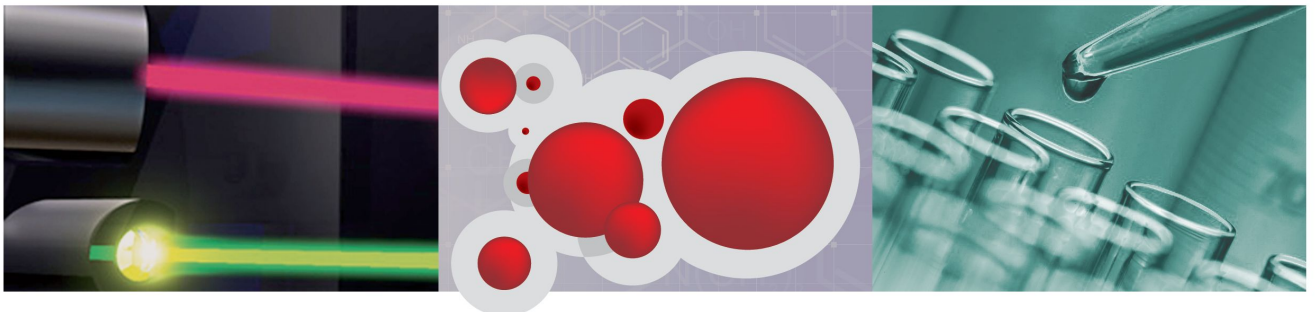


Luminex[®]

xPONENT[®] Protocol Set Up Instructions for Salmonella Serotyping Assay

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xPONENT[®] Protocol Set Up Instructions for Salmonella Serotyping Assay

PN 89-30000-00-422 Rev B

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89-30000-00-187 (Rev B.)

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89-30000-00-403 Rev. A

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Purpose

This document provides the user with instructions for creating the run protocols in xPONENT® 3.1 Software, for use with the Salmonella Serotyping Assay.



Please refer to *xPONENT 3.1 Software Manual* for additional details on software features and navigation.

Setting Up the Protocol for the O Assay

1. From the **Protocols** tab, click on **Create New Protocol**. The **Settings** tab will display. Populate the fields under **Settings** as detailed in [Table O Assay Settings](#). Fields noted with N/A remain blank.

FIGURE 1. **Settings O Assay**

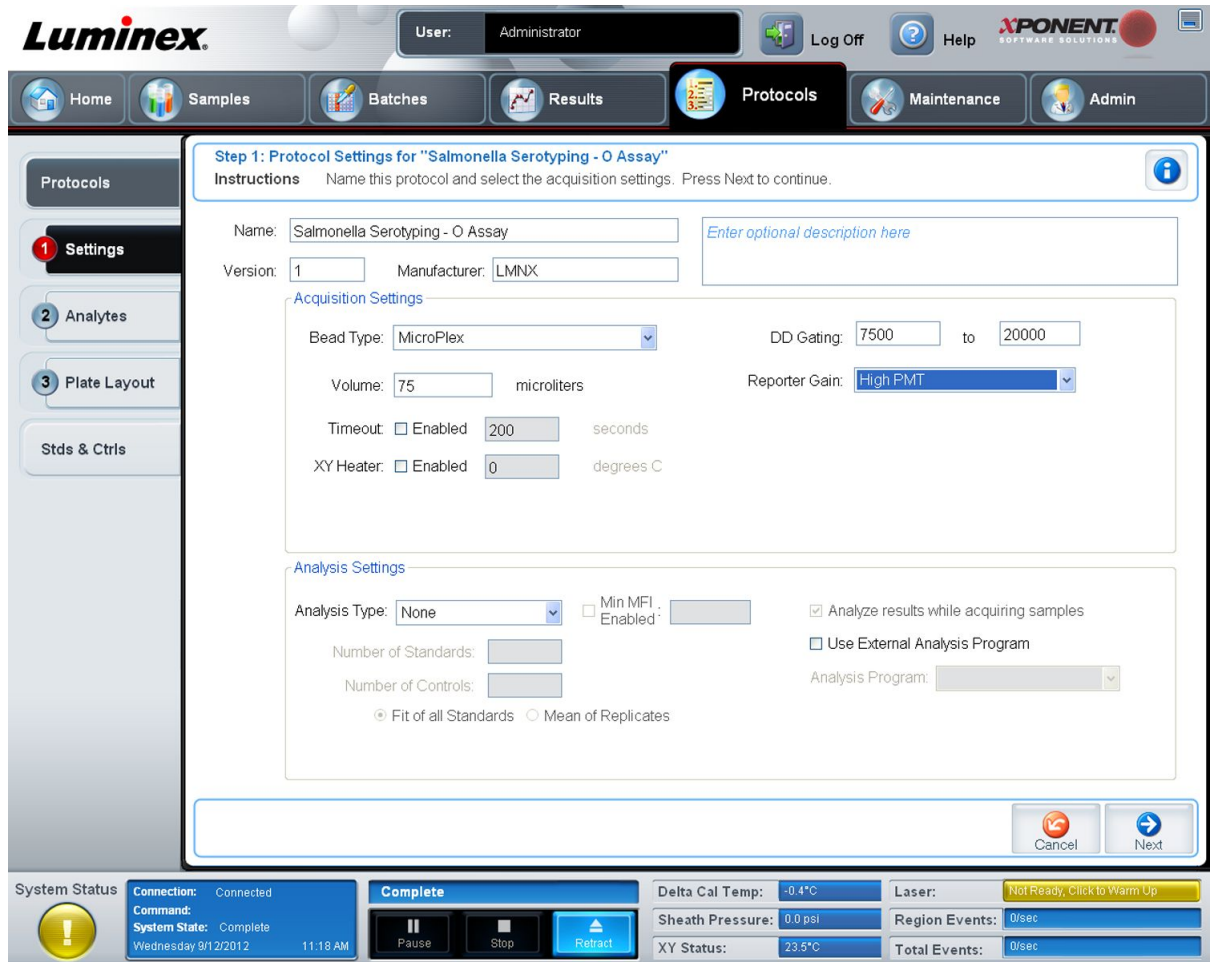


TABLE 1. **O Assay Settings**

(Protocol) Name	Enter assay name
Version (Number)	1 (or current version)
Manufacturer	LMNX
Description	Optional
Acquisition Settings	

TABLE 1. **O Assay Settings Continued**

Bead Type	MicroPlex®
(Sample) Volume	75 µL
(Sample) Timeout	Enabled: 200 sec
XY Heater	Enabled: 52°C
DD Gating	7500 - 20000 *
Reporter Gain	High PMT
Analysis Settings	
Analysis Type	None
Min MFI Enabled	N/A
Number of Standards	N/A
Number of Controls	N/A
Fit of all Standards	N/A
Mean of Replicates	N/A
Analyze results while acquiring samples	N/A
Use External Analysis Program	N/A
Analysis Program	N/A

* Specified gate settings apply to Luminex® systems only.



- Click **Next** to navigate to the **Analytes** tab. Select the bead regions and enter the corresponding analyte information as detailed in [Table O Assay Analysis](#).

FIGURE 2. **Analytes O Assay**



TABLE 2. **O Assay Analysis**

Name	Analysis Type	Units	Count	Region
B	No Analysis	-	50	64
C1	No Analysis	-	50	66
C2	No Analysis	-	50	68
D	No Analysis	-	50	62
E	No Analysis	-	50	70
G	No Analysis	-	50	72
Paratyphi A	No Analysis	-	50	60



- Click **Next** to navigate to the **Plate Layout** tab. Leave Plate Layout at default settings. Click **Save**.

FIGURE 3. Plate Layout O Assay

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Home Samples Batches Results **Protocols** Maintenance Admin

Step 3: Plate Layout for "Salmonella Serotyping - O Assay"

Instructions Select wells to add samples and maintenance commands to the plate.

Well	Type	ID	Dilution

Replicate Count: 1 Grouping: 1 1 1 2 2 2

Commands: AlcoholFlush

Plate: 1

Direction: [Wavy Arrow] [Straight Arrow]

Plate Navigation: [Grid]

Off Plate Area: [R 1] [A]

System Status: Connection: Connected **Complete** Command: System State: Complete Wednesday 9/12/2012 11:22 AM

Delta Cal Temp: -0.4°C Laser: Not Ready, Click to Warm Up

Sheath Pressure: 0.0 psi Region Events: 0/sec

XY Status: 23.6°C Total Events: 0/sec

Setting Up the Protocol for the H Assay

1. From the **Protocols** tab, click on **Create a New Protocol**. The **Settings** tab will display. Populate the fields under **Settings** as detailed in [Table H Assay Settings](#). Fields noted with N/A remain blank.

FIGURE 4. **Settings H Assay**

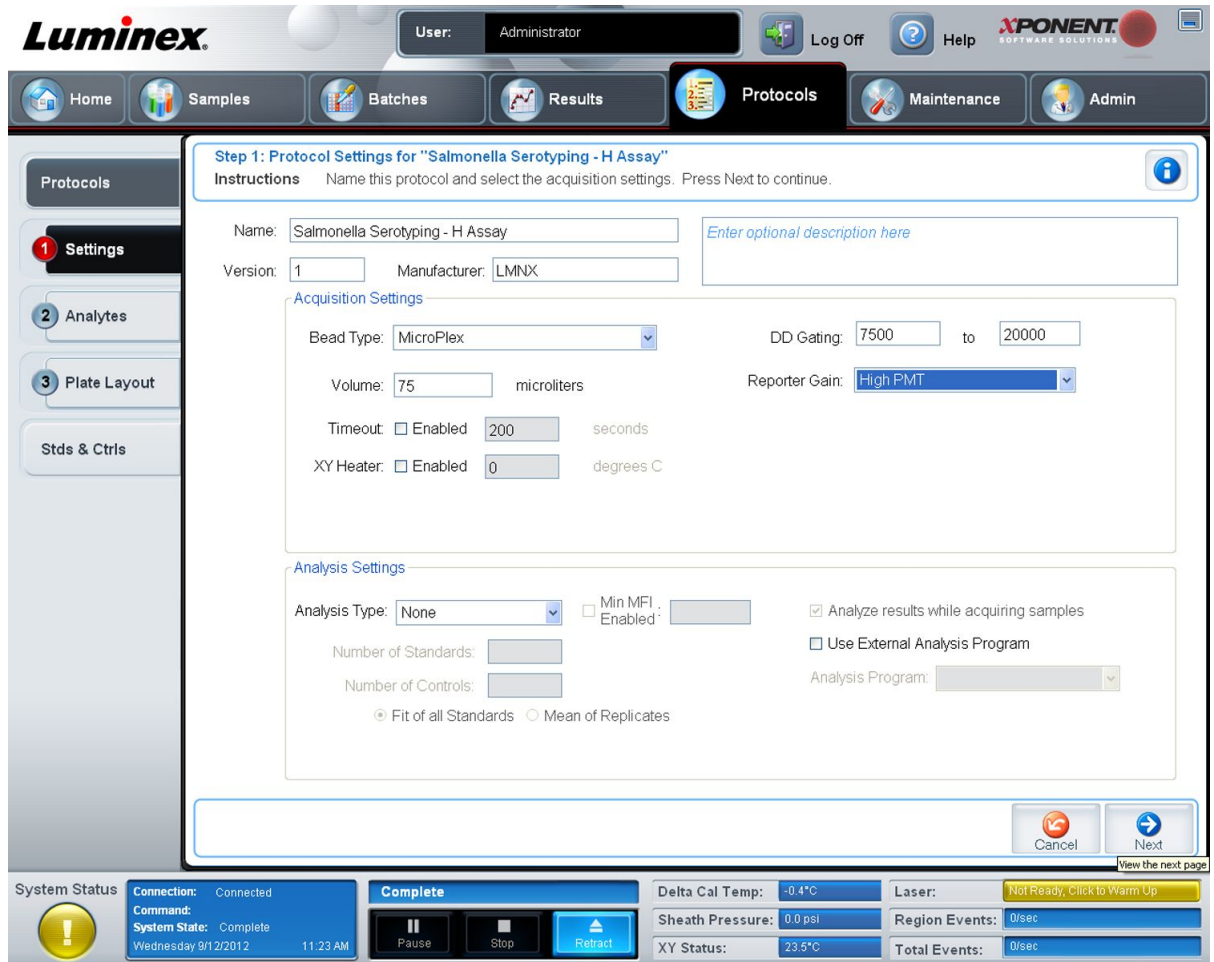


TABLE 3. **H Assay Settings**

(Protocol) Name	Enter assay name
Version (Number)	1 (or current version)
Manufacturer	LMNX
Description	Optional
Acquisition Settings	

TABLE 3. H Assay Settings *Continued*

Bead Type	MicroPlex [®]
(Sample) Volume	75 µL
(Sample) Timeout	Enabled: 200 sec
XY Heater	Enabled: 52°C
DD Gating	7500-20000 *
Reporter Gain	High PMT
Analysis Settings	
Analysis Type	None
Min MFI Enabled	N/A
Number of Standards	N/A
Number of Controls	N/A
Fit of all Standards	N/A
Mean of Replicates	N/A
Analyze results while acquiring samples	N/A
Use External Analysis Program	N/A
Analysis Program	N/A

* Specified gate settings apply to Luminex[®] systems only.



- Click **Next** to navigate to the **Analytes** tab. Select the bead regions and enter the corresponding analyte information as detailed in [Table H Assay Analysis](#).

FIGURE 5. **Analytes H Assay**



TABLE 4. **H Assay Analysis**

Name	Analysis Type	Units	Count	Region
a	No Analysis	-	50	10
b	No Analysis	-	50	11
C	No Analysis	-	50	12
d	No Analysis	-	50	13
j	No Analysis	-	50	14
e h	No Analysis	-	50	15
i	No Analysis	-	50	16

TABLE 4. **H Assay Analysis Continued**

Name	Analysis Type	Units	Count	Region
k	No Analysis	-	50	17
r	No Analysis	-	50	18
z10	No Analysis	-	50	19
z	No Analysis	-	50	20
z29	No Analysis	-	50	22
z6	No Analysis	-	50	23
Y	No Analysis	-	50	24
L-complex	No Analysis	-	50	25
V	No Analysis	-	50	26
z28	No Analysis	-	50	29
EN-complex	No Analysis	-	50	30
X	No Analysis	-	50	31
z15	No Analysis	-	50	32
1-complex	No Analysis	-	50	34
2	No Analysis	-	50	35
5	No Analysis	-	50	36
6	No Analysis	-	50	38
7	No Analysis	-	50	39
G-complex	No Analysis	-	50	40
f	No Analysis	-	50	41
m/g m	No Analysis	-	50	42
m/m t	No Analysis	-	50	43
P	No Analysis	-	50	44
s	No Analysis	-	50	45
t-1	No Analysis	-	50	47
z51	No Analysis	-	50	49
z4-complex	No Analysis	-	50	51
z24	No Analysis	-	50	53

3. Click **Next** to navigate to the **Plate Layout** tab. Leave Plate Layout at default settings. Click **Save**.



FIGURE 6. Plate Layout H Assay

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Home Samples Batches Results **Protocols** Maintenance Admin

Protocols

1 Settings
2 Analytes
3 Plate Layout
Stds & Ctrl

Step 3: Plate Layout for "Salmonella Serotyping - H Assay"

Instructions Select wells to add samples and maintenance commands to the plate.

Well	Type	ID	Dilution

Command Sequence: Plate 1

Replicate Count: 1 Grouping: 1 1 1 2 2 2

Commands: AlcoholFlush

Plate: 1

Direction:

Plate Navigation: Off Plate Area: R 1, A

Buttons: Cancel, Back, Save

System Status: Connection: Connected, Command: Complete, System State: Complete, Wednesday 9/12/2012 11:29 AM

Complete:

Delta Cal Temp: -0.2°C Laser: Not Ready, Click to Warm Up

Sheath Pressure: 0.0 psi Region Events: 0/sec

XY Status: 23.5°C Total Events: 0/sec

Setting Up the Protocol for the AT Assay

1. From the **Protocols** tab, click on **Create New Protocol**. The **Settings** tab will display. Populate the fields under **Settings** as detailed in [Table AT Assay Settings](#). Fields noted with N/A remain blank.

FIGURE 7. Settings AT Assay

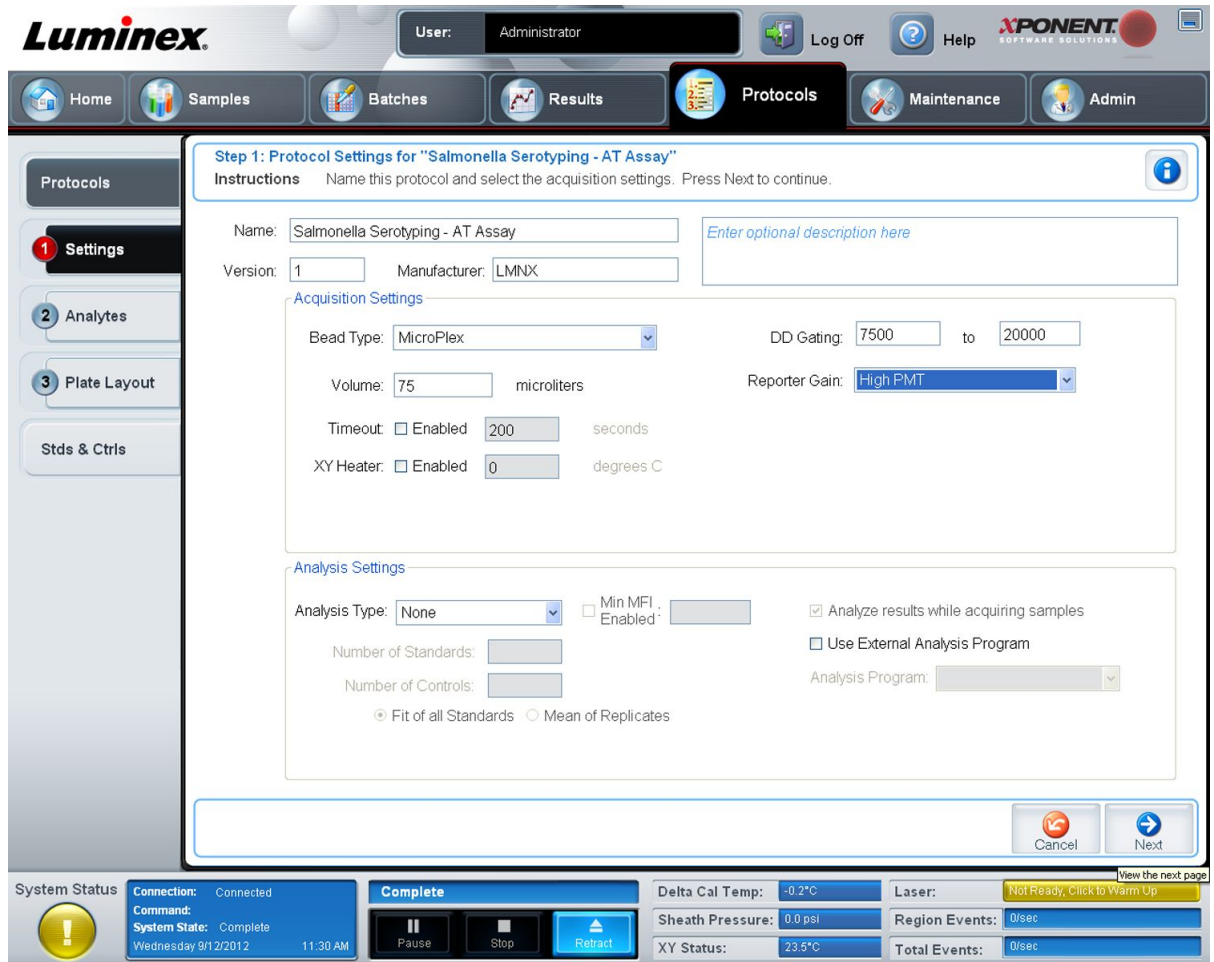


TABLE 5. AT Assay Settings

(Protocol) Name	Enter assay name
Version (Number)	1 (or current version)
Manufacturer	LMNX
Description	Optional
Acquisition Settings	

TABLE 5. AT Assay Settings Continued

Bead Type	MicroPlex®
(Sample) Volume	75 µL
(Sample) Timeout	Enable: 200 sec
XY Heater	Enabled: 52°C
DD Gating	7500 - 20000 *
Reporter Gain	High PMT
Analysis Settings	
Analysis Type	None
Min MFI Enabled	N/A
Number of Standards	N/A
Number of Controls	N/A
Fit of all Standards	N/A
Mean of Replicates	N/A
Analyze results while acquiring samples	N/A
Use External Analysis Program	N/A
Analysis Program	N/A

* Specified gate settings apply to Luminex® systems only.

- Click **Next** to navigate to the **Analytes** tab. Select the bead regions and enter the corresponding analyte information as detailed in [Table AT Assay Analysis](#).

FIGURE 8. **Analytes AT Assay**

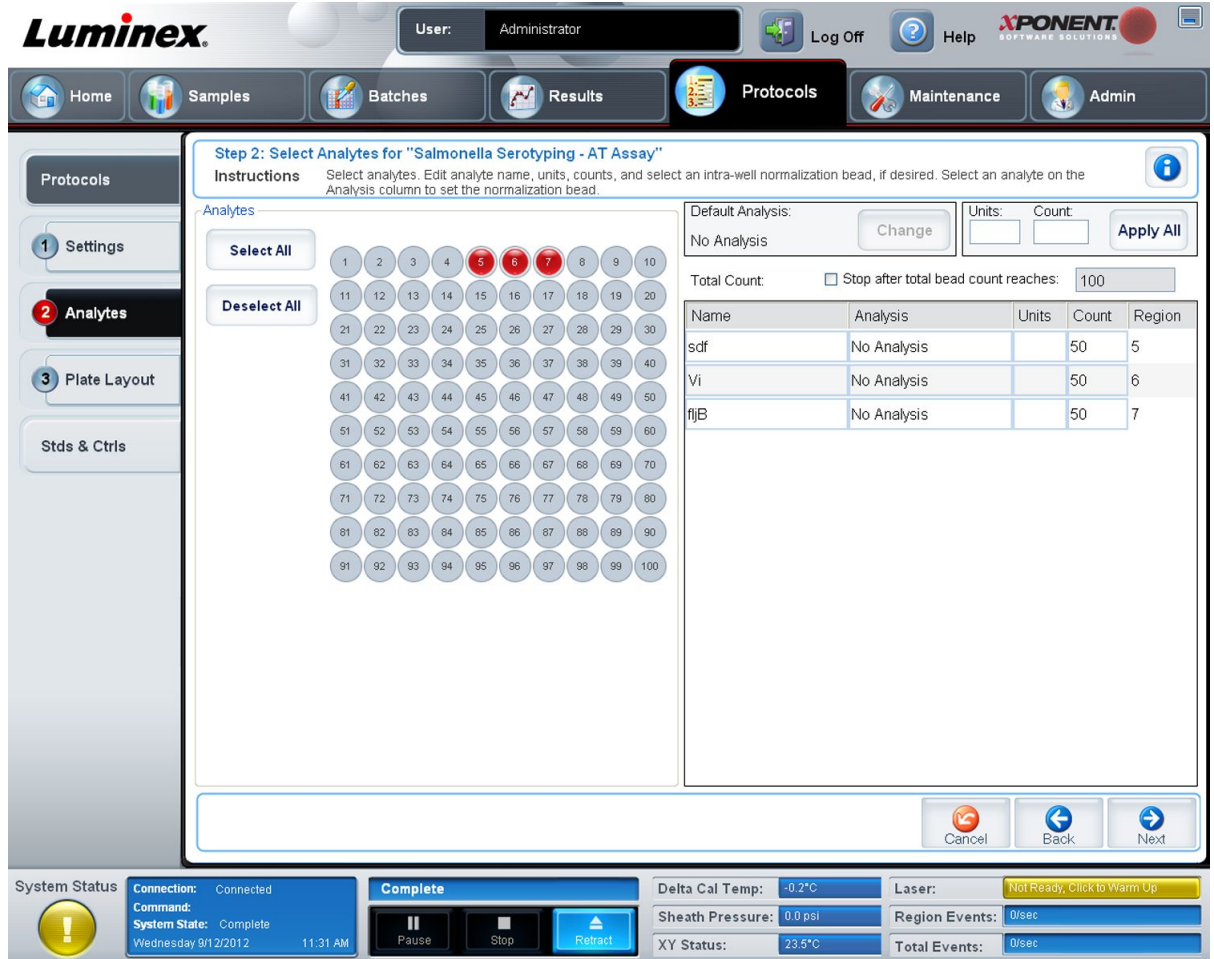


TABLE 6. **AT Assay Analysis**

Name	Analysis Type	Units	Count	Region
sdf	No Analysis	-	50	5
Vi	No Analysis	-	50	6
fliB	No Analysis	-	50	7

- Click **Next** to navigate to the **Plate Layout** tab. Leave Plate Layout at default settings. Click **Save**.



FIGURE 9. Plate Layout AT Assay

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Home Samples Batches Results **Protocols** Maintenance Admin

Protocols

1 Settings
2 Analytes
3 Plate Layout
Stds & Ctrl

Step 3: Plate Layout for "Salmonella Serotyping - AT Assay"

Instructions Select wells to add samples and maintenance commands to the plate.

Well	Type	ID	Dilution

Command Sequence: Plate 1

Replicate Count: 1 Grouping: 1 1 1 2 2 2

Commands: AlcoholFlush

Plate: 1

Direction:

Plate Navigation: Off Plate Area: R 1, A

Buttons: Cancel, Back, Save

System Status: Connection: Connected, Command: Complete, System State: Complete, Wednesday 9/12/2012 11:32 AM

Complete:

Delta Cal Temp: -0.2°C Laser: Not Ready, Click to Warm Up

Sheath Pressure: 0.0 psi Region Events: 0/sec

XY Status: 23.6°C Total Events: 0/sec