.9%	0.79	5.06		
T18-0309	12800	42.0%	0.36	4.64	4.85	6.1%
T18-0310	12800	53.8%	0.49	6.31		
T18-0310	25600	25.2%	0.23	5.85	6.08	5.4%
T18-0411	6400	65.3%	0.48	3.07		
T18-0411	12800	38.6%	0.24	3.08	3.08	0.2%
T18-0412	12800	48.2%	0.31	3.94		
T18-0412	25600	22.2%	0.14	3.64	3.79	5.5%
T18-0452	6400	61.5%	1.05	6.73		
T18-0452	12800	29.9%	0.45	5.76	6.24	10.9%
T18-0453	6400	62.1%	1.02	6.53		
T18-0453	12800	29.4%	0.40	5.06	5.79	18.0%
T18-0456	6400	53.4%	1.08	6.90		
T18-0456	12800	32.0%	0.57	7.31	7.10	4.1%
T18-0484	6400	63.8%	1.58	10.12		
T18-0484	12800	29.6%	0.56	7.22	8.67	23.7%
T18-0485	6400	59.8%	1.33	8.48		
T18-0485	12800	27.8%	0.60	7.67	8.08	7.2%
T18-0489	3200	69.5%	1.93	6.16		
T18-0489	6400	45.6%	0.91	5.85	6.00	3.7%
T18-0489	12800	20.6%	0.49	6.30	6.07	5.2%
T18-0491	3200	59.6%	1.32	4.21		
T18-0491	6400	33.9%	0.70	4.45	4.33	3.9%
T18-0553	6400	65.2%	1.45	9.26		
T18-0553	12800	38.8%	0.60	7.70	8.48	13.0%
T18-0554	1600	58.6%	1.14	1.82		
T18-0554	3200	27.3%	0.40	1.28	1.55	24.6%
T18-0555	1600	59.1%	1.10	1.75		
T18-0555	3200	28.6%	0.47	1.52	1.64	10.2%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-0557	1600	68.6%	1.50	2.40		
T18-0557	3200	45.4%	0.75	2.41	2.41	0.3%
T18-0562	1600	61.5%	1.26	2.02		
T18-0562	3200	35.6%	0.54	1.73	1.87	10.9%
T18-0563	1600	61.7%	1.18	1.90		
T18-0563	3200	33.6%	0.55	1.75	1.83	5.4%
T18-0564	3200	58.3%	1.34	4.28		
T18-0564	6400	33.1%	0.63	4.05	4.17	3.9%
T18-0565	3200	68.0%	1.64	5.25		
T18-0565	6400	41.4%	0.64	4.12	4.69	17.1%
T18-0601	3200	66.3%	1.41	4.50		
T18-0601	6400	36.0%	0.63	4.01	4.26	8.1%
T18-0602	3200	64.0%	1.18	3.76		
T18-0602	6400	29.0%	0.43	2.76	3.26	21.7%
T18-0724	1600	57.7%	1.16	1.85		
T18-0724	3200	29.9%	0.47	1.50	1.67	14.8%
T18-0725	1600	61.7%	1.33	2.13		
T18-0725	3200	38.0%	0.61	1.96	2.04	5.7%
T18-0726	1600	64.2%	1.86	2.98		
T18-0726	3200	48.2%	1.03	3.30	3.14	7.1%
T18-0727	3200	62.2%	1.35	4.32		
T18-0727	6400	29.2%	0.46	2.91	3.62	27.5%
T18-0729	1600	63.6%	1.82	2.91		
T18-0729	3200	49.8%	1.09	3.49	3.20	12.7%
T18-0729	6400	23.7%	0.39	2.50	2.99	23.5%
T18-0735	3200	63.8%	1.54	4.93		
T18-0735	6400	42.5%	0.70	4.46	4.69	7.1%
T18-0736	6400	48.8%	0.70	4.46		
T18-0736	12800	22.5%	0.29	3.71	4.09	13.0%
T18-0737	800	63.9%	1.55	1.24		
T18-0737	1600	48.0%	0.85	1.36	1.35	6.4%
T18-0737	3200	30.9%	0.46	1.46		5.4%
T18-0738	1600	63.3%	1.15	1.83		
T18-0738	3200	38.3%	0.51	1.62	1.72	8.9%
T18-0738	6400	22.4%	0.29	1.85	1.74	9.7%
T18-0739	1600	66.7%	1.74	2.79		
T18-0739	3200	44.5%	0.75	2.40	2.59	10.7%
T18-0739	6400	26.1%	0.38	2.43	2.41	1.1%
T18-0740	1600	65.4%	1.25	1.99		

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-0740	3200	39.9%	0.53	1.70	1.85	11.3%
T18-0741	3200	60.2%	1.34	4.28		
T18-0741	6400	34.8%	0.53	3.38	3.83	16.6%
T18-0742	3200	67.9%	1.39	4.45		
T18-0742	6400	40.7%	0.54	3.48	3.97	17.3%
T18-0743	3200	53.9%	0.74	2.38		
T18-0743	6400	26.5%	0.30	1.91	2.15	15.6%
T18-0744	3200	56.8%	0.65	2.07		
T18-0744	6400	27.2%	0.30	1.93	2.00	4.8%
T18-0745	3200	65.0%	1.09	3.49		
T18-0745	6400	36.5%	0.43	2.76	3.12	16.6%
T18-0746	3200	69.6%	0.93	2.96		
T18-0746	6400	32.7%	0.36	2.29	2.63	18.0%
T18-0747	3200	68.8%	1.91	6.11		
T18-0747	6400	44.3%	0.74	4.75	5.43	17.7%
T18-0748	3200	66.0%	1.28	4.10		
T18-0748	6400	36.0%	0.47	3.01	3.55	21.7%
T18-0749	1600	48.6%	0.63	1.01		
T18-0749	3200	23.5%	0.26	0.84	0.92	13.3%
T18-0750	1600	56.6%	0.64	1.03		
T18-0750	3200	25.8%	0.29	0.92	0.97	8.0%
T18-0751	3200	52.7%	0.81	2.60		
T18-0751	6400	23.4%	0.37	2.34	2.47	7.3%
T18-0752	3200	57.7%	1.13	3.63		
T18-0752	6400	28.1%	0.46	2.92	3.27	15.4%
T18-0754	3200	69.4%	1.37	4.38		
T18-0754	6400	43.7%	0.64	4.12	4.25	4.4%
T18-0755	3200	68.7%	1.34	4.27		
T18-0755	6400	46.7%	0.69	4.44	4.36	2.8%
T18-0756	1600	67.3%	1.27	2.03		
T18-0756	3200	32.6%	0.48	1.54	1.78	19.3%
T18-0757	1600	63.4%	1.38	2.22		
T18-0757	3200	33.1%	0.54	1.72	1.97	17.7%
T18-0758	1600	61.5%	1.13	1.81		
T18-0758	3200	33.4%	0.47	1.49	1.65	13.5%
T18-0759	1600	64.3%	1.40	2.23		
T18-0759	3200	33.2%	0.56	1.81	2.02	15.0%
T18-0760	1600	70.0%	0.99	1.59		
T18-0760	3200	36.0%	0.42	1.34	1.46	11.8%
T18-0761	1600	67.3%	0.90	1.44		

T18-0761	3200	33.7%	0.40	1.28	1.36	8.3%
Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-0762	1600	59.0%	0.72	1.15		
T18-0762	3200	26.3%	0.33	1.04	1.10	7.0%
T18-0763	1600	67.0%	0.90	1.44		
T18-0763	3200	33.3%	0.39	1.26	1.35	9.5%
T18-0765	1600	66.4%	1.27	2.03		
T18-0765	3200	42.8%	0.56	1.79	1.91	9.0%
T18-0766	1600	62.4%	0.81	1.30		
T18-0766	3200	40.0%	0.42	1.34	1.32	2.0%
T18-0767	800	66.8%	1.29	1.03		
T18-0767	1600	48.7%	0.67	1.07	1.05	2.4%
T18-0768	1600	56.9%	0.87	1.39		
T18-0768	3200	35.7%	0.45	1.44	1.41	2.2%
T18-0769	1600	59.8%	0.97	1.55		
T18-0769	3200	30.7%	0.38	1.23	1.39	16.3%
T18-0770	1600	63.1%	0.83	1.33		
T18-0770	3200	39.3%	0.41	1.31	1.32	1.4%
T18-0969	400	66.1%	1.12	0.45		
T18-0969	800	50.1%	0.63	0.50	0.48	8.4%
T18-0969	1600	22.7%	0.29	0.46	0.48	5.9%
T18-0978	3200	66.6%	1.10	3.51		
T18-0978	6400	29.6%	0.43	2.74	3.12	17.3%
T18-0979	3200	70.0%	0.99	3.18		
T18-0979	6400	36.0%	0.38	2.40	2.79	20.0%
T18-0980	800	65.4%	1.05	0.84		
T18-0980	1600	42.7%	0.58	0.93	0.89	7.4%
T18-0980	3200	20.4%	0.33	1.06	1.00	8.8%
T18-0982	800	68.7%	1.18	0.95		
T18-0982	1600	56.8%	0.82	1.31	1.13	22.9%
T18-0982	3200	26.9%	0.40	1.28	1.29	1.8%
T18-0983	1600	64.0%	0.82	1.30		
T18-0983	3200	33.0%	0.34	1.10	1.20	12.0%
T18-0984	800	47.8%	0.73	0.58		
T18-0984	1600	21.8%	0.32	0.52	0.55	8.6%
T18-0988	3200	59.0%	0.94	3.01		
T18-0988	6400	33.5%	0.45	2.86	2.94	3.6%
T18-0989	3200	66.8%	1.25	4.00		
T18-0989	6400	36.4%	0.41	2.61	3.30	29.8%
T18-1041	3200	58.0%	0.97	3.10		
T18-1041	6400	25.3%	0.42	2.69	2.90	9.9%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-1042	3200	69.5%	1.45	4.63		
T18-1042	6400	38.0%	0.53	3.36	4.00	22.4%
T18-1042	12800	26.0%	0.38	4.82	4.09	25.2%
T18-1043	800	55.5%	0.90	0.72		
T18-1043	1600	31.0%	0.48	0.77	0.75	5.3%
T18-1044	800	63.4%	1.11	0.89		
T18-1044	1600	35.1%	0.49	0.78	0.83	9.7%
T18-1045	1600	55.9%	0.91	1.45		
T18-1045	3200	27.5%	0.44	1.42	1.44	1.6%
T18-1046	1600	63.5%	1.12	1.79		
T18-1046	3200	36.4%	0.50	1.61	1.70	7.3%
T18-1438	1600	65.4%	1.64	2.62		
T18-1438	3200	45.9%	0.82	2.61	2.62	0.3%
T18-1439	1600	68.1%	1.55	2.49		
T18-1439	3200	48.2%	0.75	2.39	2.44	2.9%
T18-1439	6400	22.6%	0.33	2.11	2.25	8.6%
T18-1440	3200	29.3%	0.56	1.80	1.93	9.7%
T18-1440	3200	29.3%	0.56	1.80	1.93	9.7%
T18-1441	1600	60.4%	1.17	1.87		
T18-1441	3200	37.4%	0.51	1.62	1.75	9.9%
T18-1454	3200	68.0%	1.99	6.38		
T18-1454	6400	32.2%	0.55	3.52	4.95	40.9%
T18-1455	1600	66.0%	1.88	3.01		
T18-1455	3200	45.2%	0.82	2.62	2.82	9.7%
T18-1457	3200	61.2%	1.49	4.76		
T18-1457	6400	39.8%	0.68	4.37	4.56	6.0%
T18-1498	3200	69.1%	2.43	7.79		
T18-1498	6400	53.4%	1.11	7.13	7.46	6.3%
T18-1498	12800	20.5%	0.43	5.52	6.32	18.0%
T18-1499	6400	63.2%	1.65	10.56		
T18-1499	12800	32.5%	0.62	7.88	9.22	20.6%
T18-1500	1600	58.3%	1.32	2.12		
T18-1500	3200	33.6%	0.63	2.03	2.07	3.2%
T18-1518	400	59.0%	0.87	0.35		
T18-1518	800	31.6%	0.47	0.38	0.36	5.5%
T18-1639	800	61.0%	0.86	0.69		
T18-1639	1600	28.2%	0.41	0.65	0.67	4.3%
T18-1640	800	65.1%	0.96	0.77		
T18-1640	1600	34.3%	0.47	0.75	0.76	1.5%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-1945	1600	63.7%	0.77	1.24		
T18-1945	3200	38.3%	0.47	1.49	1.36	13.3%
T18-1946	1600	68.5%	1.01	1.62		
T18-1946	3200	39.9%	0.46	1.48	1.55	6.5%
T18-1950	6400	64.9%	0.90	5.74		
T18-1950	12800	35.3%	0.41	5.27	5.50	6.0%
T18-1953	3200	60.1%	0.71	2.28		
T18-1953	6400	23.3%	0.34	2.17	2.22	3.5%
T18-1954	3200	62.8%	0.84	2.69		
T18-1954	6400	21.9%	0.29	1.83	2.26	26.7%
T18-1990	1600	60.1%	0.82	1.31		
T18-1990	3200	36.9%	0.46	1.49	1.40	8.9%
T18-1991	1600	67.9%	0.99	1.59		
T18-1991	3200	38.3%	0.44	1.39	1.49	9.2%
T18-1992	3200	51.2%	0.65	2.08		
T18-1992	6400	27.4%	0.37	2.35	2.21	8.4%
T18-1993	3200	56.6%	0.70	2.24		
T18-1993	6400	26.2%	0.31	2.00	2.12	8.1%
T18-1994	3200	63.5%	0.90	2.89		
T18-1994	6400	39.9%	0.50	3.19	3.04	6.8%
T18-1995	1600	59.7%	0.81	1.30		
T18-1995	3200	42.1%	0.52	1.68	1.49	18.2%
T18-1996	1600	66.6%	1.00	1.60		
T18-1996	3200	46.5%	0.58	1.86	1.73	10.8%
T18-1998	800	60.1%	0.77	0.62		
T18-1998	1600	24.7%	0.30	0.48	0.55	18.3%
T18-2000	3200	69.3%	0.82	2.61		
T18-2000	6400	43.8%	0.36	2.32	2.46	8.3%
T18-2000	12800	23.2%	0.11	1.46	1.89	32.0%
T18-2012	1600	60.0%	0.87	1.40		
T18-2012	3200	34.9%	0.47	1.50	1.45	5.2%
T18-2013	1600	62.9%	0.65	1.04		
T18-2013	3200	38.9%	0.31	0.98	1.01	4.5%
T18-2013	6400	21.4%	0.08	0.51	0.74	44.6%
T18-2014	3200	68.2%	1.13	3.60		
T18-2014	6400	50.3%	0.68	4.34	3.97	13.1%
T18-2014	12800	22.9%	0.34	4.39	4.37	0.8%
T18-2015	6400	52.6%	0.47	3.04		
T18-2015	12800	24.0%	0.13	1.62	2.33	43.2%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-2016	1600	56.5%	0.79	1.27		
T18-2016	3200	33.0%	0.45	1.43	1.35	8.7%
T18-2017	1600	69.3%	0.82	1.31		
T18-2017	3200	46.1%	0.39	1.25	1.28	3.2%
T18-2017	6400	29.7%	0.20	1.29	1.27	2.3%
T18-2018	3200	62.0%	0.92	2.95		
T18-2018	6400	32.9%	0.45	2.86	2.90	2.3%
T18-2019	3200	65.6%	0.71	2.28		
T18-2019	6400	44.6%	0.37	2.37	2.33	2.8%
T18-2019	12800	22.3%	0.10	1.25	1.81	43.7%
T18-2031a	1600	53.8%	0.36	0.58		
T18-2031a	3200	23.2%	0.14	0.43	0.51	21.0%
T18-2031b	1600	58.5%	0.38	0.60		
T18-2031b	3200	30.3%	0.17	0.56	0.58	5.8%
T18-2031b	6400	20.1%	0.12	0.78	0.67	23.8%
T18-2032a	800	57.6%	0.41	0.33		
T18-2032a	1600	27.8%	0.16	0.26	0.29	16.5%
T18-2032b	800	59.7%	0.39	0.31		
T18-2032b	1600	35.7%	0.20	0.33	0.32	3.2%
T18-2032b	3200	21.2%	0.13	0.41	0.37	15.8%
T18-2272	3200	69.2%	1.92	6.16		
T18-2272	6400	37.0%	0.71	4.54	5.35	21.4%
T18-2273	3200	61.3%	1.36	4.37		
T18-2273	6400	35.2%	0.70	4.49	4.43	1.9%
T18-2351	3200	66.8%	1.09	3.48		
T18-2351	6400	39.9%	0.52	3.32	3.40	3.2%
T18-2352	3200	64.6%	1.20	3.85		
T18-2352	6400	38.7%	0.61	3.93	3.89	1.6%
T18-2353	3200	56.4%	0.80	2.57		
T18-2353	6400	32.9%	0.42	2.71	2.64	3.8%
T18-2354	3200	51.4%	0.83	2.66		
T18-2354	6400	31.3%	0.52	3.30	2.98	15.3%
T18-2355	800	65.3%	1.04	0.83		
T18-2355	1600	52.4%	0.72	1.16	0.99	23.2%
T18-2355	3200	25.9%	0.33	1.07	1.11	5.7%
T18-2356	800	67.6%	1.33	1.07		
T18-2356	1600	40.9%	0.65	1.03	1.05	2.2%
T18-2360	12800	69.3%	1.19	15.22		
T18-2360	25600	39.0%	0.54	13.71	14.46	7.4%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-2362	6400	66.0%	0.45	2.91		
T18-2362	12800	37.9%	0.20	2.61	2.76	7.6%
T18-2363	6400	69.3%	0.52	3.35		
T18-2363	12800	41.5%	0.26	3.29	3.32	1.3%
T18-2389	6400	61.3%	0.58	3.71		
T18-2389	12800	35.7%	0.26	3.37	3.54	6.8%
T18-2390	6400	65.1%	0.60	3.87		
T18-2390	12800	36.8%	0.26	3.34	3.60	10.5%
T18-2391	6400	54.6%	0.47	2.98		
T18-2391	12800	29.6%	0.22	2.76	2.87	5.5%
T18-2392	6400	62.5%	0.55	3.54		
T18-2392	12800	37.2%	0.26	3.37	3.46	3.5%
T18-2393	12800	50.1%	0.41	5.21		
T18-2393	25600	24.8%	0.18	4.66	4.93	7.8%
T18-2394	12800	55.2%	0.44	5.65		
T18-2394	25600	24.9%	0.18	4.57	5.11	14.9%
T18-2395	1600	67.5%	0.72	1.15		
T18-2395	3200	44.7%	0.35	1.11	1.13	3.0%
T18-2395	6400	21.8%	0.16	1.04	1.07	4.6%
T18-2396	1600	69.4%	0.71	1.13		
T18-2396	3200	56.2%	0.45	1.45	1.29	17.7%
T18-2396	6400	28.2%	0.20	1.28	1.37	9.2%
T18-2397	3200	53.7%	0.45	1.45		
T18-2397	6400	29.1%	0.21	1.36	1.41	4.5%
T18-2398	3200	64.5%	0.59	1.89		
T18-2398	6400	44.9%	0.33	2.10	2.00	7.3%
T18-2398	12800	21.5%	0.16	2.02	2.06	2.7%
T18-2406	400	54.0%	0.34	0.14		
T18-2406	800	29.6%	0.17	0.13	0.13	2.2%
T18-2407	400	55.8%	0.34	0.14		
T18-2407	800	29.3%	0.17	0.13	0.13	2.1%
T18-2412	6400	66.7%	0.61	3.91		
T18-2412	12800	43.2%	0.25	3.16	3.53	15.0%
T18-2413	6400	65.6%	0.50	3.19		
T18-2413	12800	32.9%	0.19	2.46	2.82	18.3%
T18-2414	3200	63.6%	0.54	1.72		
T18-2414	6400	39.1%	0.21	1.36	1.54	16.5%
T18-2415	3200	67.7%	0.54	1.72		
T18-2415	6400	43.7%	0.26	1.66	1.69	2.3%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T18-2416	12800	58.8%	0.44	5.65		
T18-2416	25600	27.7%	0.14	3.53	4.59	32.8%
T18-2485	6400	50.5%	0.37	2.37		
T18-2485	12800	22.5%	0.16	2.09	2.23	8.8%
T18-2486	6400	53.0%	0.53	3.39		
T18-2486	12800	28.0%	0.28	3.59	3.49	3.9%
T18-2566	1600	66.9%	1.35	2.15		
T18-2566	3200	55.9%	0.89	2.85	2.50	19.6%
T18-2566	6400	25.6%	0.36	2.28	2.56	15.7%
T18-2567	3200	62.5%	0.90	2.88		
T18-2567	6400	28.3%	0.40	2.56	2.72	8.4%
T18-2569	1600	68.4%	1.12	1.79		
T18-2569	3200	45.1%	0.58	1.85	1.82	2.2%
T18-2571	1600	62.6%	0.90	1.44		
T18-2571	3200	26.0%	0.38	1.21	1.33	12.2%
T18-2573	1600	66.5%	1.04	1.66		
T18-2573	3200	39.8%	0.52	1.65	1.65	0.4%
T19-0049	6400	62.9%	0.82	5.27		
T19-0049	12800	39.6%	0.40	5.13	5.20	2.0%
T19-0050	6400	66.9%	1.06	6.82		
T19-0050	12800	40.2%	0.44	5.65	6.23	13.2%
T19-0051	12800	52.6%	0.59	7.54		
T19-0051	25600	25.7%	0.25	6.48	7.01	10.6%
T19-0052	12800	50.7%	0.60	7.68		
T19-0052	25600	20.4%	0.23	6.01	6.85	17.3%
T19-0053	1600	67.8%	1.19	1.90		
T19-0053	3200	56.4%	0.75	2.41	2.15	16.8%
T19-0053	6400	25.7%	0.31	2.00	2.20	13.3%
T19-0054	3200	63.1%	1.03	3.29		
T19-0054	6400	38.3%	0.49	3.15	3.22	2.9%
T19-0055	3200	71.7%	1.49	4.78		
T19-0055	6400	45.7%	0.55	3.49	4.14	22.0%
T19-0055	12800	22.4%	0.28	3.59	3.54	1.8%
T19-0056	3200	66.3%	1.19	3.80		
T19-0056	6400	44.9%	0.58	3.72	3.76	1.5%
T19-0057	400	66.3%	0.93	0.37		
T19-0057	800	45.6%	0.49	0.39	0.38	2.9%
T19-0058	800	58.7%	0.68	0.54		
T19-0058	1600	29.9%	0.32	0.51	0.53	4.0%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T19-0059	1600	65.1%	0.90	1.43		
T19-0059	3200	47.6%	0.52	1.65	1.54	9.9%
T19-0059	6400	21.8%	0.22	1.43	1.54	9.9%
T19-0060	1600	67.6%	0.92	1.47		
T19-0060	3200	48.5%	0.51	1.64	1.56	8.1%
T19-0060	6400	23.5%	0.27	1.71	1.68	2.8%
T19-0061	6400	62.2%	0.90	5.77		
T19-0061	12800	42.8%	0.47	6.00	5.89	2.8%
T19-0062	6400	67.3%	0.89	5.69		
T19-0062	12800	43.5%	0.43	5.49	5.59	2.5%
T19-0063	12800	56.0%	0.71	9.11		
T19-0063	25600	23.6%	0.26	6.64	7.87	22.1%
T19-0064	12800	60.9%	0.70	8.92		
T19-0064	25600	29.3%	0.30	7.65	8.29	10.9%
T19-0065	12800	58.8%	0.79	10.08		
T19-0065	25600	25.1%	0.27	7.01	8.55	25.4%
T19-0066	12800	60.8%	0.70	8.90		
T19-0066	25600	26.7%	0.28	7.12	8.01	15.7%
T19-0067	3200	68.9%	1.27	4.07		
T19-0067	6400	43.6%	0.48	3.07	3.57	19.9%
T19-0067	12800	21.2%	0.24	3.04	3.06	0.6%
T19-0068	6400	52.2%	0.54	3.44		
T19-0068	12800	23.2%	0.25	3.22	3.33	4.6%
T19-0070	3200	58.4%	0.64	2.06		
T19-0070	6400	28.9%	0.30	1.89	1.97	6.0%
T19-0159	1600	54.7%	1.14	1.83		
T19-0159	3200	43.1%	0.79	2.53	2.18	22.9%
T19-0159	6400	21.9%	0.40	2.58	2.56	1.3%
T19-0160	1600	67.0%	1.43	2.29		
T19-0160	3200	48.0%	0.74	2.38	2.34	3.0%
T19-0185	400	63.3%	0.92	0.37		
T19-0185	800	40.1%	0.48	0.38	0.38	2.9%
T19-0187	1600	61.0%	0.86	1.37		
T19-0187	3200	32.6%	0.39	1.25	1.31	6.6%
T19-0191	3200	49.2%	0.89	2.86		
T19-0191	6400	28.6%	0.51	3.23	3.05	8.6%
T19-0192	3200	62.6%	0.94	3.01		
T19-0192	6400	36.6%	0.38	2.41	2.71	15.7%
T19-0192	12800	22.5%	0.21	2.68	2.55	7.6%

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T19-0193	1600	67.7%	1.81	2.90		
T19-0193	3200	43.3%	0.88	2.82	2.86	1.9%
T19-0193	6400	21.0%	0.44	2.80	2.81	0.5%
T19-0194	1600	62.4%	1.32	2.12		
T19-0194	3200	54.0%	1.04	3.31	2.72	31.1%
T19-0194	6400	22.7%	0.37	2.34	2.83	24.4%
T19-0194b	1600	66.2%	1.40	2.24		
T19-0194b	3200	46.1%	0.76	2.44	2.34	5.8%
T19-0196	3200	42.1%	0.59	1.89		
T19-0196	6400	24.0%	0.33	2.14	2.02	8.7%
T19-0197	6400	62.0%	1.05	6.75		
T19-0197	12800	52.0%	0.80	10.22	8.49	28.9%
T19-0198	12800	49.4%	0.74	9.44		
T19-0198	25600	21.2%	0.30	7.72	8.58	14.2%
T19-0199	12800	50.8%	0.77	9.92		
T19-0199	25600	21.5%	0.33	8.45	9.18	11.3%
T19-0201	6400	40.5%	0.60	3.82		
T19-0201	12800	22.3%	0.34	4.35	4.08	9.3%
T19-0202	3200	62.2%	1.12	3.58		
T19-0202	6400	48.2%	0.71	4.55	4.06	16.9%
T19-0202	12800	20.5%	0.29	3.77	4.16	13.3%
T19-0244	12800	52.0%	0.65	8.34		
T19-0244	25600	20.4%	0.25	6.32	7.33	19.5%
T19-0245	12800	60.4%	0.88	11.30		
T19-0245	25600	24.5%	0.30	7.57	9.43	27.9%
T19-0246	12800	62.3%	0.89	11.37		
T19-0246	25600	27.3%	0.32	8.13	9.75	23.5%
T19-0247	1600	66.7%	1.09	1.74		
T19-0247	3200	42.0%	0.51	1.63	1.69	4.5%
T19-0247	6400	20.6%	0.26	1.64	1.64	0.1%
T19-0267	1600	62.8%	1.11	1.78		
T19-0267	3200	33.7%	0.54	1.74	1.76	1.6%
T19-0268	1600	66.5%	0.97	1.56		
T19-0268	3200	39.5%	0.53	1.68	1.62	5.5%
T19-0316	12800	58.5%	0.43	5.45		
T19-0316	25600	28.8%	0.17	4.36	4.90	15.8%
T19-0317	12800	38.4%	0.43	5.56		
T19-0317	25600	23.1%	0.29	7.54	6.55	21.3%
T19-0318	12800	44.8%	0.47	6.07		

T19-0318	25600	22.1%	0.28	7.04	6.56	10.5%
Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T19-0319	12800	44.8%	0.45	5.73		
T19-0319	25600	20.2%	0.20	5.18	5.46	7.2%
T19-0320	12800	38.2%	0.43	5.55		
T19-0320	25600	22.0%	0.27	6.96	6.26	15.9%
T19-0321	12800	47.0%	0.55	7.10		
T19-0321	25600	23.2%	0.27	6.80	6.95	3.0%
T19-0323	1600	51.2%	0.62	0.98		
T19-0323	3200	28.7%	0.33	1.06	1.02	4.9%
T19-0324	1600	53.7%	0.69	1.11		
T19-0324	3200	27.1%	0.33	1.06	1.08	3.2%
T19-0450	6400	65.7%	0.70	4.49		
T19-0450	12800	32.4%	0.33	4.29	4.39	3.2%
T19-0451	6400	65.9%	0.70	4.51		
T19-0451	12800	35.5%	0.36	4.61	4.56	1.6%
T19-0452	1600	72.7%	0.85	1.37		
T19-0452	3200	36.7%	0.37	1.18	1.27	10.2%
T19-0459	12800	47.5%	0.49	6.21		
T19-0459	25600	22.8%	0.26	6.77	6.49	6.0%
T19-0460	6400	69.2%	0.77	4.92		
T19-0460	12800	33.1%	0.33	4.22	4.57	10.9%
T19-0461	6400	60.7%	0.65	4.17		
T19-0461	12800	24.4%	0.28	3.56	3.86	11.2%
T19-0462	6400	58.5%	0.59	3.76		
T19-0462	12800	22.3%	0.24	3.09	3.42	13.8%
T19-0463	1600	56.1%	0.59	0.94		
T19-0463	3200	22.2%	0.26	0.83	0.88	8.8%
T19-0464	1600	60.5%	0.61	0.98		
T19-0464	3200	28.3%	0.29	0.93	0.96	4.2%
T19-0465	6400	56.8%	0.56	3.55		
T19-0465	12800	25.0%	0.28	3.61	3.58	1.0%
T19-0469	6400	67.1%	1.14	7.27		
T19-0469	12800	43.0%	0.55	7.07	7.17	2.0%
T19-0578	6400	69.8%	1.02	6.55		
T19-0578	12800	41.9%	0.47	5.98	6.27	6.4%
T19-0603	12800	49.9%	0.60	7.68		
T19-0603	25600	20.3%	0.25	6.50	7.09	11.8%
T19-0605	3200	51.9%	0.63	2.02		
T19-0605	6400	21.5%	0.27	1.70	1.86	11.9%
T19-0645	3200	65.0%	1.01	3.24		

Sample ID	Assay Dilution	% inhibition [1-(A/Amax)]	Concentration (ng PbTx-3 eq/ml)	Sample ppm corrected for dilution	Mean ppm	%CV of adjacent dilutions
T19-0645	6400	33.0%	0.48	3.10	3.17	3.1%
T19-0646	3200	68.5%	1.07	3.42		
T19-0646	6400	41.7%	0.47	3.02	3.22	8.6%
T19-0647	6400	65.6%	1.03	6.59		
T19-0647	12800	27.9%	0.43	5.47	6.03	13.1%
T19-0648	6400	67.4%	1.02	6.55		
T19-0648	12800	40.4%	0.46	5.83	6.19	8.2%
T19-0691	1600	60.4%	0.66	1.05		
T19-0691	3200	28.6%	0.30	0.97	1.01	5.5%
T19-0693	12800	53.6%	0.56	7.11		
T19-0693	25600	22.8%	0.26	6.63	6.87	4.9%
T19-0694	12800	51.2%	0.50	6.43		
T19-0694	25600	22.5%	0.23	5.76	6.10	7.7%
T19-0695	6400	69.9%	0.87	5.55		
T19-0695	12800	36.5%	0.37	4.76	5.15	10.9%
T19-0814	12800	46.8%	0.52	6.71		
T19-0814	25600	24.7%	0.30	7.78	7.25	10.4%
T19-0816	12800	51.0%	0.51	6.53		
T19-0816	25600	24.9%	0.25	6.37	6.45	1.7%
T19-0817	12800	52.5%	0.52	6.62		
T19-0817	25600	20.0%	0.20	5.10	5.86	18.4%
T19-0818	3200	53.8%	0.64	2.05		
T19-0818	6400	27.2%	0.32	2.05	2.05	0.2%
T19-0819	3200	65.2%	0.86	2.75		
T19-0819	6400	30.7%	0.34	2.18	2.46	16.5%
T19-0821	6400	59.1%	0.68	4.37		
T19-0821	12800	26.3%	0.27	3.50	3.94	15.6%
T19-0822	12800	49.3%	0.52	6.69		
T19-0822	25600	20.6%	0.22	5.74	6.21	10.9%
T19-0823	12800	49.8%	0.53	6.78		
T19-0823	25600	23.7%	0.25	6.42	6.60	3.9%