Title: Asparaginase Ammonia Assay for Specific Activity

Author/Date: [redacted]

Approvals/Date: [redacted]

SOP Reference: 22702, 22100, 21531	Supersedes: Revision 00

Purpose: This SOP describes the measurement of L-asparaginase enzymatic activity with the ammonia assay using Nessler's reagent in a 96-well plate format. Ammonia is released and measured after reacting with Nessler's reagent by the absorbance at 405 nm when L-asparagine (substrate) is mixed with L-asparaginase (enzyme). The enzyme and substrate are mixed at 37ºC and the reaction is stopped when cold TCA is added. The reaction mixture of enzyme substrate and TCA is then added to a plate containing Nessler's reagent (mercuric iodide mixture). After the plate is developed, it is read by the plate reader. Ammonium sulfate is used as the source of the ammonia for the standard curve. The activity is measured in micromoles of ammonia per unit time per mg of asparaginase.

Scope: This procedure applies to BDP personnel responsible for determining enzyme activity of Asparaginase by the ammonia assay described in this procedure.

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5. Asparaginase Ammonia Assay Sample Preparation, Form 22150-01

1.0 Authority and Responsibility

1.1 The Director, Biopharmaceutical Quality Control (BQC) has the authority to define this procedure.

1.2 BQC personnel are responsible for training laboratory personnel on this procedure and documenting this training to Biopharmaceutical Quality Assurance (BQA).

1.3 BQC is responsible for the implementation of this procedure.

1.4 BQA is responsible for quality oversight of this procedure.

2.0 Materials and Equipment

2.1 Nessler's Reagent Solution (BDP PN 30353), or equivalent approved BDP PN.

2.2 Ammonium Sulfate (BDP PN 10019) or equivalent approved BDP PN.

2.3 Sodium Phosphate, Monobasic, Monohydrate crystal (BDP PN 10057) or equivalent approved BDP PN.

2.4 5% (w/v) Trichloroacetic acid (TCA) (BDP PN 30359).

2.5 L-Asparagine, Monohydrate (BPN PN 10320).

2.6 Bovine Serum Albumin (BSA) (BDP PN 30222).

2.7 10N Sodium Hydroxide (BDP PN 10105).

2.8 [redacted] Reference Standard Lot [redacted] or equivalent.

2.9 Milli-Q H_2O, Direct Q H_2O, or reagent grade.

2.10 Sterile 0.22 micron filter device (BDP PN 20194).

2.11 Incubator set to 37°C.

2.12 Calibrated pH Meter.

2.13 Nalgene bottles, 1L (BDP PN 20160), 250 mL (BDP PN 20161), 125 mL (BDP PN 20159).

2.14 Graduated cylinder, 1L.

2.15 Water Bath set to 37°C.

2.16 Two calibrated thermometers, one for the incubator and one for the water bath.

2.17 Plastic rack for holding 50 mL conical tube in water bath.

2.18 Labsystems iEMS Microtiter Plate Reader MF with Ascent software version 2.4.2, Model Number 1401 (BDP MEF 66160), or equivalent.

2.19 96-well plates (BDP PN 20050).

2.20 Reservoir Trays (BDP PN 20481).

2.21 15 mL conical tubes (BDP PN 20006), 50 mL conical tubes (BDP PN 20140).

2.22 WFI water (BDP PN 30295), or equivalent, approved BDP PN.

2.23 Decrimper for removing vial lids.

2.24 Calibrated Timer.

2.25 Tray containing ice for incubation of TCA plate.

2.26 Corning Cryovials (BDP PN 20007).

2.27 Calibrated Single channel pipettes (L-200, L-1000).

2.28 Calibrated Multichannel pipettes.

2.29 Pipette tips 2-250 µL (BDP PN 21767) and 1-1000 µL (BDP PN 20769) or equivalent approved BDP PN.

2.30 Vortex mixer.

3.0 Procedures

3.1 Preparation of Water Bath and Incubator

3.1.1 Turn on the plate reader.

3.1.2 Turn on the water bath and the incubator.

3.1.3 Set the water bath to 37°C ± 2°C.

3.1.4 Set the incubator to 37°C ± 2°C.

3.1.5 Place a calibrated thermometer in the incubator.

3.1.6 Place a rack for holding 50 mL conical tubes in the water bath.

3.1.7 Record the temperature of the incubator in the equipment logbook as per ***SOP 21531, Equipment Logs***.

3.1.8 Label a 50 mL conical tube H_2O and fill with 20 mL of Milli-Q or Direct-Q water. Place a thermometer in the tube and place the tube in the water bath.

3.1.9 In a second 50 mL conical tube, place 20 mL of L-asparagine and place in the water bath. See step 3.2.5.

3.1.10 Incubate the L-asparagine and water tubes at 37ºC ± 2°C for 5-10 minutes.

3.2 Preparation of Buffers, Solutions and W. Asparaginase Reference Standard

NOTE: All solutions must be given a log number and recorded in the QC Solutions logbook. Label the solution bottles with the log number, initials, date prepared, and expiration date. Refer to ***SOP 22702, Solutions Used in BQC***.

3.2.1 0.1M Sodium Phosphate pH 7.0 (Buffer is stable for 6 months at room temperature).

3.2.1.1 To prepare 1 liter of buffer add 13.8 grams of sodium phosphate to a 1L graduated bottle. Add 800 mL of Milli-Q, or Direct-Q H_2O. Stir until visually clear.

3.2.1.2 Measure pH with a calibrated pH meter. Adjust the pH to 7.0 with 10 N Sodium Hydroxide. Add Milli-Q or Direct-Q water to achieve a final volume of 1L.

3.2.1.3 Sterile filter buffer with 0.22 micron filter.

3.2.2 10 mM Sodium Phosphate pH 7.0 (Buffer is stable for 7 days at room temperature).

In a 1L graduated cylinder, add 100 mL of 0.1M Sodium phosphate pH 7.0 and 900 mL of Milli-Q or Direct-Q water. Stir until visually clear.

3.2.3 100 mM Ammonium Sulfate (Stable for 2 days at 2-8ºC)

Weigh 1.32 grams of Ammonium sulfate. Add 10 mM Sodium phosphate pH 7.0 to achieve a final volume of 100 mL. Stir until visually clear.

3.2.4 1 mg/mL BSA in 10 mM Sodium phosphate (Stable for 5 days at 2-8ºC).

3.2.4.1 Weigh 200 mg BSA. Add 10 mM Sodium phosphate pH 7.0 achieve a final volume of 200 mL. Stir until visually clear.

3.2.4.2 Transfer 15 mL to a 50 mL conical tube and place on ice.

3.2.5 0.08M L-asparagine with 1 mg/mL BSA (Stable for 2 days at 2-8ºC)

3.2.5.1 Weigh 1.2 grams of L-asparagine. Add 10 mM Sodium phosphate pH 7.0 with 1 mg/mL BSA to achieve a final volume of 100 mL.

3.2.5.2 Vortex 15-20 minutes or until all solid material is dissolved.

3.2.6 Preparation of [redacted] Reference Standard

3.2.6.1 Reference Standard Stock Aliquot Preparation

- Thaw a vial of lyophilized [redacted] reference standard at room temperature for 10-20 minutes in a Biological Safety Cabinet.
- Remove the lid using a decrimper, and add 1 mL of WFI water.

- Pipet up and down until solution is visually clear. **Do Not Vortex.**
- Store as 50 µL aliquots at ≤ -70°C.

3.2.6.2 Reference Standard Dilution

NOTE: When using a frozen aliquot, thaw at room temperature no more than 30 minutes prior to starting enzymatic reaction. Once completely thawed, store on ice until starting enzymatic reaction. Record dilutions on Form 22150-01 (Attachment 5).

- Dilute [redacted] Reference Standard **30-fold** (1:30) by adding a 33.3 µL [redacted] to 966.7 µL of ice cold 1 mg/mL BSA in 10 mM Sodium phosphate (this can be prepared in a 2 mL cyrovial).
- Dilute [redacted] Reference Standard **80-fold** (1:80) by adding 25 µL of the 30-fold dilution to 1975 µL of ice cold 1 mg/mL BSA in 10 mM Sodium phosphate (this can be prepared in a 2 mL cryovial).

3.3 Preparation of 96-well plates: TCA Plate, Nessler Plate, Ammonia Standard Plate, and Reaction Plate

3.3.1 TCA Plate

Label a 96-well plate “TCA Plate.” In each well, pipette 30 µL of 5% TCA. Place the plate in the ice tray.

3.3.2 Nessler’s Plate

Label a second 96-well plate “Nessler plate.” Prepare 30 mL of Nessler’s Reagent by adding 3 mL of Nessler’s Reagent to a 50 mL conical tube containing 27 mL Milli-Q or Direct-Q H_2O and vortex vigorously for 10-20 seconds. Transfer solution to a reservoir tray. Pipette 200 µL into each well of the 96-well plate. Hold at room temperature.

3.3.3 Preparation of Ammonia Standard Plate

3.3.3.1 Label a series of 15 mL conical tubes or 2 mL cyrovials 64, 32, 16, 14, 12, 8, 6, 4, 2, 0 µmole ammonia. Prepare a series of standards according to the table below.

3.3.3.2 Add 1 mL of 1 mg/mL BSA in 10 mM Sodium phosphate buffer to a 15 mL conical tube or 2 mL cryovial.

3.3.3.3 Next, add the appropriate amount of water according to the table.

3.3.3.4 Finally, add the appropriate volume of 100 mM Ammonium sulfate.

3.3.3.5 Place the lid on each 15 mL conical tube or 2 mL cryovial and vortex for 10 – 20 seconds.

Table of Ammonia Standards

µmoles of Ammonia	1 mg/mL BSA in 10 mM sodium phosphate (mL)	WFI Water	100 mM Ammonium sulfate (µL)
0 (blank)	1.0 mL	1.0 mL	0
64	1.0 mL	680 µL	320 µL
32	1.0 mL	840 µL	160 µL
16	1.0 mL	920 µL	80 µL
14	1.0 mL	930 µL	70 µL
12	1.0 mL	940 µL	60 µL
8	1.0 mL	960 µL	40 µL
6	1.0 mL	970 µL	30 µL
4	1.0 mL	980 µL	20 µL
2	1.0 mL	990 µL	10 µL

3.3.3.6 Prepare a 96-well plate with the 0, 2, 4, 6, 8, 12, 14, 16, 32, 64 Ammonia standards. Label the plate “Standards.” Pipette 200 µL of each standard to the appropriate wells. Store at room temperature. See Ammonia Standard Plate below.

Ammonia Standards Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F	0 µmole standard			16 µmole standard			8 µmole standard			2 µmole standard		
G	64 µmole standard			14 µmole standard			6 µmole standard			Empty		
H	32 µmole standard			12 µmole standard			4 µmole standard			Empty		

3.3.4 Reaction Plate

3.3.4.1 Label a 96-well plate "Reaction Plate." Transfer 1 mg/mL BSA 10 mM sodium phosphate buffer into a reservoir tray.

3.3.4.2 Pipet 100 µL to wells 1-3 of Row B (negative control).

3.3.4.3 Pipet 50 µL to wells 4-12 of Row B. Place Reaction Plate in 37ºC ± 2ºC incubator for 5-10 minutes.

3.4 Preparation of [redacted]

NOTE: Asparaginase should be reconstituted (lyophilized samples) or thawed (frozen samples) no more than 30 minutes prior to starting the enzymatic reaction. Asparaginase test samples should be reconstituted or thawed and diluted as per the assay profile. Record dilutions on Form 22150-01 (Attachment 5).

When performing release or stability testing, the measured protein concentration obtained by BQC for the test article should be used rather than the nominal labeled concentration.

3.4.1 Pipet 50 µL of each diluted sample in triplicate to the appropriate wells of Row B. (Example: Transfer [redacted] Reference Standard into wells 4 - 6 of Row B; transfer sample 1 into wells 7 - 9 of Row B; transfer sample 2 into wells 10-12 of Row B). See Reaction Plate below. Place the Reaction Plate in a 37 ± 2°C incubator for 5-10 minutes.

Reaction Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	10 mM Sodium phosphate with BSA 100 µL			[redacted]		Ref. Std. 100 µL	[redacted]	#1 100 µL		[redacted]	#2 100 µL	
C												
D												
E												
F												
G												
H												

3.5 Enzymatic Reaction Time Course in the Incubator

NOTE: During the time course, it is crucial that the reaction plate be removed and returned to the incubator as quickly as possible in order to minimize assay temperature fluctuations.

3.5.1 Remove the reaction plate from the incubator. Transfer 0.08M L-asparagine with BSA to a reservoir tray. Using a multichannel pipette with 12 tips, pipette 100 µL of 0.08 M L-asparagine with BSA into the entire Row B of the reaction plate. It is imperative that a 12-channel pipette is used to ensure the addition of L-asparagine is added to each sample simultaneously.

3.5.2 Pipette up and down several times to thoroughly mix contents.

3.5.3 Return the plate to the incubator and start the timer.

3.6 Stopping the Enzymatic Reaction with Cold TCA

3.6.1 At **5** minutes, remove the reaction plate from the incubator. Using the multichannel pipette with 12 tips, transfer 30 µL from Row B of the reaction plate into Row A of the TCA plate which is located on ice. Pipet up and down to 2-3 times. Return the plate to the incubator.

3.6.2 At **10** minutes, remove the reaction plate from the incubator. Using the multichannel pipette with 12 tips, transfer 30 µL from Row B of the reaction plate into Row B of the TCA plate, which is located on ice. Pipet up and down 2-3 times. Return the plate to the incubator.

3.6.3 At **20** minutes, remove the reaction plate from the incubator. Using the multichannel pipette with 12 tips, transfer 30 µL from Row B of the reaction plate into Row C of the TCA plate, which is located on ice. Pipet up and down 2-3 times. Return the plate to the incubator.

3.6.4 At **30** minutes, remove the reaction plate from the incubator. Using the multichannel pipette with 12 tips, transfer 30 µL from Row B of the reaction plate into Row D of the TCA plate, which is located on ice. Pipet up and down 2-3 times. Return the plate to the incubator.

3.6.5 At **40** minutes, remove the reaction plate from the incubator. Using the multichannel pipette with 12 tips, transfer 30 µL from Row B of the reaction plate into Row E of the TCA plate, which is located on ice. Pipet up and down 2-3 times. (See table below)

TCA Plate Containing Completed Time Course

	1	2	3	4	5	6	7	8	9	10	11	12
A	10 mM Na Phos			Ref. BSA - 5 min		#1	[redacted] STD. - 5 min		#2	[redacted] 5 min		
B	10 mM Na Phos			BSA - 10 min			[redacted] REF. STD. – 10 min		#1	[redacted] 10 min		#2
C	10 mM Na Phos			BSA - 20 min			[redacted] REF. STD. – 20 min		#1	[redacted] 20 min		#2
D	10 mM Na Phos			BSA - 30 min			[redacted] REF. STD. – 30 min		#1	[redacted] 30 min		#2
E	10 mM Na Phos			BSA - 40 min			[redacted] REF. STD. – 40 min		#1	[redacted] 40 min		#2
F												
G												
H												

3.7 Adding Ammonia Standards to the TCA Plate

3.7.1 Between the 30 and 40-minute time points, transfer standards to the TCA plate. Transfer 30 µL from Rows F through H of the Ammonia standards plate (see step 3.3.3.6) to Rows F through H of the TCA plate. Mix well by pipetting up and down 2-3 times.

3.7.2 Wells G 10-12 and H 10-12 should remain empty. See the diagram below for the identity of the sample in each well of the TCA plate.

TCA Plate with Time Course and Standards

	1	2	3	4	5	6	7	8	9	10	11	12
	A Phos BSA - 5 min			[redacted] Ref. STD. - 5 min			[redacted] 5 min			[redacted] #2 - 5 min		
B	10 mM Na Phos BSA - 10 min			[redacted] REF. STD. – 10 min			[redacted] #1 10 min			[redacted] #2 10 min		
C	10 mM Na Phos BSA - 20 min			[redacted] REF. STD. – 20 min			[redacted] #1 – 20 min			[redacted] #2 20 min		
D	10 mM Na Phos BSA - 30 min			[redacted] REF. STD. – 30 min			[redacted] #1 – 30 min			[redacted] #2 30 min		
E	10 mM Na Phos BSA - 40 min			[redacted] REF. STD. – 40 min			[redacted] #1 40 min			[redacted] #2 40 min		
F	0 umole STD			16 umole STD			8 umole STD			2 umoleSTD		
G	64 umole STD			14 umole STD			6 umole STD			Empty		
H	32 umole STD			12 umole STD			4 umole STD			Empty		

3.8 Transfer the contents of the TCA plate to the Nessler Plate

3.8.1 Using a multichannel pipette with 12 tips, transfer 20 µL from Row A of the TCA plate to Row A of the Nessler plate. Mix by pipetting up and down 2-3 times.

3.8.2 Transfer 20 µL from Row B of the TCA plate to Row B of the Nessler plate. Mix by pipetting up and 2-3 times.

3.8.3 Repeat transfer for rows C through H.

3.8.4 Read the absorbance of the Nessler plate at 405 nm. The plate should be read within 6-8 minutes of the addition of the samples to the Nessler plate.

3.9 Reading the Plate

3.9.1 At the PC Plate Reader workstation, open the Ascent software.

3.9.2 Open the file containing the template. Refer to ***SOP 22100, Operation of Labsystems iEMS Microtiter Plate Reader/Dispenser,*** to label the template and read the plate.

3.9.3 Save the Data using the QC request number as the file name (Example: QC12345.see) to the Ascent folder.

3.9.4 Print Ascent Asparaginase Results and attach it to the QC Test Request.

4.0 Data Analysis, Acceptance Criteria

Paste the Absorbance 405 nm data from the Ascent file into the Asparaginase Microsoft Excel Template.

4.1 Data Analysis

4.1.1 Go to Windows “Start.” Select programs and click on “Microsoft Excel.”

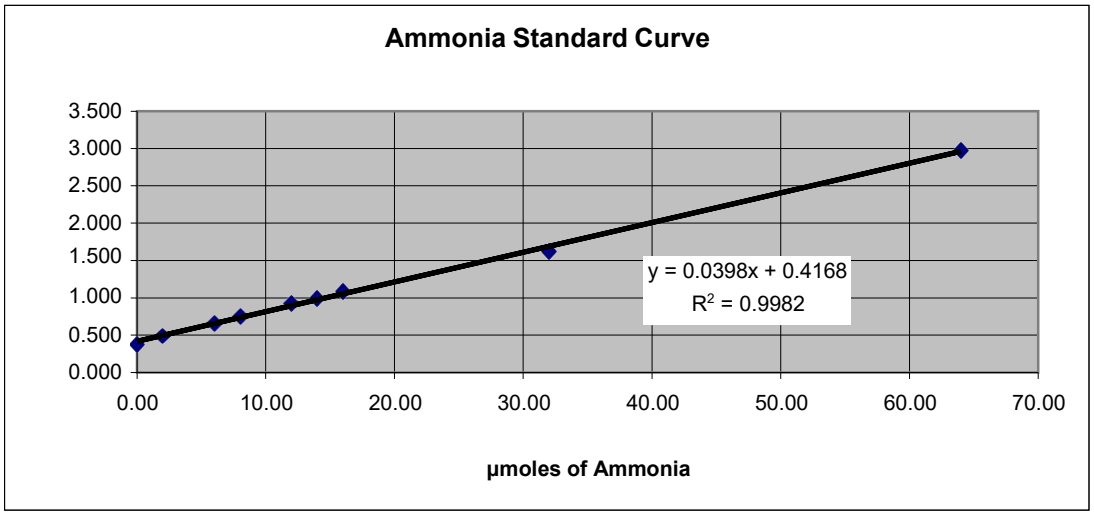
4.1.2 Click on “My Computer” and select “Quality Control on ‘bdpmaster’(I:).”

4.1.3 Open the “Quality Control” folder, then the “QC_Public” folder. Click on the “Asparaginase Results” folder.

4.1.4 Open the Asparaginase Microsoft Excel Template. Fill in top portion of the template with QC request number, Reference standard lot number, sample name, and sample lot number. Copy and paste the Ascent data to the “96-Well Nessler Plate.” Update the “Nessler Plate Well Contents” with appropriate sample and lot number identification (Attachment 1).

4.1.5 The standard curve graph, which is a plot of the mean absorbance at 405 nm versus the Ammonia concentration in µmoles, is plotted automatically. See example graph below.

Standard Curve of Absorbance at 405 nm as a Function of Ammonia Amount



4.1.6 The standard curve equation must be updated manually. Delete the existing equation, right click on the standard curve line, and then select "Format Trendline." Select the "Options tab." Check the "Display Equation on chart box" and the "Display R-squared value on chart box." Click "OK." The standard curve equation is now updated (Attachment 2).

4.1.7 Time point data calculations are generated automatically (Attachment 3).

4.2 Acceptance Criteria for Ammonia Standard Curve and Time Point Assay Data

4.2.1 The slope of the standard curve must be at least 0.0371 and no greater than 0.0569 (0.047 $\pm$ 3 Standard Deviations). The R^2 value must be at least 0.96. A standard curve not meeting these criteria must be repeated.

4.2.2 The R^2 value of the plotted Asparaginase test samples must also be at least 0.96. A standard curve not meeting this criterion must be repeated.

4.2.3 The %CV for each point of the Ammonia Standard Curve must not exceed 25%. One data point may be removed from the standard curve if the %CV exceeds 25%. If more than one point in the standard curve exceeds 25%, the assay must be repeated. The %CV of each calculated time point for the W. Asparaginase reference standard and [redacted] test samples must not exceed 25%. If any of the

values from the Time Point Assay Data exceed 25%, the assay must be repeated.

If the above criteria are not met, the assay must be repeated.

5.0 Calculations and Graphing

5.1 The equation generated from the Ammonia standard curve is used to calculate the mean result for the sample data. In the example shown above, the standard curve equation is y = 0.0398x + 0.4168. To calculate the mean result for each sample time point, solve for **x** and insert the mean absorbance at 405 nm for **y**. **X = (Y - 0.4168)/0.0398.**

5.2 Using Excel, enter the time in one column (5, 10, 20, 30 and 40 minutes) and the calculated mean results in another column (see Attachment 4).

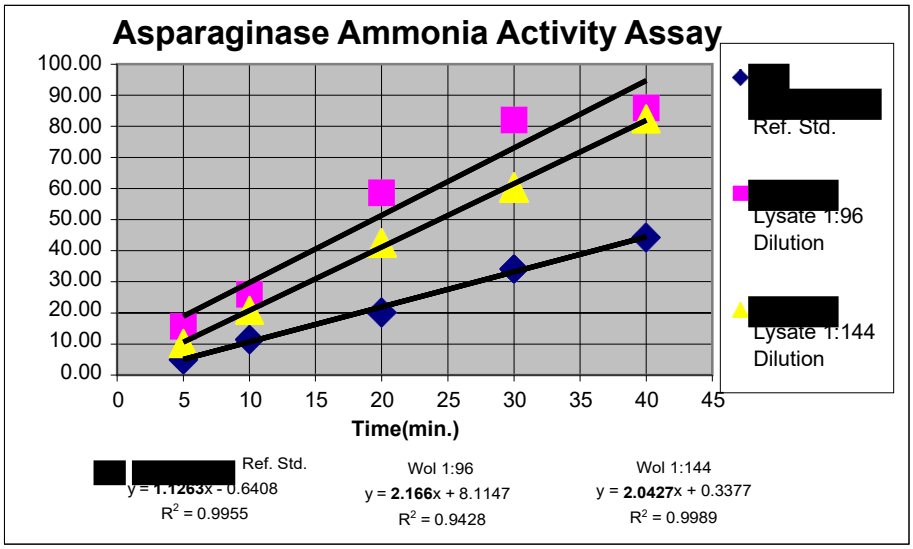
5.3 Enter the data from the buffer blank with BSA (10 mM sodium phosphate with BSA) and the data for the Asparaginase samples (see example below).

Time Course Data

Time (minutes)	Buffer	[redacted] REF. STD.	[redacted] #1	[redacted] #2
5	0.91	4.96	15.67	10.21
10	0.71	11.44	25.90	20.75
20	0.86	20.19	58.53	42.47
30	0.58	34.19	81.99	60.29
40	0.52	44.28	85.91	82.45

5.4 Graph the calculated values for each sample versus time. See the graph below. The resulting equation is used to calculate the activity. The 1st number in the equation (example y = **2.0427**x + 0.337) is the slope of activity as a function of time.

Asparaginase Activity as a Function of Time



5.5 The activity (IU/mL) is calculated by multiplying the slope by the dilution factor. See calculation below.

[redacted] REF. STD	.	SLOPE	X	4800 DILUTION FACTOR	=	ACTIVITY
[redacted] Test Sample		SLOPE	X	? DILUTION FACTOR	=	ACTIVITY

To calculate the activity per milligram of Asparaginase, divide the activity per milliliter by the concentration of the original sample (see **NOTE**, section 3.4). Example: Determined activity = 5,200 IU/mL, original concentration = 25 mg/mL. Divide 5,200 IU/mL by 25 mg/mL = 208 IU/mg. Print the graph with calculated activity (Attachment 4).

6.0 Documentation

6.1 Print Ascent Asparaginase Results and attach to QC Test Request.

6.2 Print Nessler Plate Well Contents with appropriate sample and lot number identification and attach to QC Test Request (Attachment 1).

6.3 Print the Ammonia Standard Curve Data and Graph and attach to QC Test Request (Attachment 2).

6.4 Print Time Point Data Calculations and attach to QC Test Request (Attachment 3).

6.5 Print the Graph with Calculated Activity and attach to QC Test Request (Attachment 4).

6.6 Record the assay preparations and assay specifications on Form 22150-01, Asparaginase Ammonia Assay Sample Preparation (Attachment 5) and attach to QC Test Request.

7.0 References

7.1 **SOP 21531, Equipment Logs**

7.2 **SOP 22702, Solutions Used in BQC**

7.3 **SOP 22100, Operation of Labsystems iEMS Microtiter Plate Reader/ Dispenser**

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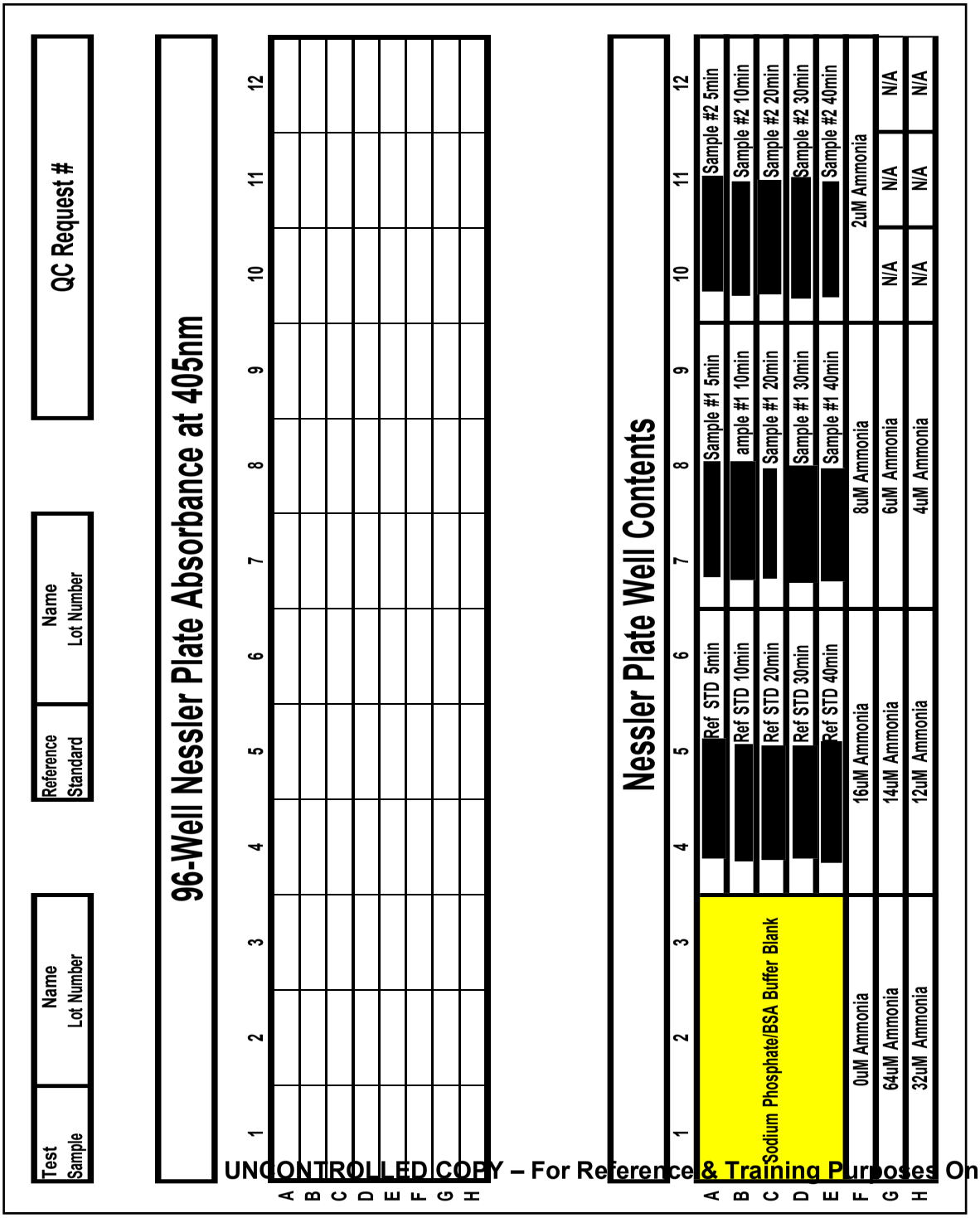
ATTACHMENT 1

Sample Nessler Plate Well Contents



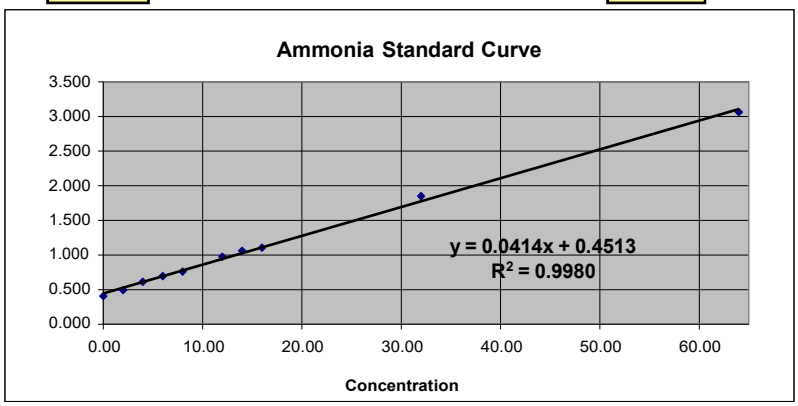
ATTACHMENT 2

Sample Ammonia Standard Curve Data and Graph

	A405nm	Conc.	Result	CV-%
	0.432		-0.27	
	0.396		-1.14	
	0.390		-1.28	
Mean	0.406	0.00	-0.90	20.00%
	0.489		1.10	
	0.486		1.02	
	0.504		1.46	
Mean	0.493	2.00	1.19	19.43%
	0.624		4.34	
	0.606		3.91	
	0.605		3.88	
Mean	0.612	4.00	4.04	6.35%
	0.704		6.26	
	0.694		6.02	
	0.686		5.83	
Mean	0.695	6.00	6.03	3.59%
	0.756		7.51	
	0.761		7.63	
	0.759		7.58	
Mean	0.759	8.00	7.57	0.80%

	A405nm	Conc.	Result	CV-%
	0.978		12.84	
	0.981		12.91	
	0.964		12.50	
Mean	0.974	12.00	12.75	1.71%
	1.066		14.95	
	1.062		14.85	
	1.045		14.45	
Mean	1.058	14.00	14.75	1.82%
	1.107		15.94	
	1.109		15.98	
	1.096		15.67	
Mean	1.104	16.00	15.86	1.06%
	1.844		33.63	
	1.839		33.51	
	1.868		34.21	
Mean	1.850	32.00	33.78	1.10%
	3.086		63.45	
	3.048		62.54	
	3.056		62.73	
Mean	3.063	64.00	62.91	0.76%

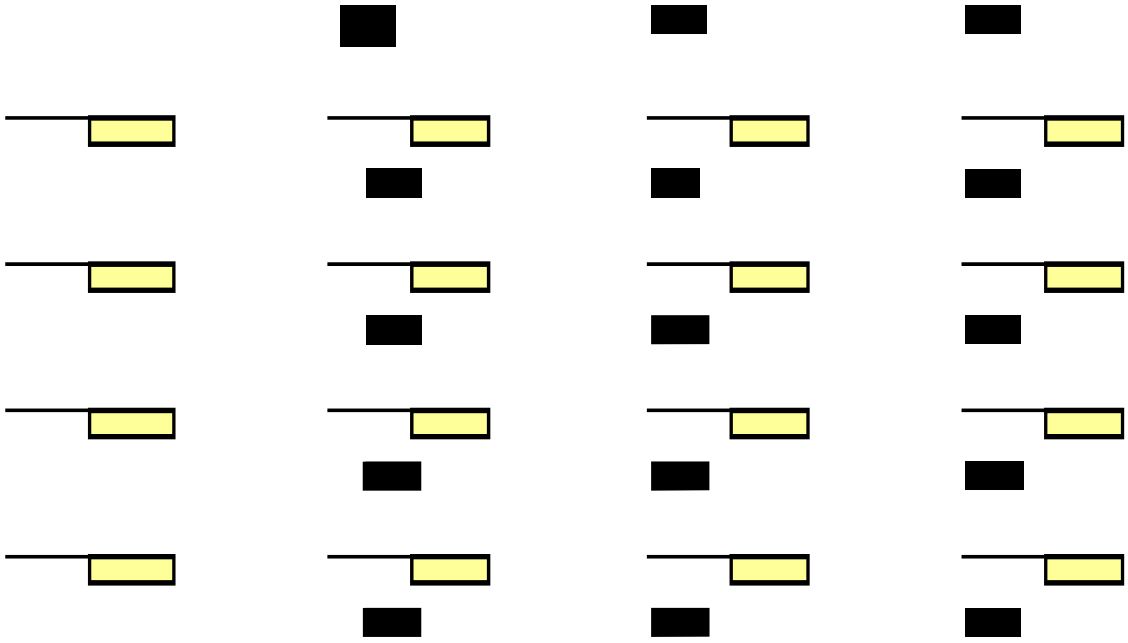
Conc.	Mean
0.00	0.406
2.00	0.493
4.00	0.612
6.00	0.695
8.00	0.759
12.00	0.974
14.00	1.058
16.00	1.104
32.00	1.850
64.00	3.063



Ammonia Standard Curve Data	
Slope	0.0416
Y Intercept	0.4434
R-Squared	0.9979

ATTACHMENT 3

Sample Time Point Data Calculations



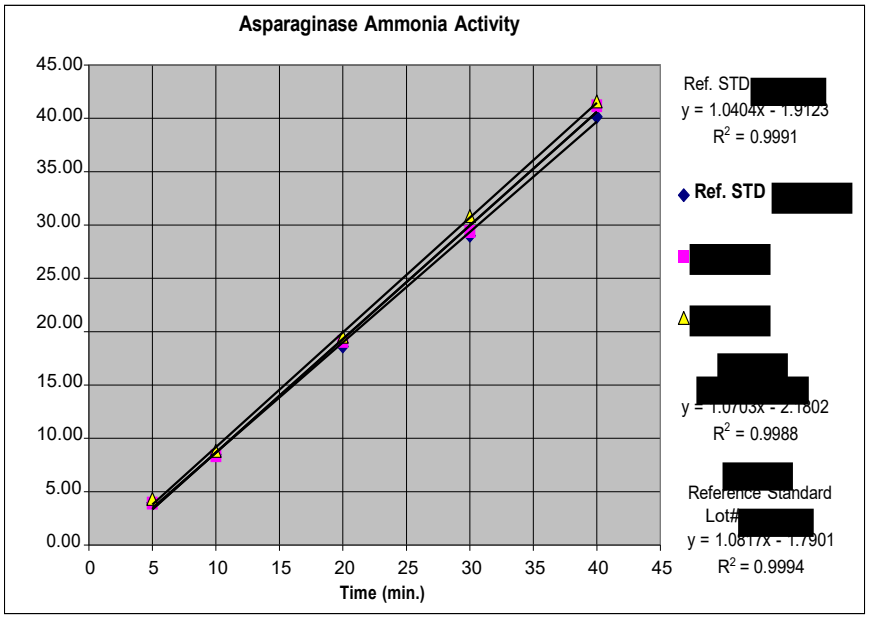
Title: Asparaginase Ammonia Assay for Specific Activity

ATTACHMENT 4

Sample Graph with Calculated Activity

Mean Result Vs. Time(min)			
Time	[redacted] 2011	[redacted]	[redacted]
5	3.95	3.91	4.30
10	8.44	8.36	8.80
20	18.60	19.08	19.52
30	28.98	29.38	30.84
40	40.14	41.17	41.56

Results				
Sample	Slope	Dilution	Activity IU/ml	Activity IU/mg
[redacted]	1.035	4800	4,968.3	198.7
[redacted]	1.065	6640	7,069.9	212.9
[redacted]	1.076	2120	2,281.4	215.2



Analyzed By:_____Date:_____

Reviewed By:_____Date: _____

ATTACHMENT 5

NCI-Frederick
Form No.: 22150-01
SOP No.: 22150
Revision 01:

Asparaginase Ammonia Assay Sample Preparation

QC Number:_____ **Analyst:**_____ **Date:** _____

Diluent:_____ **Lot#**_____ **Exp. Date:** _____

_____

Sample Dilutions

Test Sample #1
Name:_____
Lot #_____
Protein Concentration:_____mg/mL

Initial dilution:_____
Volume of diluent:_____µL Volume of test sample:_____µL

Second dilution:_____
Volume of diluent:_____µL
Volume of initial diluted test sample_____µL

Third Dilution_____
Volume of Diluent:_____µL
Volume of Second Diluent sample_____µL

Test Sample #3
Name:_____
Lot #_____
Protein Concentration:_____mg/mL

Initial dilution: _____
Volume of diluent:_____µL Volume of test sample:_____µL

Second dilution: _____
Volume of diluent:_____µL
Volume of initial diluted test sample_____µL

Third Dilution _____
Volume of Diluent:_____µL
Volume of Second Diluent sample_____µL

Test sample #2
Name:_____
Lot #_____
Protein Concentration:_____mg/mL

Initial dilution:_____
Volume of diluent:_____µL Volume of test sample:_____µL

Second dilution:_____
Volume of diluent:_____µL
Volume of initial diluted test sample_____µL

Third Dilution_____
Volume of Diluent:_____µL
Volume of Second Diluent sample_____µL

Reference Standard
Name:_____
Lot #_____
Protein Concentration:_____mg/mL

Initial dilution: _____
Volume of diluent:_____µL
Volume of test sample:_____µL

Second dilution: _____
Volume of diluent:_____µL
Volume of initial diluted test sample_____µL

Third Dilution _____
Volume of Diluent:_____µL
Volume of Second Diluent sample_____µL

ATTACHMENT 5 (Continued)

NCI-Frederick
Form No.: 22150-01
SOP No.: 22150
Revision 01:

Asparaginase Ammonia Assay Sample Preparation

QC Number:_____ **Analyst:**_____ **Date:** _____

Assay Reagents

5% TCA

Lot#_____ Exp. Date: _____

Nessler's Reagent

Lot#_____ Exp. Date: _____

Equipment

Incubator

MEF#_____Calibration Due Date:_____Initial Temperature:_____Final Temperature: _____

Water Bath

MEF#_____Calibration Due Date:_____Initial Temperature:_____Final Temperature: _____

Plate Reader

MEF#_____ Calibration Due Date: _____

Assay Specifications

Specification	Assay Data
Standard curve must have an R^2 value > 0.96	
Acceptable range of Standard Curve slope: 0.0371 – 0.0569 (0.047 $\pm$ 3 std. dev.)	
Reference Standard must have an R^2 value > 0.96	
Reference Standard activity acceptable range (4511.2 - 6103.4 IU/mL)	
Test sample(s) must have an R^2 value > 0.96	
%CV Ammonia Standard Curve $\leq$ 25%	
%CV Asparaginse Ref. Std. and Test Samples $\leq$ 25%	

Performed by/Date: _____

Reviewed by/Date: _____