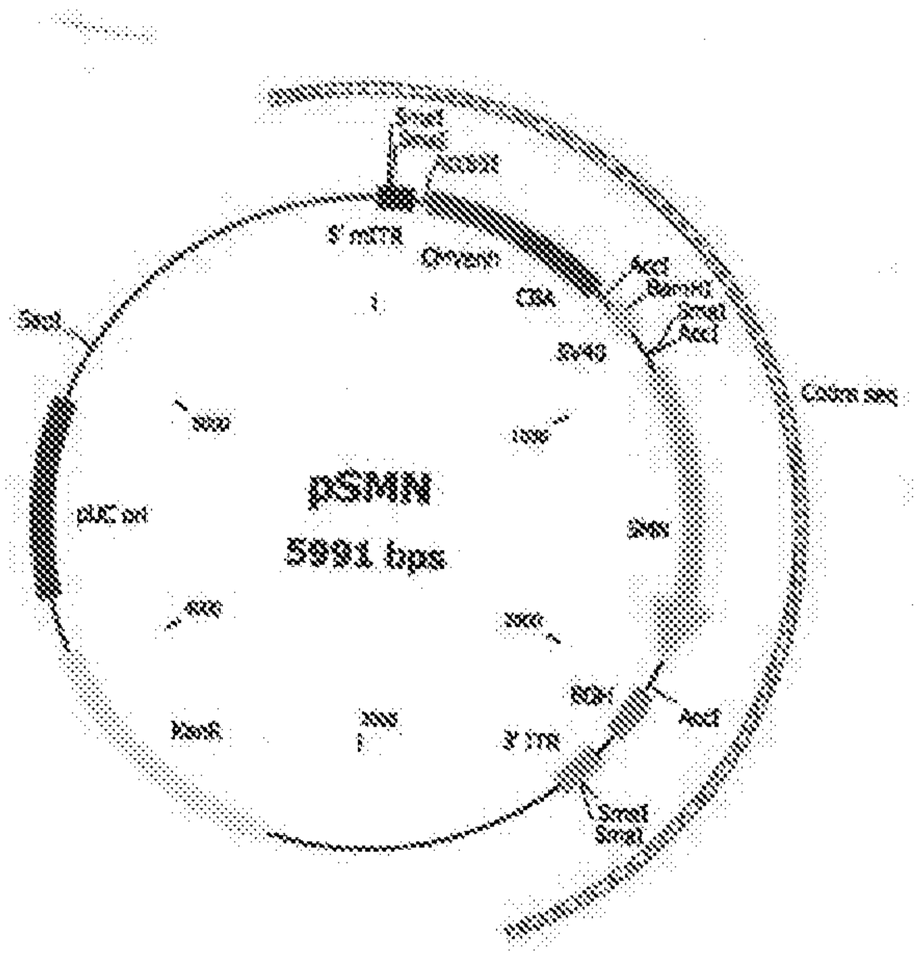


FIG. 1A pSMN map

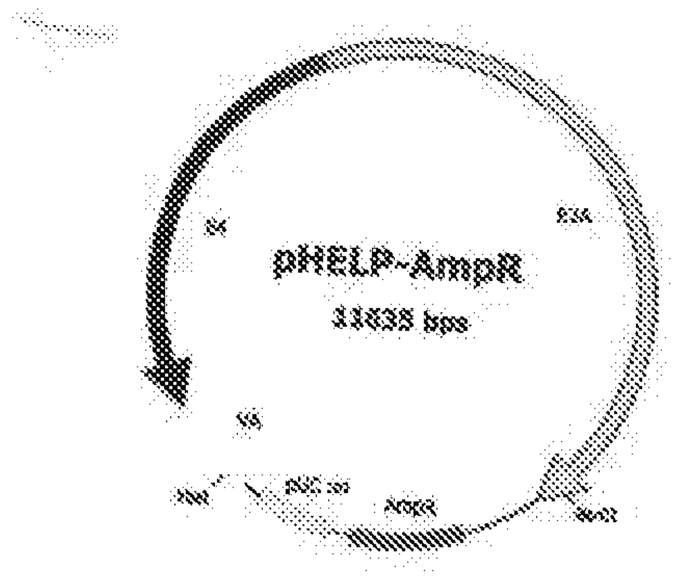


FIG. 1B pHELP map

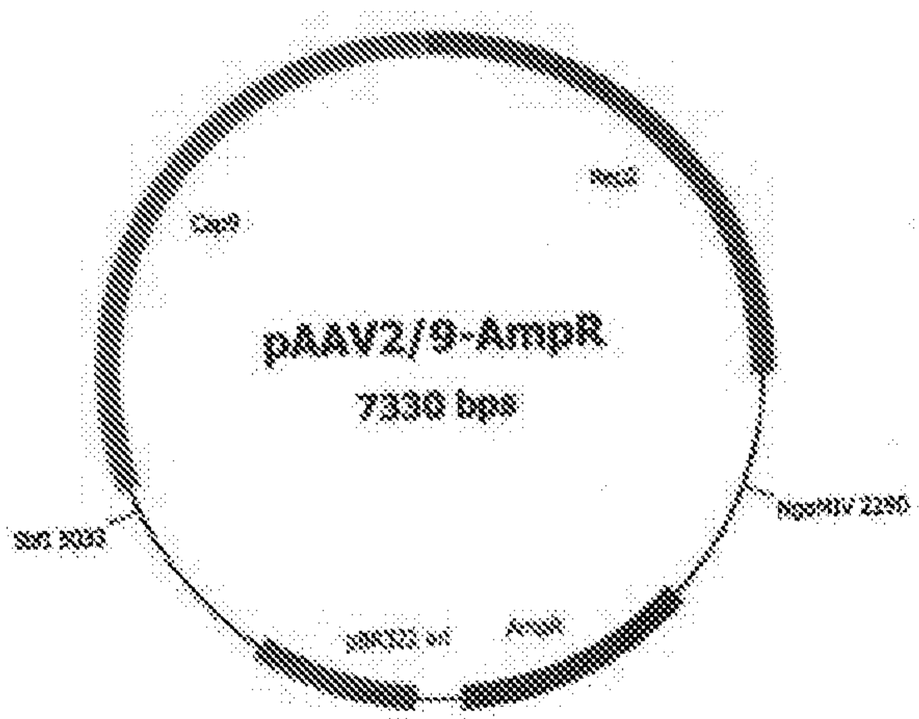


FIG. 1C pAAV2/9 map

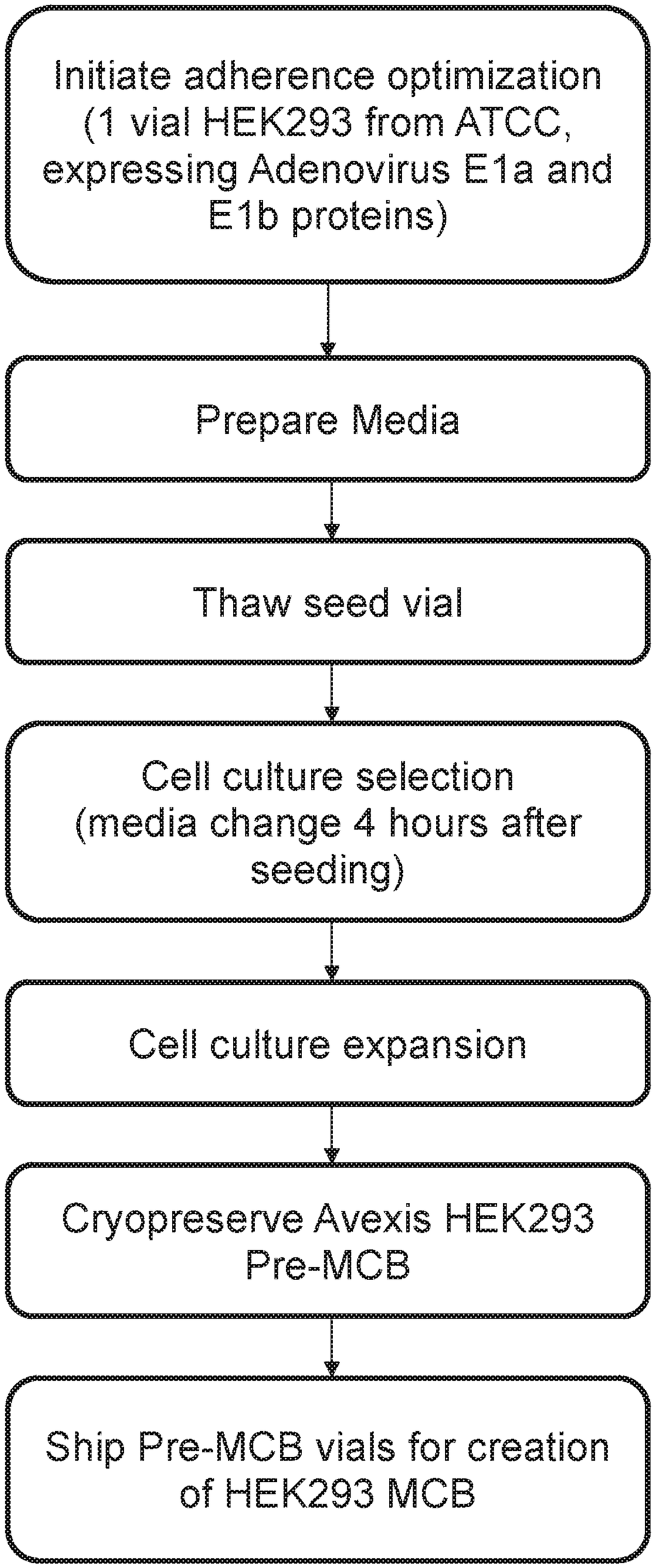

Initiate adherence optimization (1 vial HEK293 from ATCC, expressing Adenovirus E1a and E1b proteins)
Prepare Media
Thaw seed vial
Cell culture selection (media change 4 hours after seeding)
Cell culture expansion
Cryopreserve Avexis HEK293 Pre-MCB
Ship Pre-MCB vials for creation of HEK293 MCB

Figure 2

Step/Notebook Page	Date	Details				Notes
Thaw Page 3	7/22/2015	Flasks seeded	2T	Old Passage	0	Cells were not counted, but full vial from ATCC ($1x10^6$ cells) were plated. Four media changes between this step and the next.
		Seeding Density	$1x10^6$ cells	New Passage	1*	
Passage Page 5	7/27/2015	Flasks seeded	7S	Old Passage	1	Cells before splitting were 95% confluent. Adherence selection step after seeding lead to significant portion of non-adherent cells lost: ~35% cell loss by visual inspection.
		Seeding Density	1:1	New Passage	2	
Passage Page 7	7/29/2015	Flasks seeded	7S	Old Passage	2	Adherence selection step included after seeding.
		Seeding Density	1:1	New Passage	3	
Passage Page 8	7/31/2015	Flasks seeded	2 x 17S	Old Passage	3	Adherence selection step included after seeding, with much less pronounced loss of cells by visual inspection.
		Seeding Density	1:4.7	New Passage	4	
Passage Page 9	8/3/2015	Flasks seeded	8 x 17S	Old Passage	4	One media change between this step and the next.
		Seeding Density	1:4	New Passage	5	
Passage Page 10	8/6/2015	Flasks seeded	20 x 17S	Old Passage	5	One media change between this step and the next.
		Seeding Density	1:3 or 1:2	New Passage	6	
Harvest/ Cryo-preservation Pages 11-13	8/9/2015	Cell Concentration	$1x10^7$ cells/mL	Vials Filled	22	Pooled 3 cell suspensions to yield $1x10^8$ cells and $1.2x10^8$ cells.

Note: All Total Cells reported above have the unit of "cells" and indicate total cells recovered.
All Seeding Densities reported above have the unit of "cells/cm^2".
All flask sizes reported above have the unit of "cm^2".
All Cell Concentrations reported above have the unit of "cells/mL".

* Thawing was considered a passage, thus thawed cells were labelled as "passage 1".

Figure 3

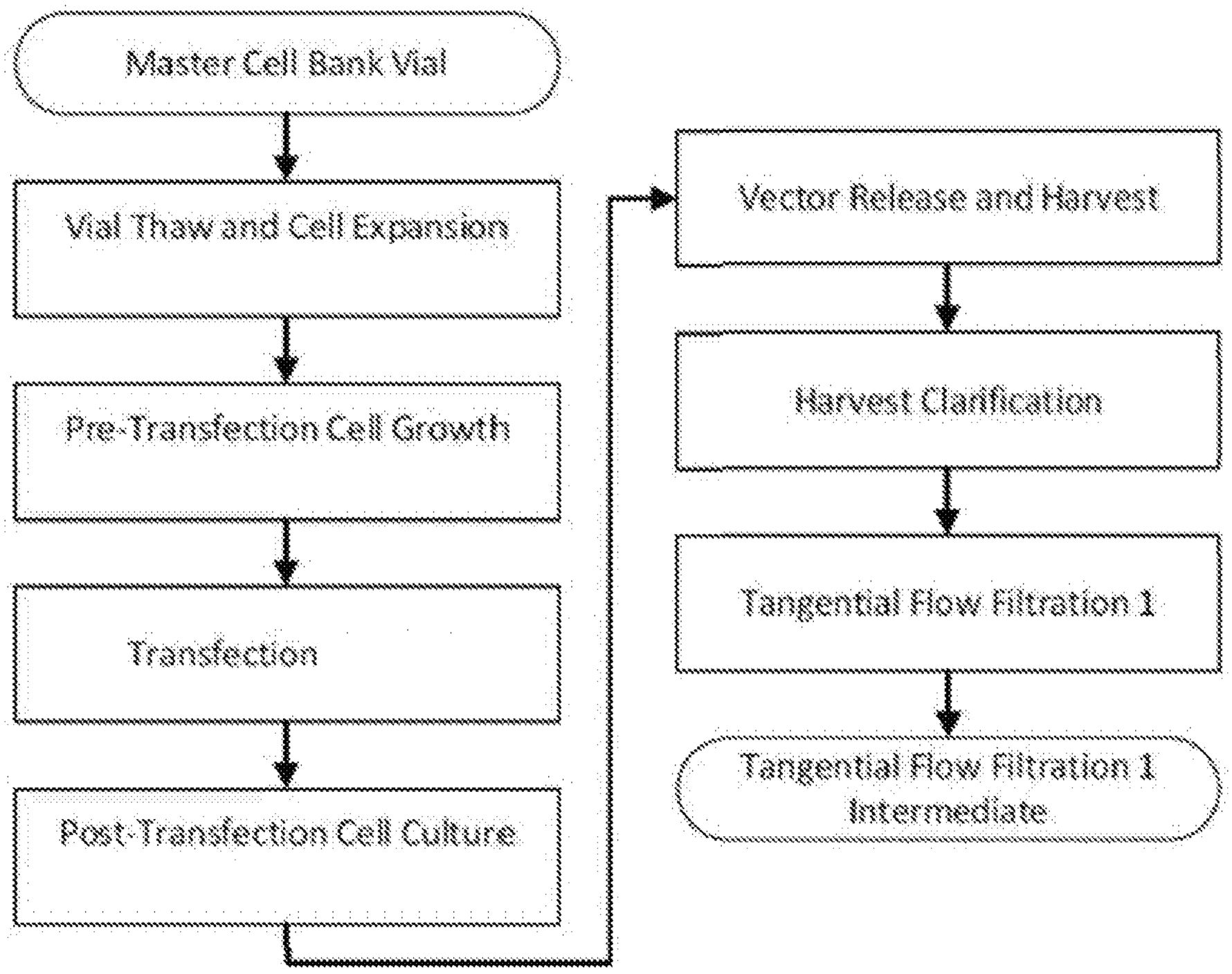

Master Cell Bank Vial
Vial Thaw and Cell Expansion
Pre-Transfection Cell Growth
Transfection
Post-Transfection Cell Culture
Vector Release and Harvest
Harvest Clarification
Tangential Flow Filtration 1
Tangential Flow Filtration 1 Intermediate

Figure 4

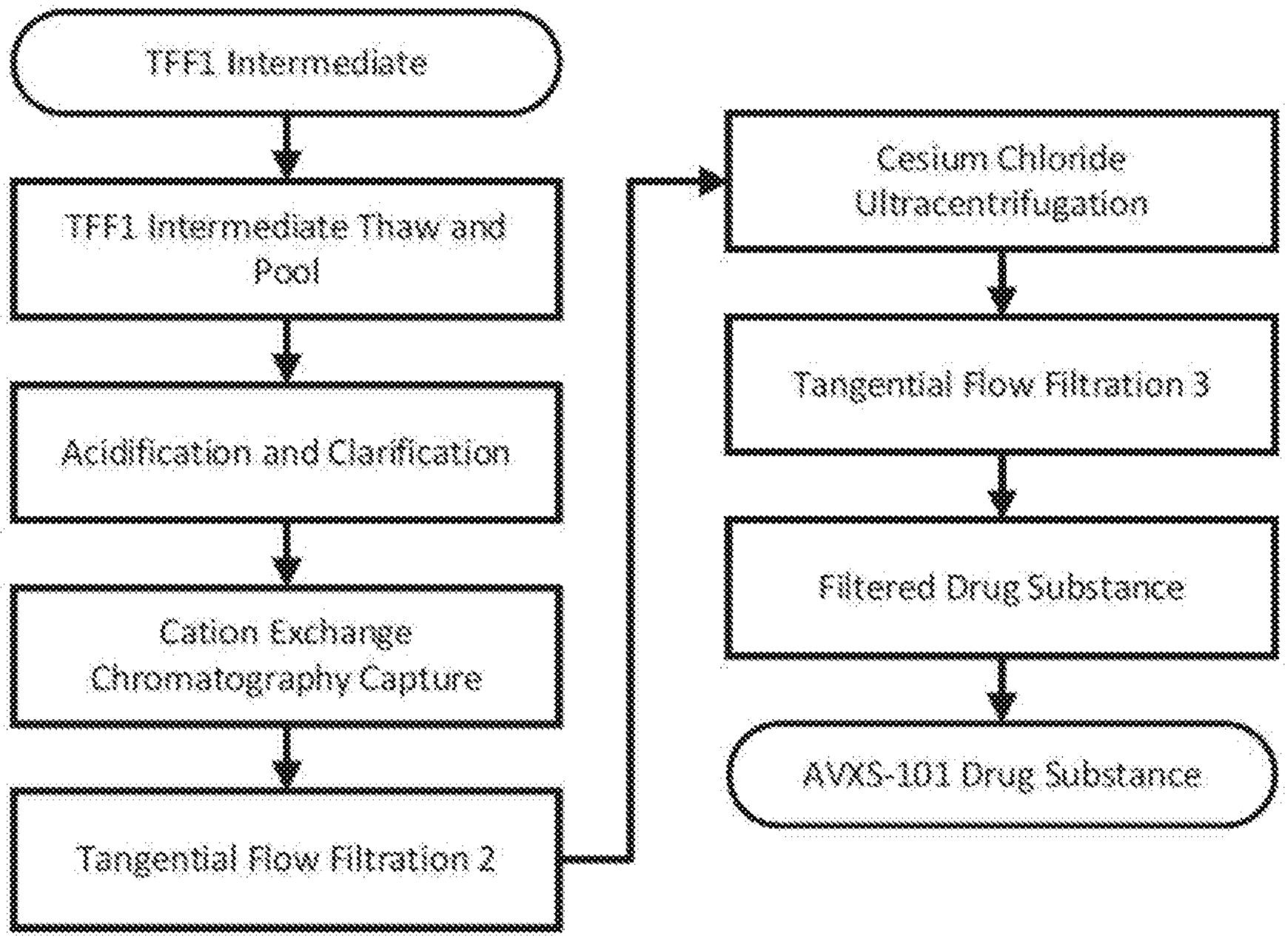

TFF1 Intermediate
TFF1 Intermediate Thaw and Pool
Acidification and Clarification
Cation Exchange Chromatography Capture
Tangential Flow Filtration 2
Cesium Chloride Ultracentrifugation
Tangential Flow Filtration 3
Filtered Drug Substance
AVXS-101 Drug Substance

Figure 5

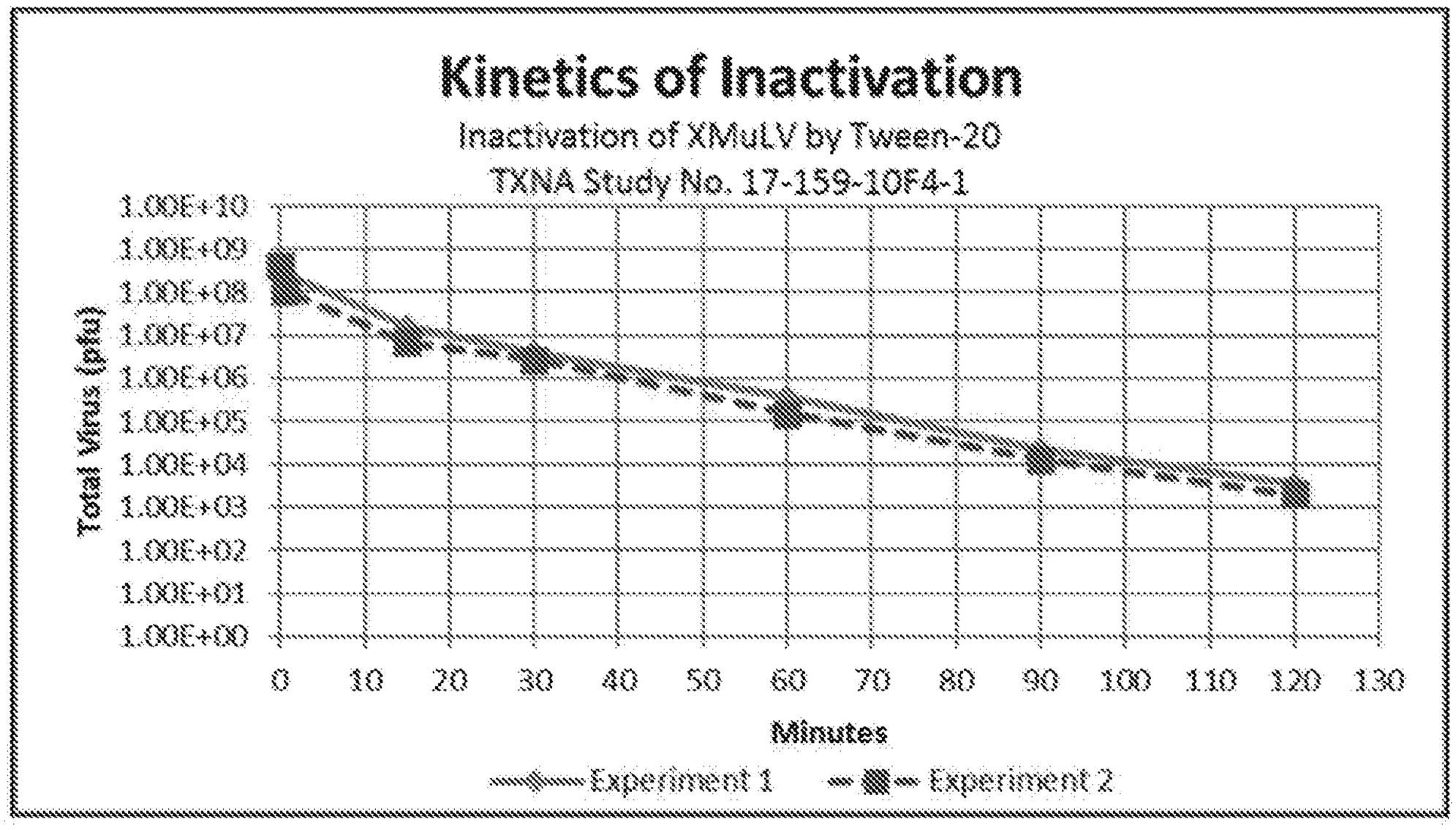

Kinetics of Inactivation
Inactivation of XMuLV by Tween-20
TXNA Study No. 17-159-10F4-1
Total Virus (pfu)
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
10
20
30
40
50
60
70
80
90
100
110
120
130
Minutes
Experiment 1
Experiment 2

Figure 6

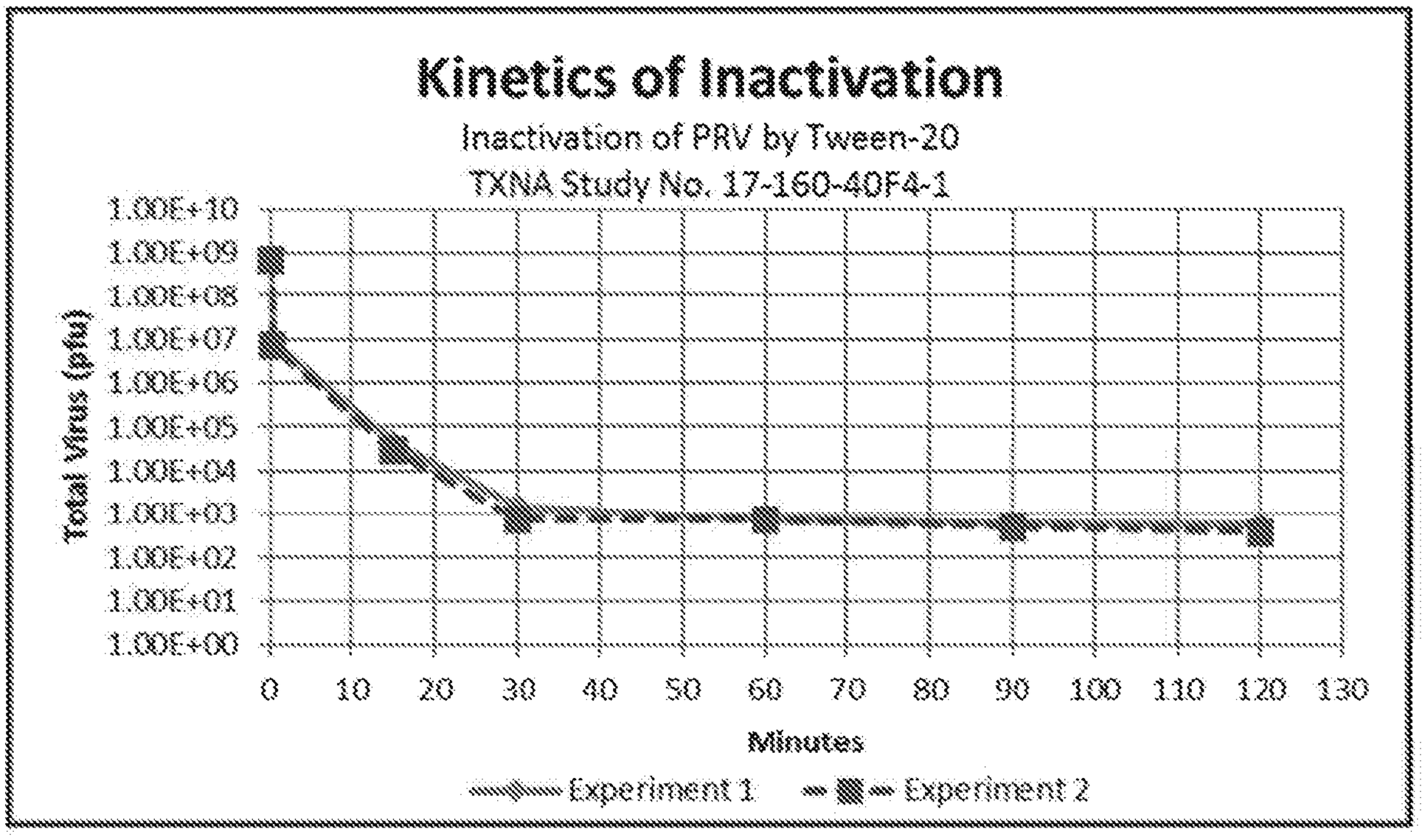

Kinetics of Inactivation
Inactivation of PRV by Tween-20
TXNA Study No. 17-160-40F4-1
Total Virus (pfu)
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
10
20
30
40
50
60
70
80
90
100
110
120
130
Minutes
Experiment 1
Experiment 2

Figure 7

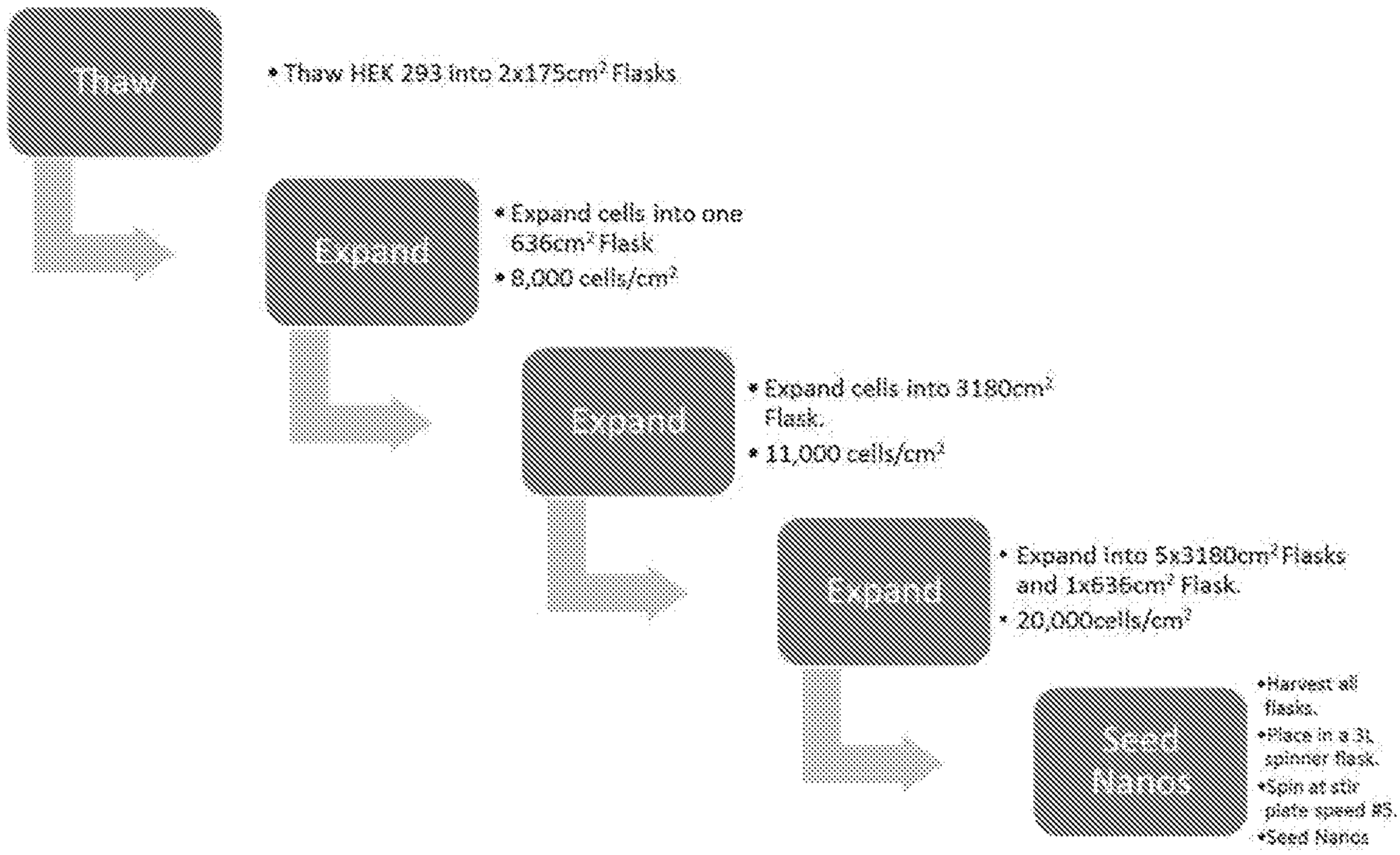

Thaw
• Thaw HEK 293 into 2x175cm² Flasks
Expand
• Expand cells into one 636cm² Flask
• 8,000 cells/cm²
Expand
• Expand cells into 3180cm² Flask.
• 11,000 cells/cm²
Expand
• Expand into 5x3180cm² Flasks and 1x636cm² Flask.
• 20,000cells/cm²
Seed Nanos
•Harvest all flasks.
•Place in a 3L spinner flask.
•Spin at stir plate speed #5.
•Seed Nanos

Figure 8

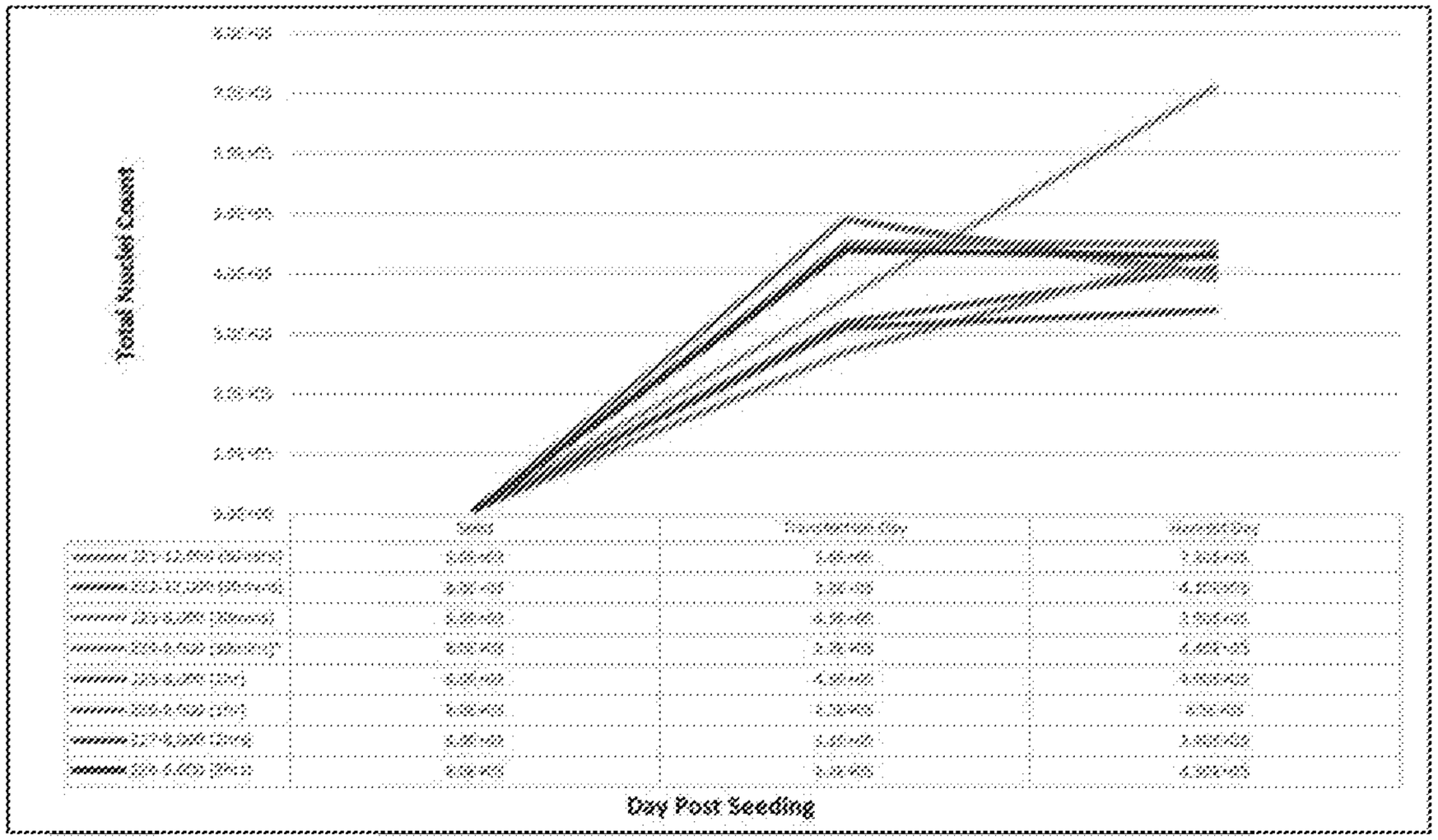
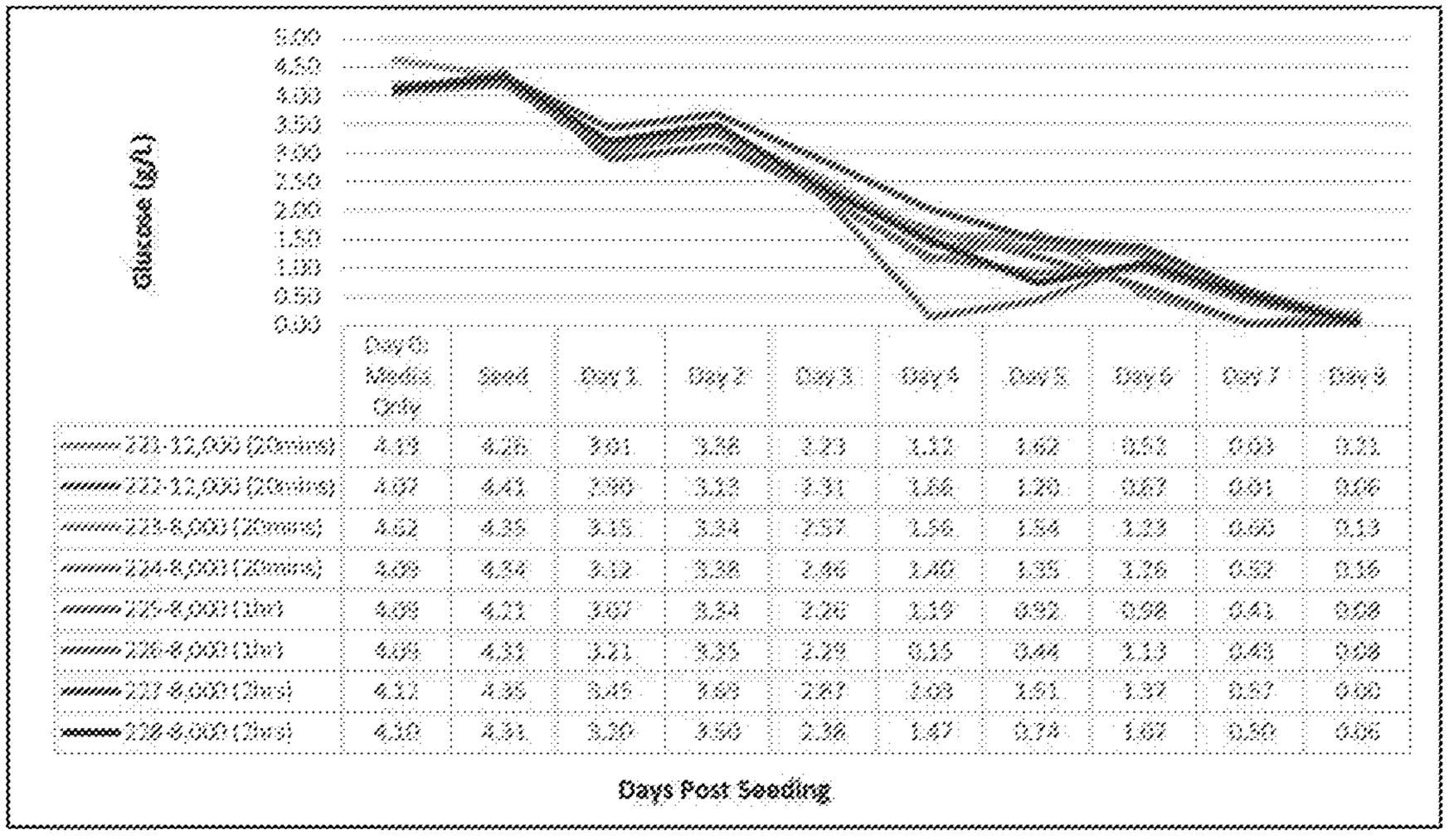
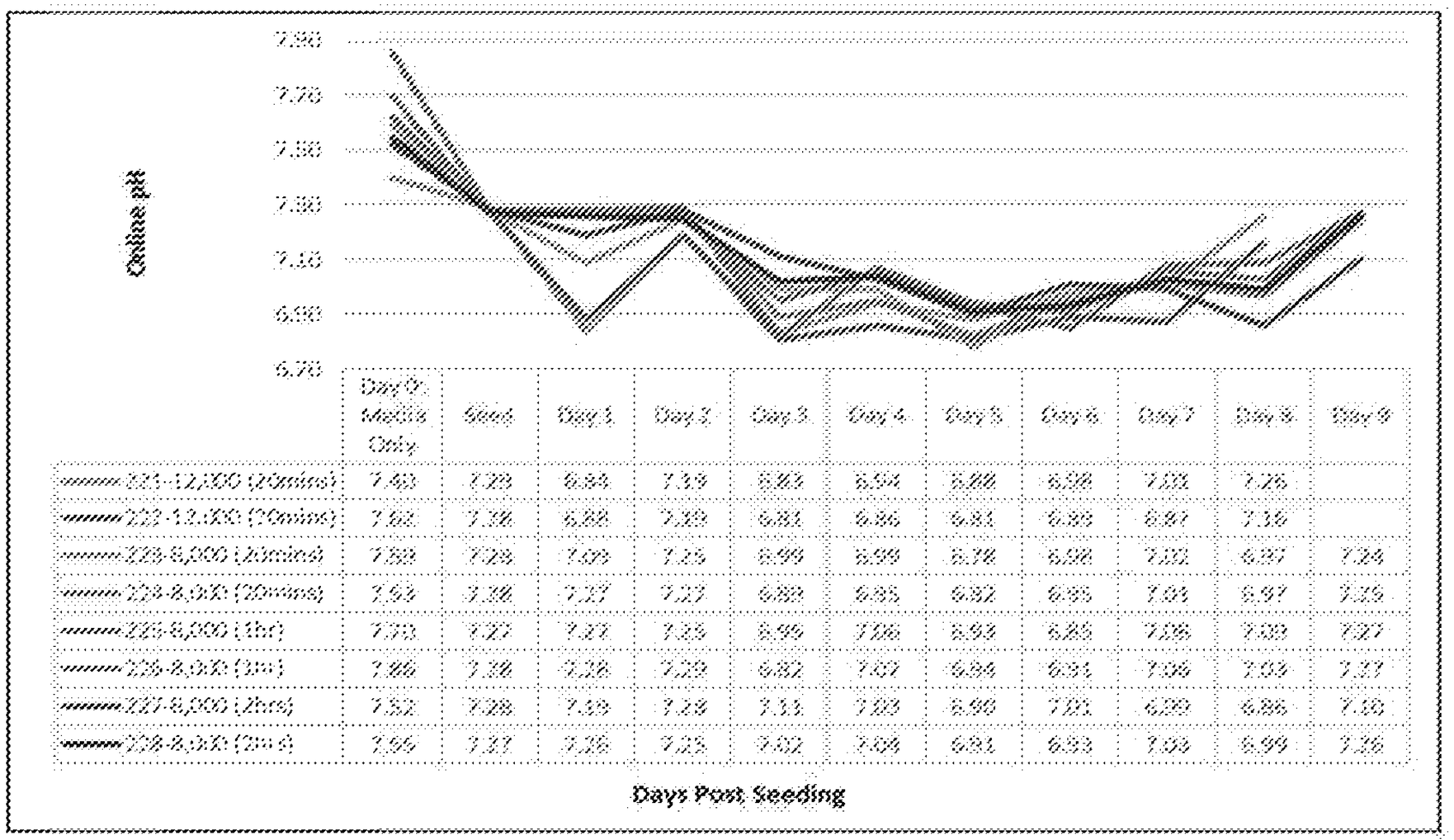


Figure 9A



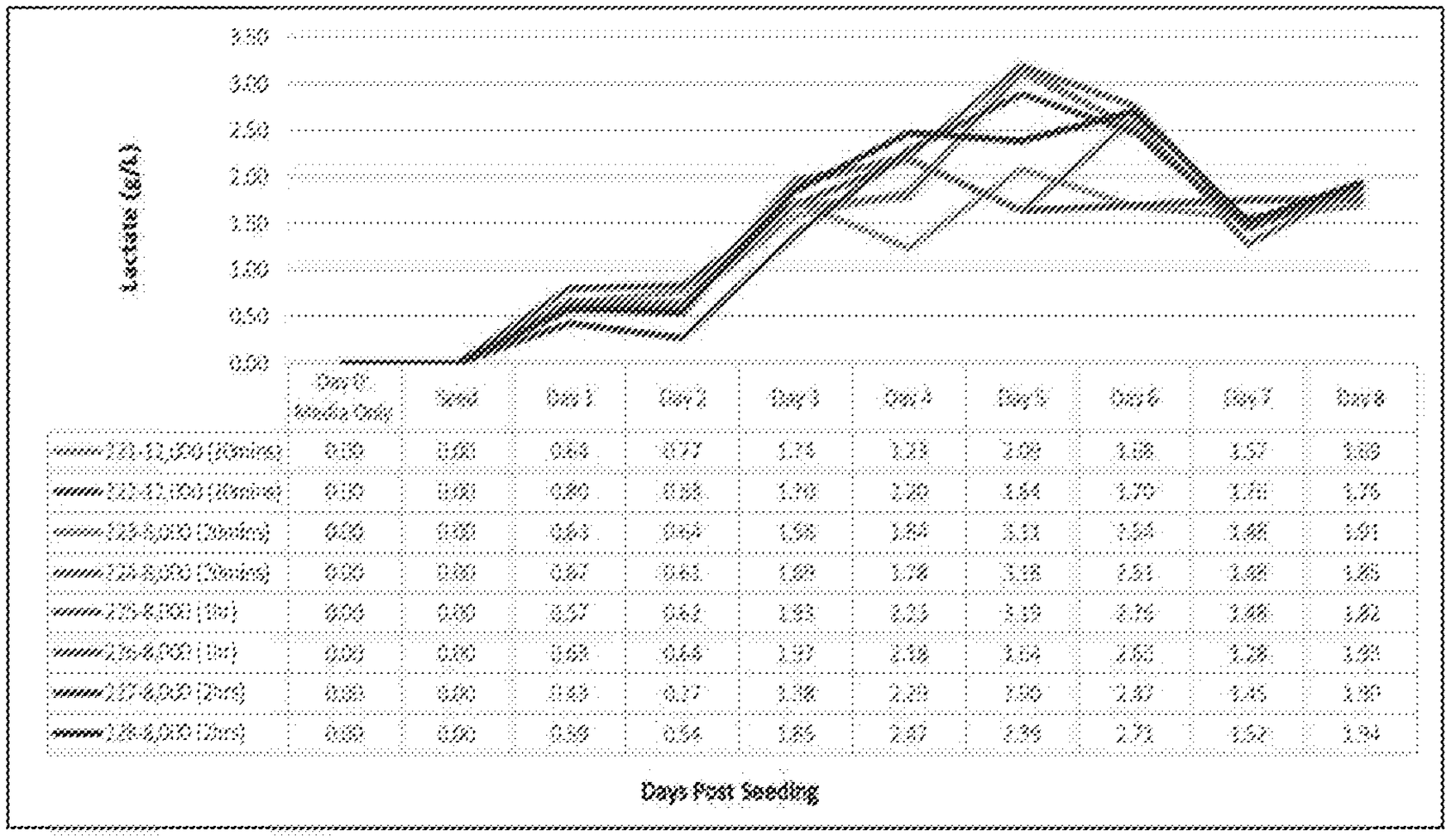
	Day 0: Media Only	Seed	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
213-12,000 (20mins)	4.19	4.26	3.61	3.38	3.23	3.12	1.62	0.51	0.03	0.21
223-12,000 (20mins)	4.07	4.41	3.90	3.53	3.31	3.66	1.26	0.67	0.01	0.06
223-8,000 (20mins)	4.62	4.35	3.15	3.34	2.57	1.56	1.54	1.23	0.66	0.13
224-8,000 (20mins)	4.09	4.34	3.12	3.38	2.66	1.40	1.35	1.26	0.52	0.16
225-8,000 (3hr)	4.09	4.21	3.67	3.24	2.26	2.19	0.92	0.58	0.41	0.08
226-8,000 (3hr)	4.09	4.31	3.21	3.35	2.29	0.15	0.44	1.13	0.43	0.08
227-8,000 (3hrs)	4.11	4.35	3.45	3.69	2.87	1.03	1.61	1.37	0.57	0.00
228-8,000 (3hrs)	4.10	4.31	3.20	3.50	2.36	1.47	0.74	1.07	0.50	0.06

Figure 9B



	Day 0: Media Only	Seed	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
221-12,500 (20mins)	7.40	7.29	6.84	7.19	6.83	6.94	6.88	6.98	7.03	7.26	
222-12,500 (1Omins)	7.62	7.36	6.88	7.29	6.81	6.86	6.81	6.89	6.87	7.16	
223-6,000 (20mins)	7.59	7.28	7.09	7.25	6.99	6.99	6.78	6.98	7.01	6.97	7.24
224-6,000 (10mins)	7.53	7.36	7.27	7.27	6.89	6.95	6.82	6.95	7.01	6.97	7.25
225-6,000 (1hr)	7.71	7.27	7.27	7.25	6.99	7.06	6.93	6.85	7.05	7.03	7.27
226-6,000 (1m)	7.66	7.36	7.28	7.29	6.82	7.07	6.94	6.91	7.06	7.03	7.27
227-6,000 (2hrs)	7.52	7.28	7.19	7.24	7.11	7.03	6.90	7.01	6.99	6.86	7.15
228-6,000 (2m s)	7.59	7.31	7.26	7.25	7.02	7.04	6.91	6.93	7.04	6.99	7.26

Figure 9C



	Day 0: Media Only	Seed	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
221-12,500 (20mins)	0.39	0.68	0.84	0.77	1.74	1.23	2.08	1.68	1.57	1.89
222-12,500 (10mins)	0.39	0.68	0.80	0.88	1.70	1.20	1.84	1.70	1.78	1.76
223-6,000 (20mins)	0.39	0.68	0.84	0.84	1.58	1.84	2.11	1.84	1.66	1.91
224-6,000 (10mins)	0.39	0.68	0.87	0.81	1.69	1.78	2.18	1.81	1.68	1.86
225-6,000 (1hr)	0.39	0.68	0.87	0.83	1.83	2.23	2.19	1.76	1.48	1.82
226-6,000 (1m)	0.39	0.68	0.88	0.84	1.87	2.18	2.04	1.83	1.38	1.91
227-6,000 (2hrs)	0.39	0.68	0.83	0.77	1.78	2.29	1.90	2.07	1.45	1.90
228-6,000 (2m s)	0.39	0.68	0.86	0.84	1.85	2.07	1.96	2.01	1.52	1.94

Figure 9D

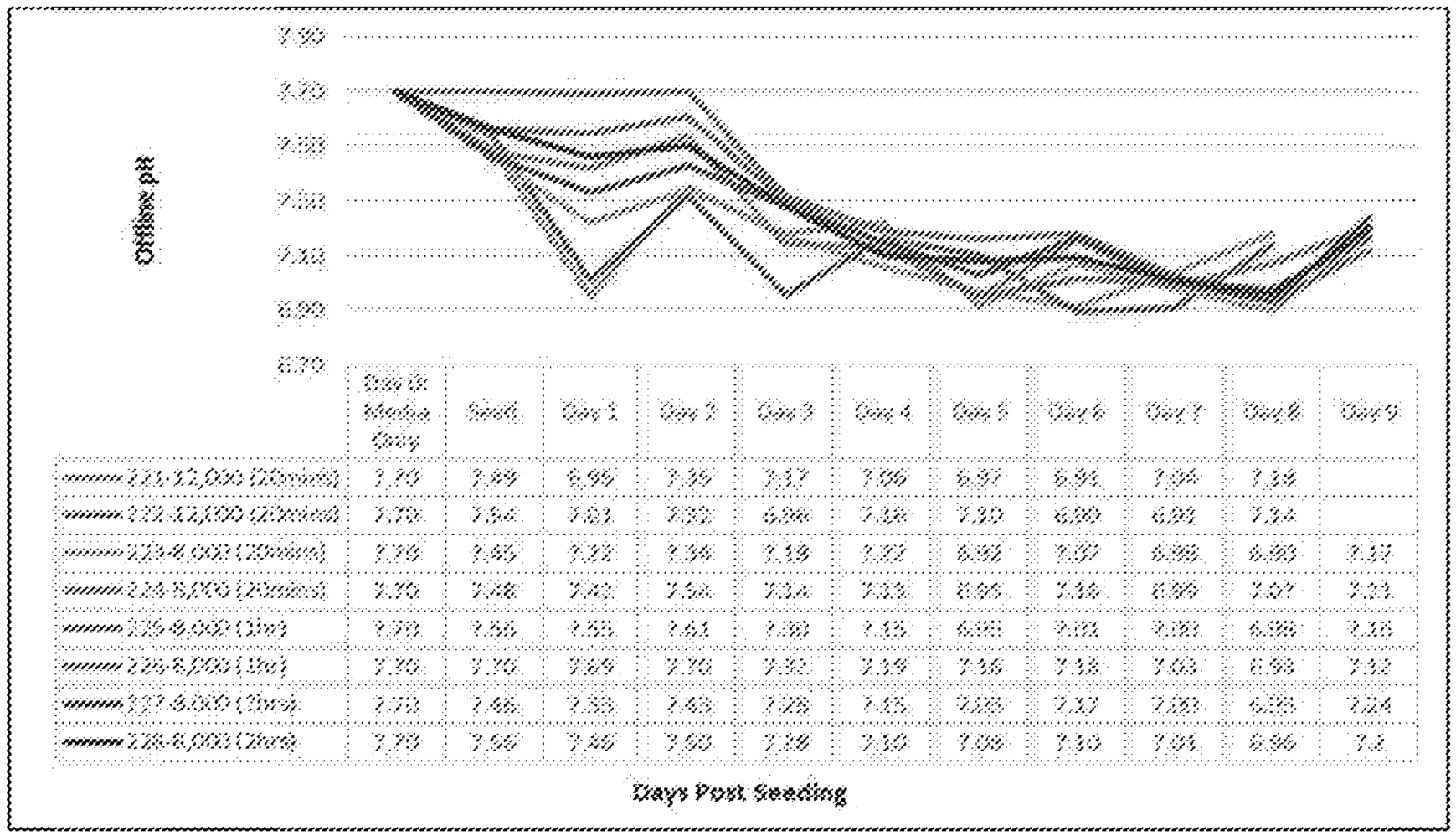
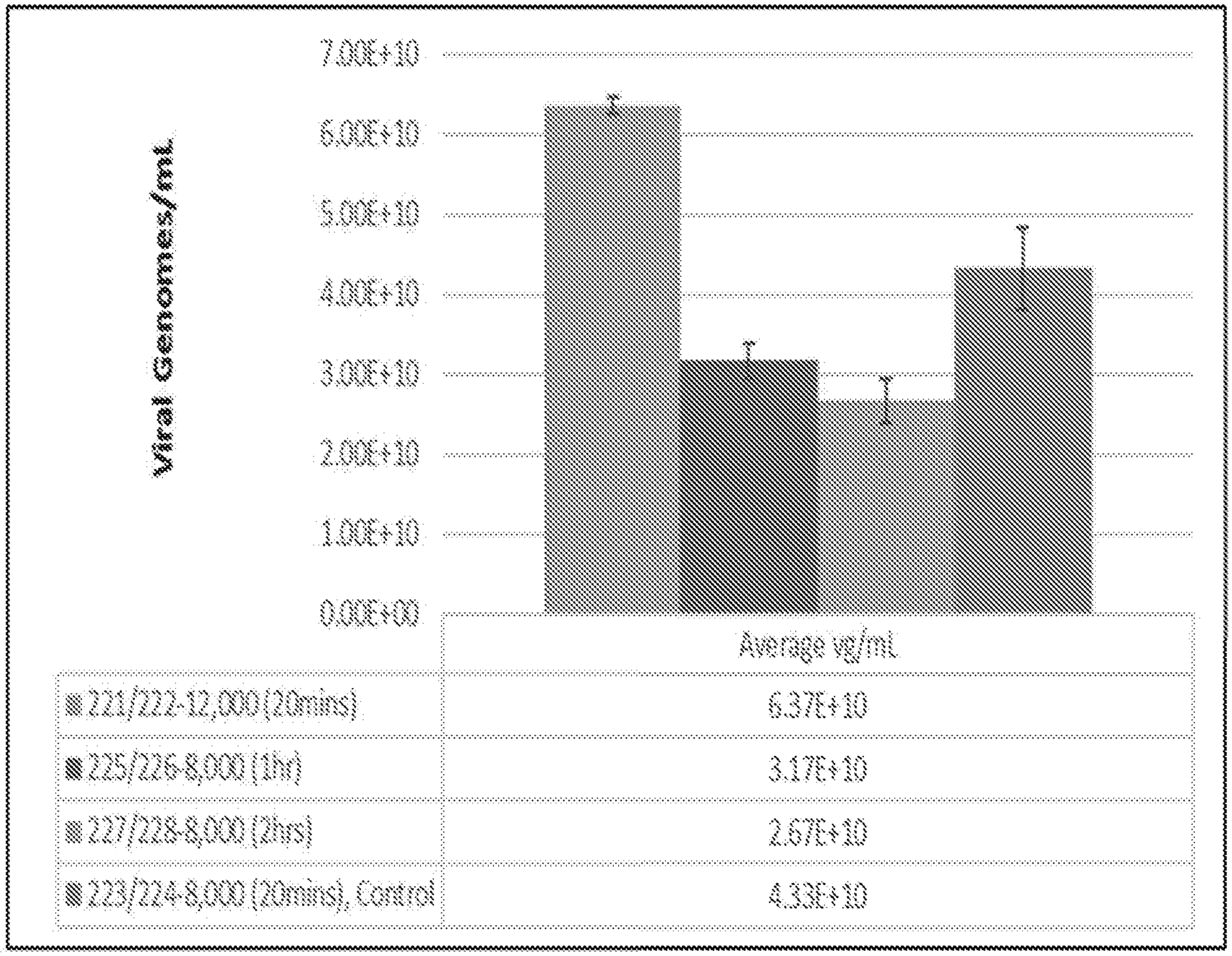

7.90
7.70
7.50
7.30
7.10
6.90
6.70
Offline pH
Day 0: Media Only
Seed
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
221-12,000 (20mins)
7.70 7.69 6.99 7.39 7.17 7.06 6.97 6.91 7.04 7.16
222-12,000 (2hrs4mins)
7.70 7.54 7.01 7.32 6.96 7.16 7.10 6.90 6.91 7.14
223-8,000 (20mins)
7.70 7.45 7.22 7.34 7.19 7.22 6.92 7.07 6.88 6.90 7.17
224-8,000 (2hrs4mins)
7.70 7.48 7.42 7.54 7.14 7.19 6.95 7.16 6.99 7.07 7.21
225-8,000 (3hr)
7.70 7.56 7.55 7.61 7.50 7.15 6.91 7.01 7.01 6.98 7.16
226-8,000 (5hr)
7.70 7.70 7.69 7.70 7.52 7.19 7.16 7.18 7.03 6.93 7.12
227-8,000 (2hrs)
7.70 7.46 7.33 7.43 7.26 7.15 7.03 7.17 7.03 6.95 7.24
228-8,000 (2hrs)
7.70 7.56 7.40 7.50 7.26 7.10 7.06 7.10 7.01 6.96 7.2
Days Post Seeding

Figure 9E



	Average vg/mL
221/222-12,000 (20mins)	6.37E+10
225/226-8,000 (1hr)	3.17E+10
227/228-8,000 (2hrs)	2.67E+10
223/224-8,000 (20mins), Control	4.33E+10

Figure 10

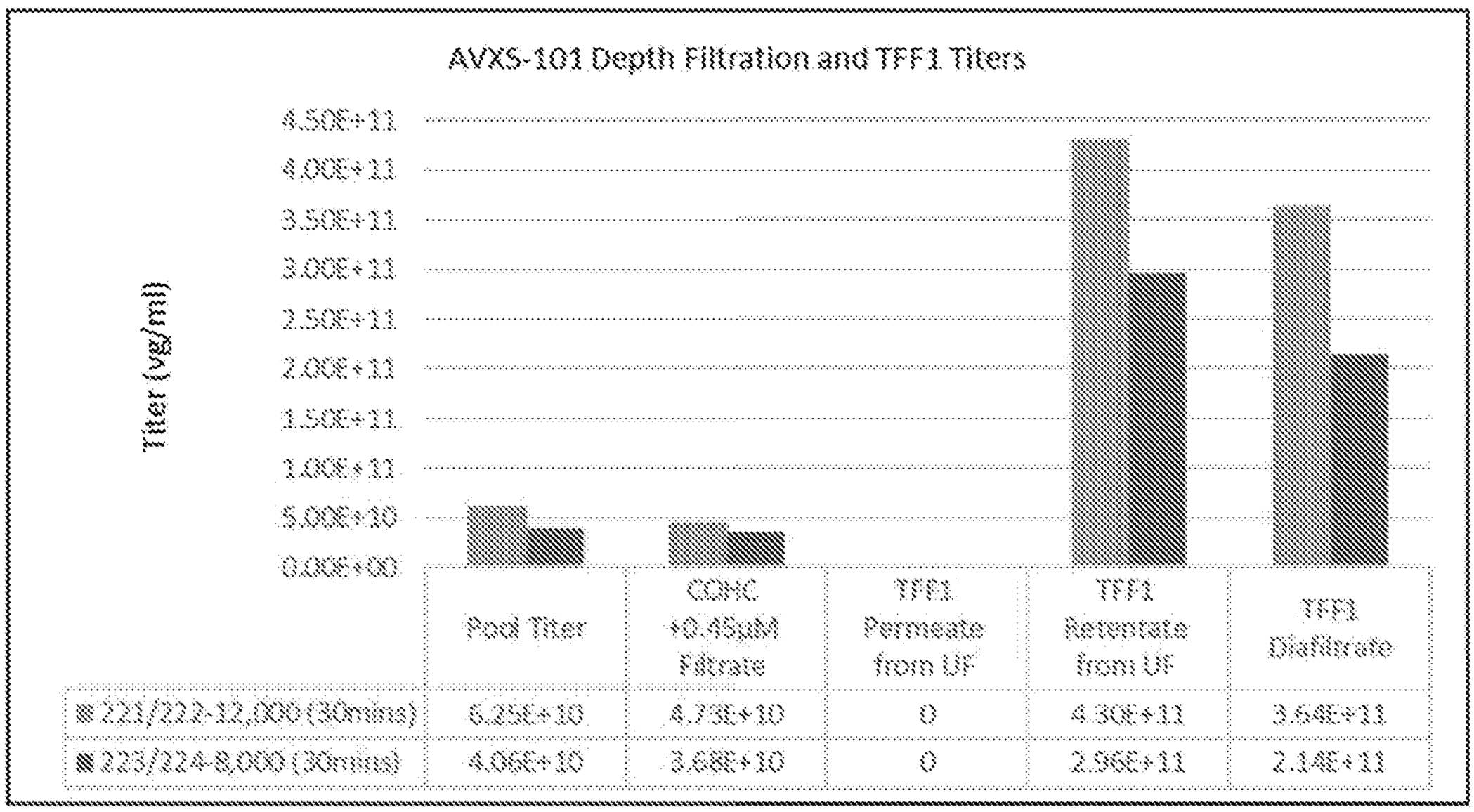

AVXS-101 Depth Filtration and TFF1 Titers
Titer (vg/ml)
4.50E+11
4.00E+11
3.50E+11
3.00E+11
2.50E+11
2.00E+11
1.50E+11
1.00E+11
5.00E+10
0.00E+00
Pool Titer
CCHC +0.45µM Filtrate
TFF1 Permeate from UF
TFF1 Retentate from UF
TFF1 Diafiltrate
221/222-12,000 (30mins) 6.25E+10 4.73E+10 0 4.30E+11 3.64E+11
223/224-8,000 (30mins) 4.06E+10 3.68E+10 0 2.96E+11 2.14E+11

Figure 11

	8,000 cells/ cm2, D5 Trans, D9 Harvest					12,000 cells/ cm2, D4 Trans, D8 Harvest				
	Volume	Titer	Total vg	Step Recovery	Total Recovery	Volume	Titer	Total vg	Step Recovery	Total Recovery
Lysate Pool	3200	4.06E+10	1.30E+14			3200	6.25E+10	2.00E+14		
post C0HC + 0.45	4000	3.68E+10	1.47E+14	113%	113%	4000	4.73E+10	1.89E+14	95%	95%
TFF1 UF Retentate	400	2.96E+11	1.18E+14	80%	91%	400	4.30E+11	1.72E+14	91%	86%
TFF1 DF Retentate	580	2.14E+11	1.24E+14	105%	96%	502	3.64E+11	1.83E+14	106%	91%
TFF1 UF Permeate	3600	<4E8	1.44E+12	1%		3600	<4.0E8	1.44E+12	1%	

Figure 12A

	8,000 cells/cm^2, D5 Transfect, D9 Harvest					12,000 cells/cm2, D4 Transfect, D8 Harvest				
	Volume	ug/mL	Total HCP ug	HCP Step Clearance	Total HCP Clearance	Volume	ug/mL	Total HCP ug	HCP Step Clearance	Total HCP Clearance
Lysate Pool	3200	67.52	216065			3200	58.38	186813		
Post C0HC+0.45	4000	49.47	197862	8.4%	8.42%	4000	35.25	140988	24.5%	24.53%
TFF1 UF Retentate	400	170.90	68358	65.5%	68.36%	400	120.09	48036	65.9%	74.29%
TFF1 DF Retentate	580	52.17	30261	55.7%	86%	502	17.48	8775	81.7%	95%
TFF1 UF Permeate	3600	29.50	106186	54%		3600	26.69	96080.4	68%	

Figure 12B

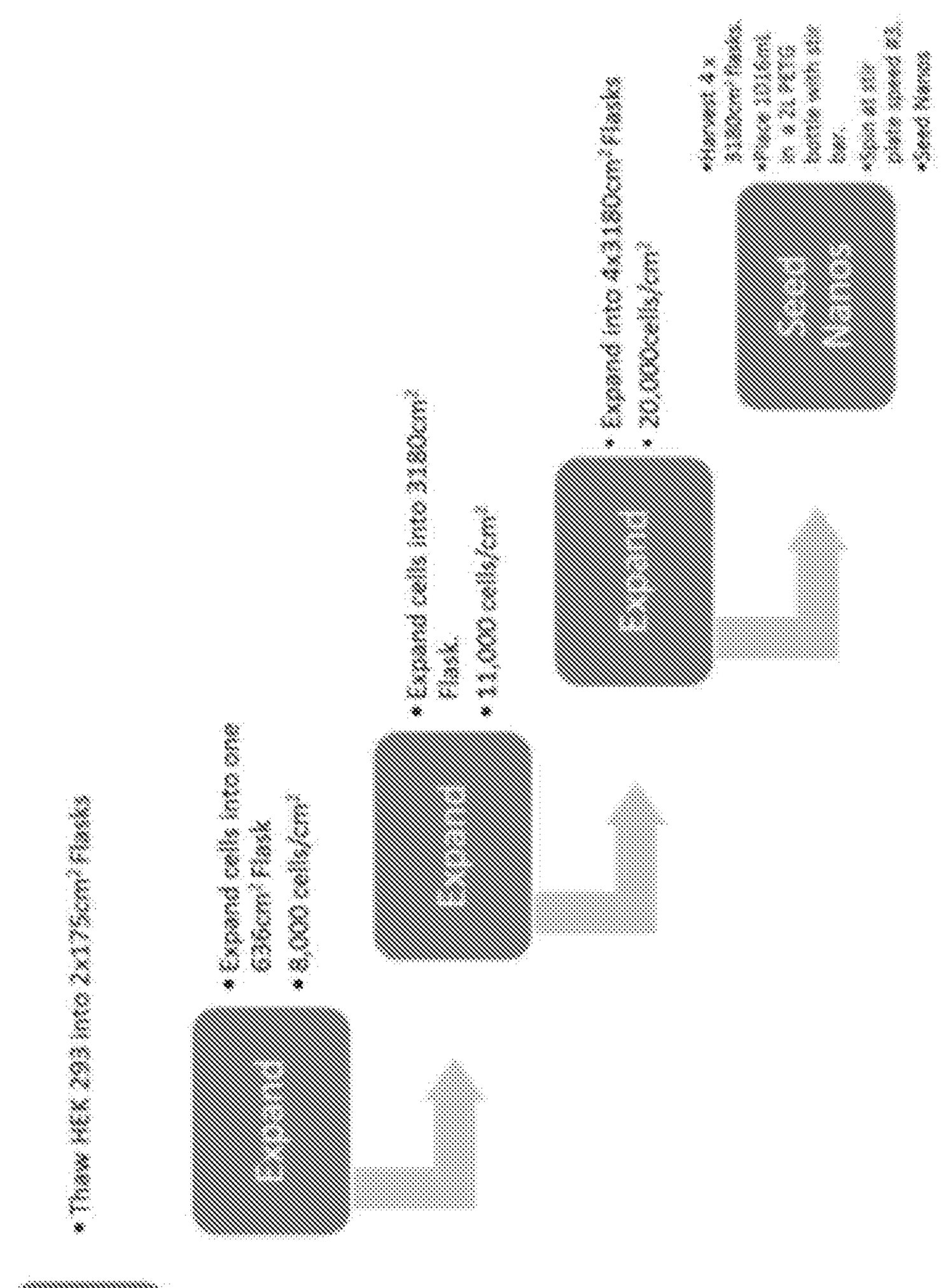
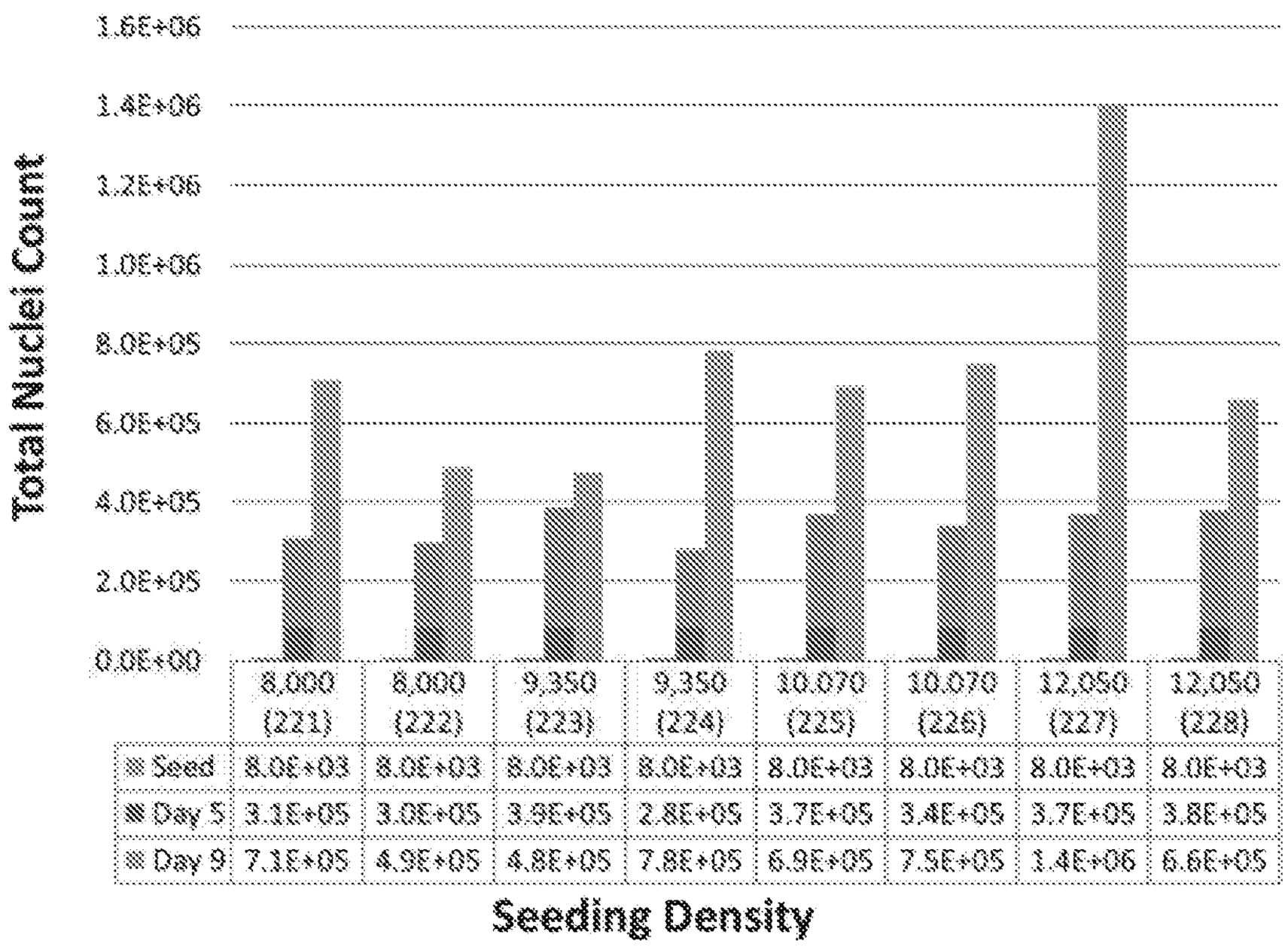
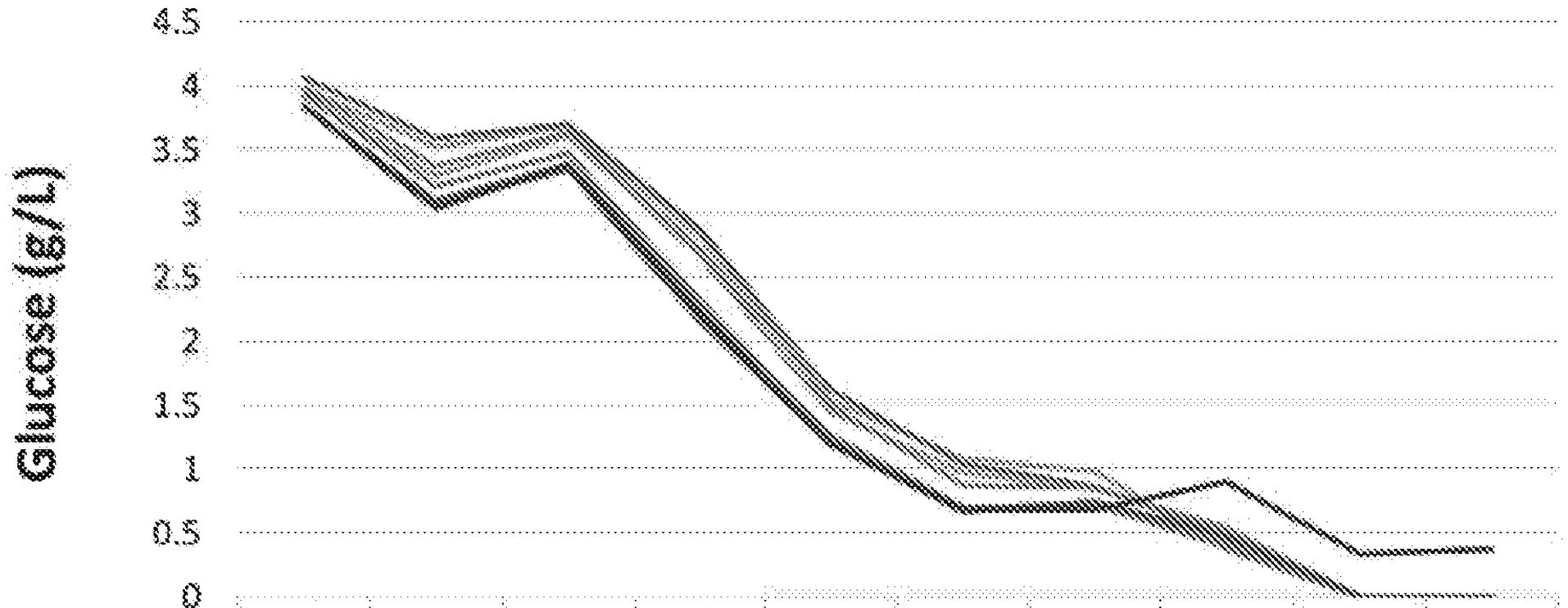

Thaw MEK 293 into 2x175cm² flasks
Expand cells into one 636cm² flask
8,000 cells/cm²
Expand
Expand cells into 312.5cm²
636cm² flask.
11,000 cells/cm²
Expand
Expand into 4x312.5cm² flasks
26,000cells/cm²
Count
Harvest 4x 312.5cm² flasks
Pool cells in a 2L vessel
Spin down cells
Spent Media

Figure 13



	8,000 (221)	8,000 (222)	9,350 (223)	9,350 (224)	10,070 (225)	10,070 (226)	12,050 (227)	12,050 (228)
Seed	8.0E+03	8.0E+03	8.0E+03	8.0E+03	8.0E+03	8.0E+03	8.0E+03	8.0E+03
Day 5	3.1E+05	3.0E+05	3.9E+05	2.8E+05	3.7E+05	3.4E+05	3.7E+05	3.8E+05
Day 9	7.1E+05	4.9E+05	4.8E+05	7.8E+05	6.9E+05	7.5E+05	1.4E+06	6.6E+05

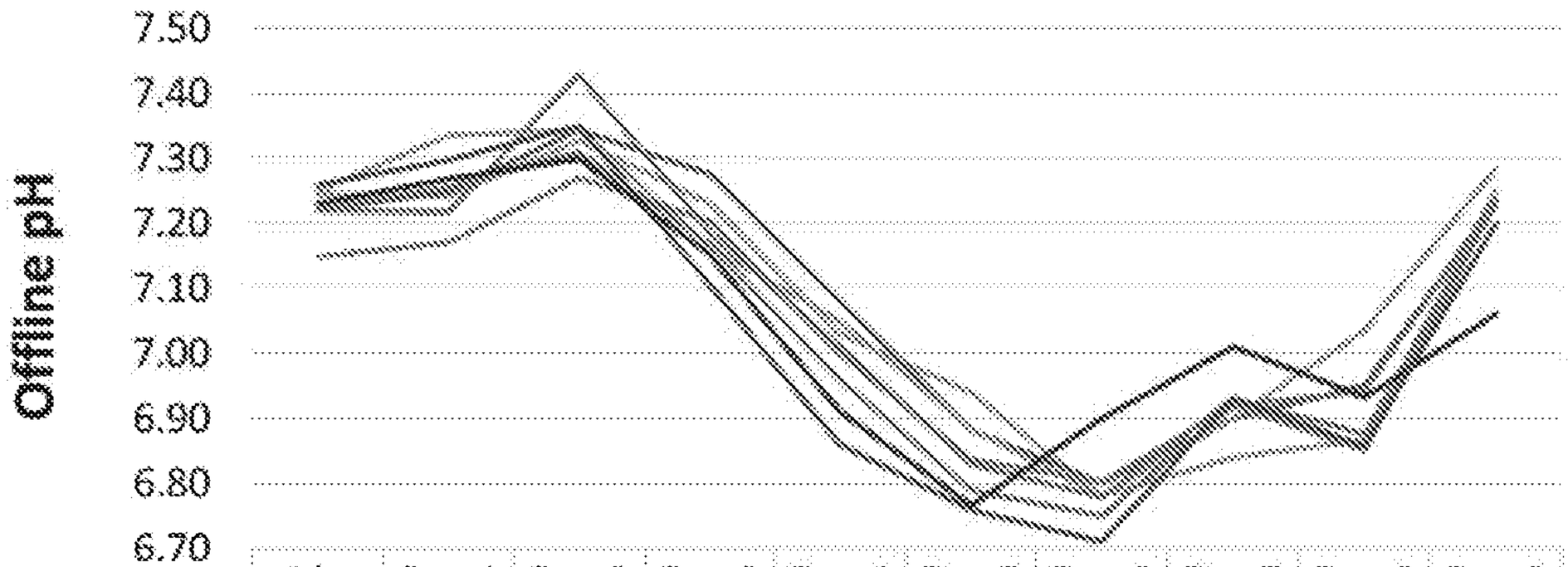
Figure 14A



	1 hr	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
8,000 (221)	3.98	3.53	3.68	2.82	1.54	1.08	0.98	0.37	0	0
8,000 (222)	4.06	3.59	3.7	2.88	1.62	1.04	0.86	0.47	0	0
9,350 (223)	3.92	3.3	3.61	2.67	1.45	0.97	0.84	0.54	0	0
9,350 (224)	3.98	3.37	3.64	2.72	1.49	0.87	0.85	0.51	0	0
10,070 (225)	3.84	3.11	3.39	2.27	1.26	0.7	0.71	0.43	0	0
10,070 (226)	3.94	3.22	3.47	2.32	1.24	0.7	0.73	0.39	0	0
12,050 (227)	3.85	3.09	3.34	2.16	1.2	0.66	0.74	0.47	0	0
12,050 (228)	3.84	3.04	3.38	2.21	1.19	0.68	0.68	0.9	0.33	0.37

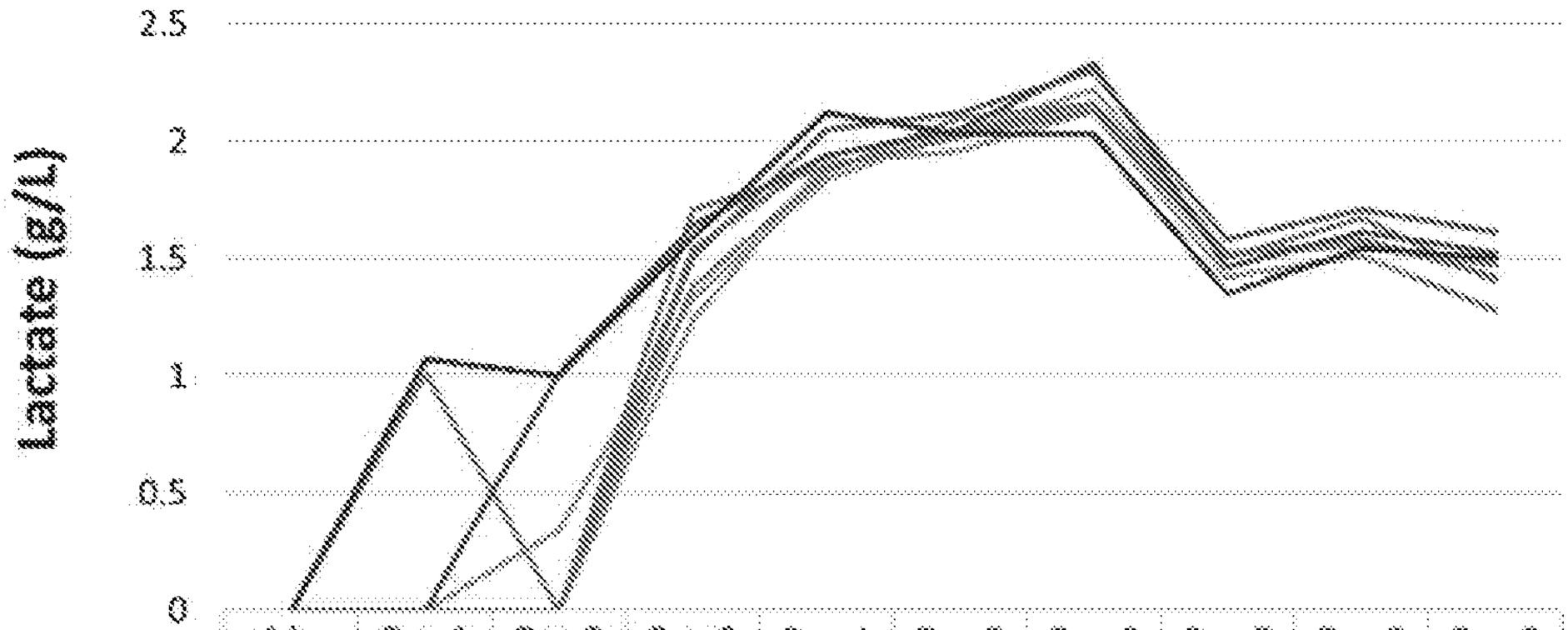
Days Post Seeding

Figure 14B



	1 hr	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
8,000 (221)	7.25	7.24	7.33	7.23	7.03	6.94	6.78	6.84	6.87	7.25
8,000 (222)	7.22	7.25	7.35	7.28	7.08	6.88	6.80	6.90	6.95	7.23
9,350 (223)	7.25	7.34	7.35	7.16	7.05	6.88	6.78	6.90	7.04	7.28
9,350 (224)	7.15	7.17	7.27	7.20	7.00	6.84	6.80	6.92	6.85	7.23
10,070 (225)	7.22	7.22	7.43	7.19	7.01	6.83	6.78	6.93	6.88	7.23
10,070 (226)	7.22	7.26	7.31	7.17	6.96	6.79	6.75	6.92	6.94	7.24
12,050 (227)	7.26	7.30	7.35	7.10	6.86	6.76	6.71	6.93	6.86	7.20
12,050 (228)	7.23	7.27	7.30	7.15	6.91	6.77	6.90	7.01	6.93	7.06

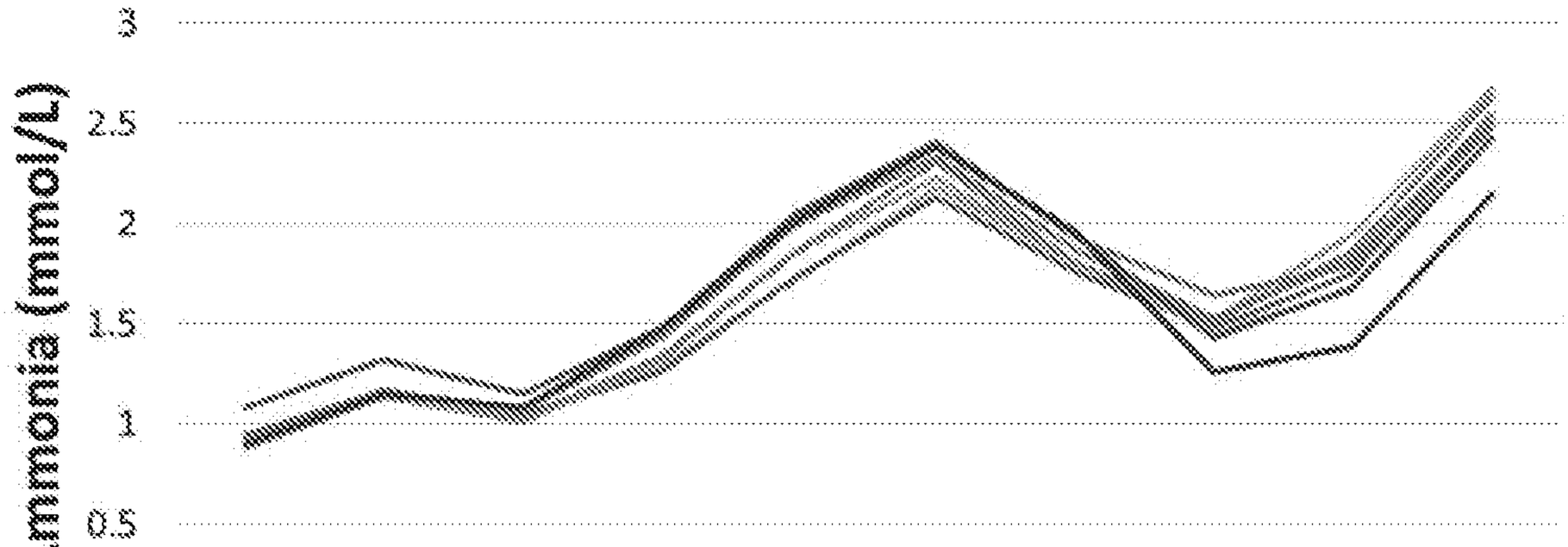
Figure 14C



	1 hr	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
8,000 (221)	0	0	0	1.24	1.91	1.96	2.13	1.42	1.51	1.28
8,000 (222)	0	0	0	1.51	2.05	2.12	2.3	1.58	1.71	1.61
9,350 (223)	0	0	0.34	1.33	1.84	2.04	2.22	1.52	1.55	1.48
9,350 (224)	0	1.01	0	1.38	1.87	2.1	2.16	1.52	1.56	1.47
10,070 (225)	0	0	0	1.54	1.94	2.04	2.16	1.51	1.58	1.42
10,070 (226)	0	0	0	1.71	1.89	2.02	2.13	1.5	1.67	1.4
12,050 (227)	0	0	1.01	1.64	1.94	2.05	2.33	1.46	1.61	1.52
12,050 (228)	0	1.07	1	1.59	2.12	2.03	2.03	1.35	1.54	1.5

Days Post Seeding

Figure 14D



	1 Hr*	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
8,000 (221)	0.92	1.16	1.02	1.29	1.73	2.18	1.8	1.52	1.95	2.67
8,000 (222)	0.94	1.17	1.05	1.26	1.74	2.13	1.76	1.48	1.75	2.55
9,350 (223)	0.9	1.14	1.01	1.32	1.86	2.23	1.81	1.46	1.67	2.54
9,350 (224)	0.92	1.17	1.03	1.34	1.87	2.31	1.8	1.51	1.86	2.63
10,070 (225)	0.9	1.16	1.07	1.43	2	2.33	1.87	1.53	1.82	2.51
10,070 (226)	0.88	1.14	1.07	1.46	2.06	2.41	1.93	1.64	1.79	2.48
12,050 (227)	1.08	1.32	1.15	1.48	2.06	2.38	1.94	1.43	1.68	2.43
12,050 (228)	0.9	1.15	1.08	1.47	2.02	2.38	1.96	1.26	1.39	2.15

Figure 14E

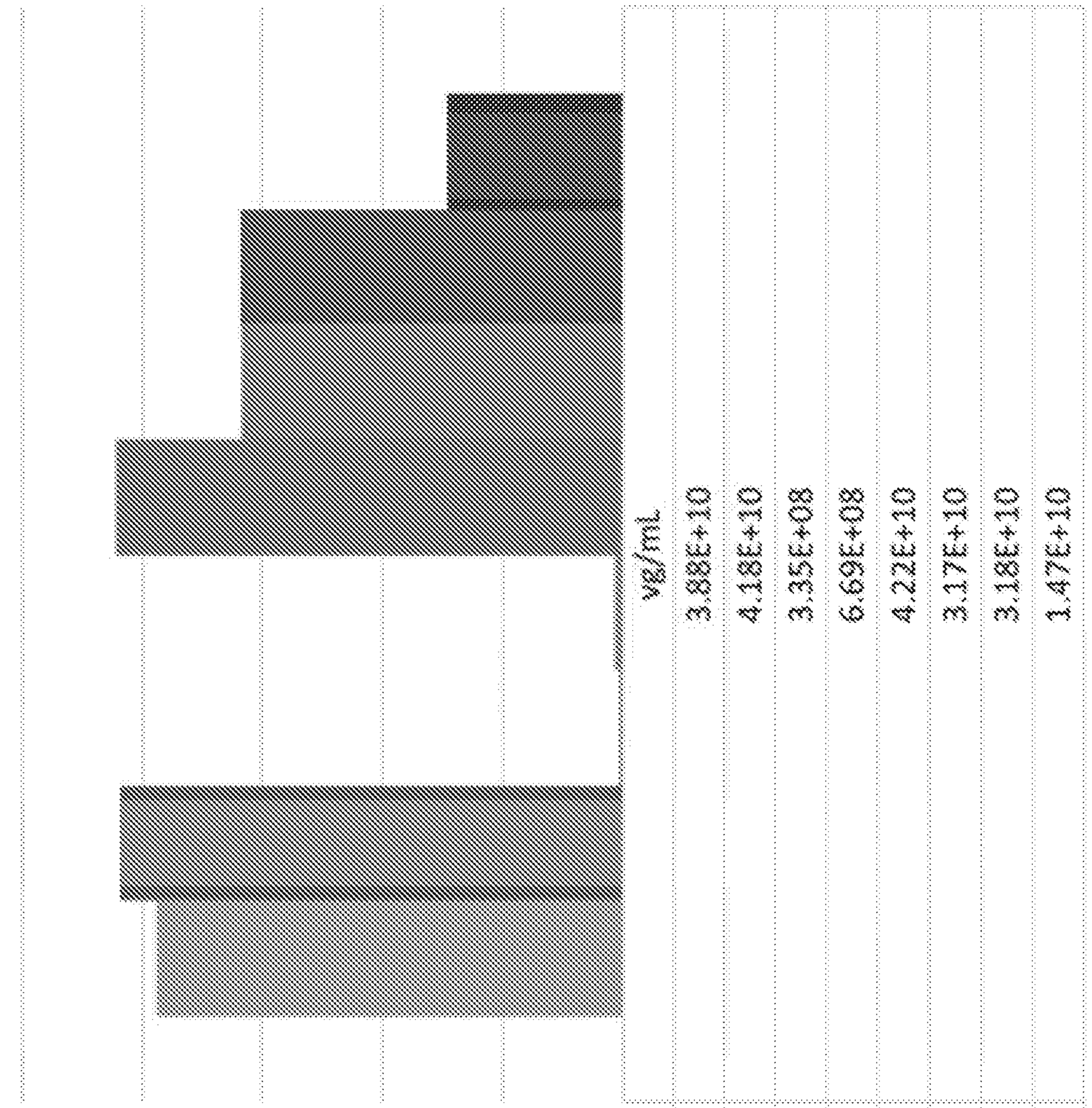

Vg/mL
3.88E+10
4.18E+10
3.35E+08
6.69E+08
4.22E+10
3.17E+10
3.18E+10
1.47E+10
Vg/mL
5.00E+10
4.00E+10
3.00E+10
2.00E+10
1.00E+10
0.00E+00
Vg/mL
8,000 cells/cm2 (2211
8,000 cells/cm2 (2221
9,350 cells/cm2 (2231
9,350 cells/cm2 (2241
10,070 cells/cm2 (2251
10,070 cells/cm2 (2261
12,050 cells/cm2 (2271
12,050 cells/cm2 (2281

Figure 15A

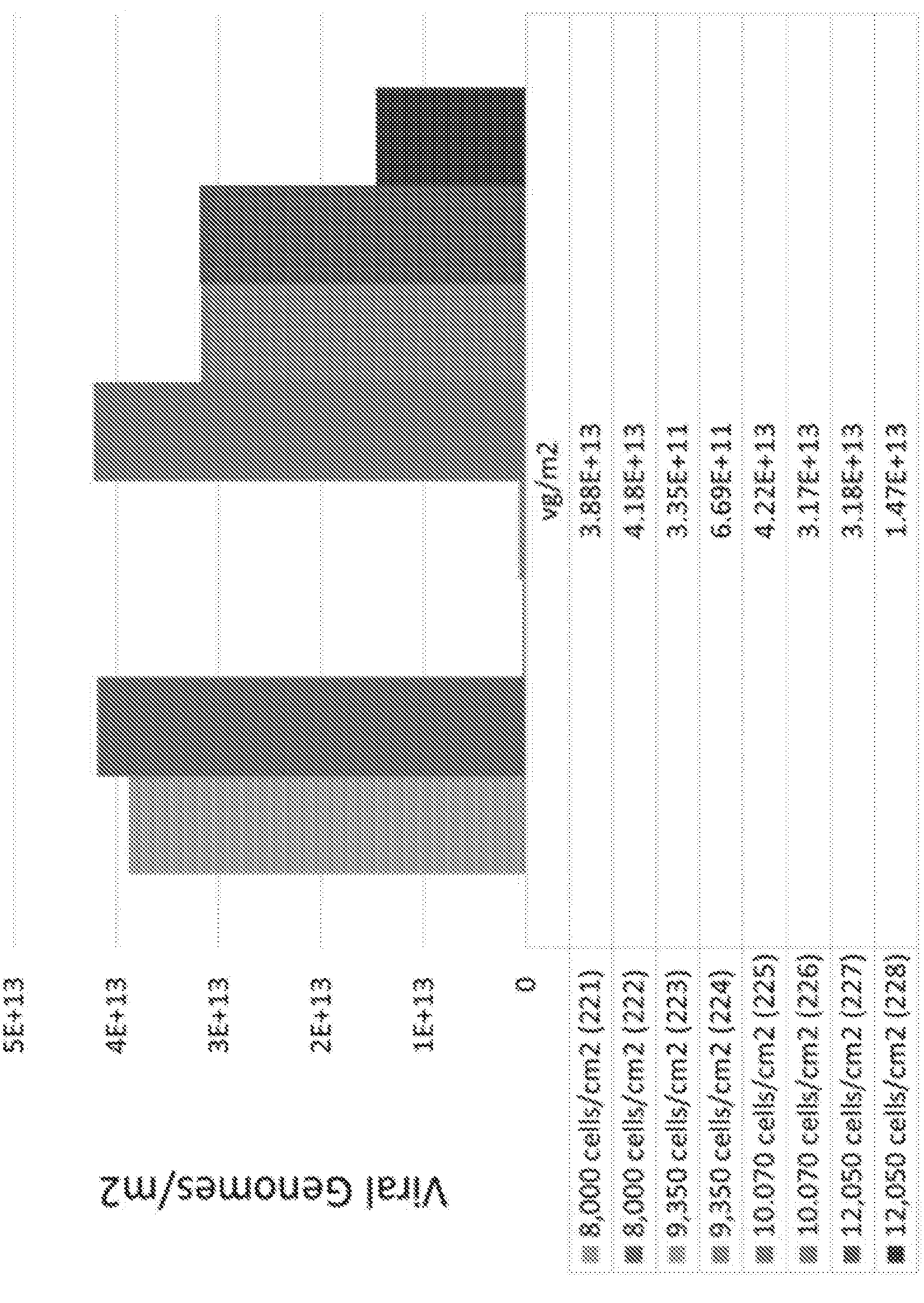

Viral Genomes/m2
0
1E+13
2E+13
3E+13
4E+13
5E+13
vg/m2
3.88E+13
4.18E+13
3.35E+11
6.69E+11
4.22E+13
3.17E+13
3.18E+13
1.47E+13
8,000 cells/cm2 (221)
8,000 cells/cm2 (222)
9,350 cells/cm2 (223)
9,350 cells/cm2 (224)
10,070 cells/cm2 (225)
10,070 cells/cm2 (226)
12,050 cells/cm2 (227)
12,050 cells/cm2 (228)

Figure 15B

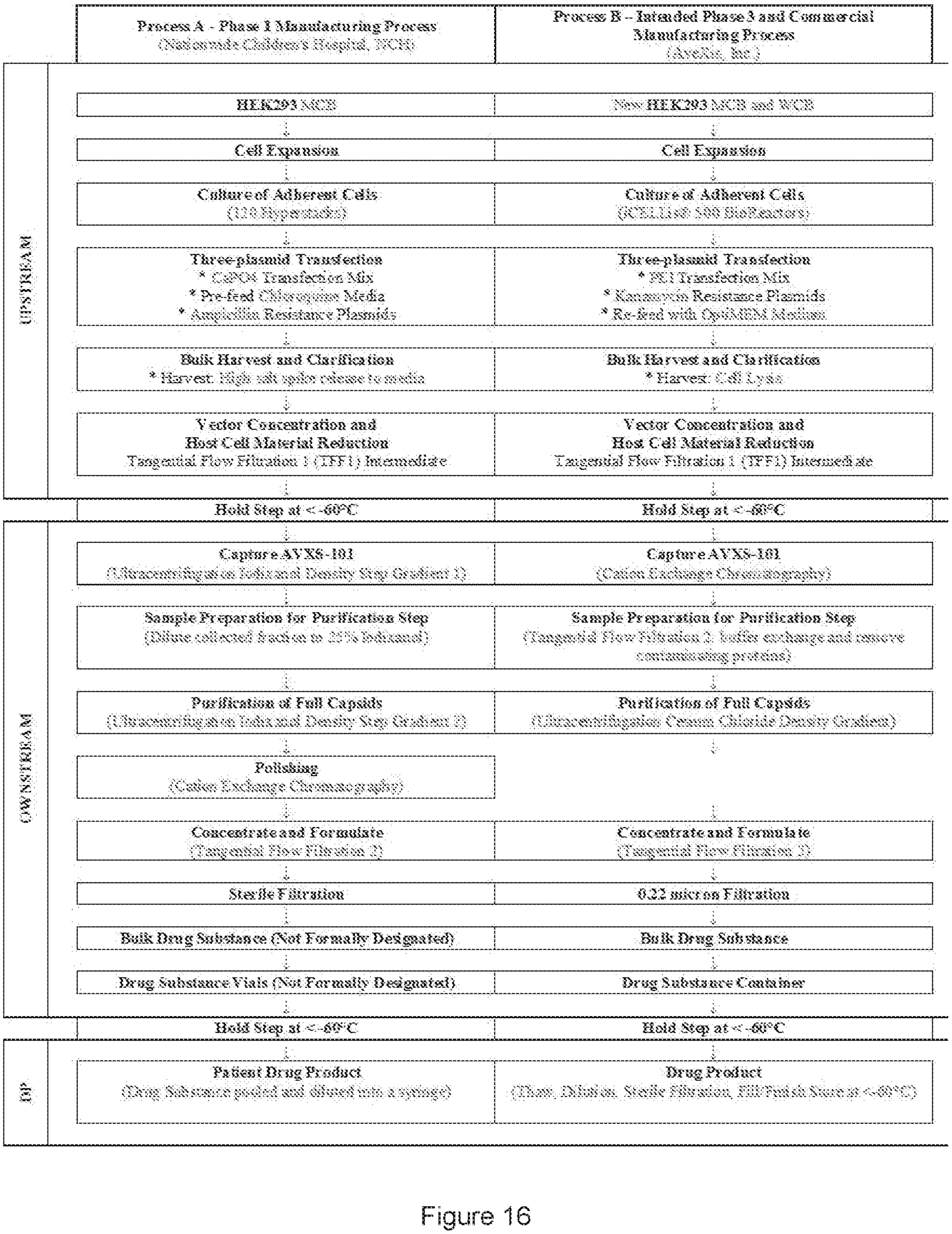

Process A - Phase 1 Manufacturing Process
(Nationwide Children's Hospital, NCH)
Process B – Intended Phase 3 and Commercial Manufacturing Process
(AveXis, Inc.)
UPSTREAM
HEK293 MCB
New HEK293 MCB and WCB
Cell Expansion
Cell Expansion
Culture of Adherent Cells
(10 Hyperstacks)
Culture of Adherent Cells
(iCELLis® 500 BioReactors)
Three-plasmid Transfection
* CaPO4 Transfection Mix
* Pre-feed Chloroquine Media
* Ampicillin Resistance Plasmids
Three-plasmid Transfection
* PEI Transfection Mix
* Kanamycin Resistance Plasmids
* Re-feed with OptiMEM Medium
Bulk Harvest and Clarification
* Harvest: High salt spike release to media
Bulk Harvest and Clarification
* Harvest: Cell Lysis
Vector Concentration and Host Cell Material Reduction
Tangential Flow Filtration 1 (TFF1) Intermediate
Vector Concentration and Host Cell Material Reduction
Tangential Flow Filtration 1 (TFF1) Intermediate
Hold Step at <-60°C
Hold Step at <-60°C
DOWNSTREAM
Capture AVXS-101
(Ultracentrifugation Iodixanol Density Step Gradient 1)
Capture AVXS-101
(Cation Exchange Chromatography)
Sample Preparation for Purification Step
(Dilute collected fraction to 25% Iodixanol)
Sample Preparation for Purification Step
(Tangential Flow Filtration 2: buffer exchange and remove contaminating proteins)
Purification of Full Capsids
(Ultracentrifugation Iodixanol Density Step Gradient 2)
Purification of Full Capsids
(Ultracentrifugation Cesium Chloride Density Gradient)
Polishing
(Cation Exchange Chromatography)
Concentrate and Formulate
(Tangential Flow Filtration 3)
Concentrate and Formulate
(Tangential Flow Filtration 3)
Sterile Filtration
0.22 micron Filtration
Bulk Drug Substance (Not Formally Designated)
Bulk Drug Substance
Drug Substance Vials (Not Formally Designated)
Drug Substance Container
Hold Step at <-60°C
Hold Step at <-60°C
DP
Patient Drug Product
(Drug Substance pooled and diluted into a syringe)
Drug Product
(Thaw, Dilution, Sterile Filtration, Fill/Finish Store at <-60°C)

Figure 16

Test Description	Test Method	Proposed Test Limit	NCH Phase I Lot NCHAAV93MN0613 Date Manufactured: 18-Dec-2013 Test Date: 13-Feb-2017 to 08-Aug-2017 (1)	AVXS-101 Lot 680156 Date Manufactured: 07-Nov-2017 Test Date: 08-Nov-2017 to 26-Dec-2017	AVXS-101 Lot 680307 Date Manufactured: 04-Dec-2017 Test Date: 05-Dec-2017 to 30-Jan-2017
Genomic Titer by ddPCR	SOP-137	$2.0x10^{13}$ – $8.0x10^{13}$ vg/mL	$1.1x10^{13}$ vg/mL (n=9) (2)	$3.7x10^{13}$ vg/mL (n=9) (2)	$4.0x10^{13}$ vg/mL (n=14) (2)
Infectious Titer	SOP-192	Report Results	$5.9x10^{9}$ IU/mL (3)	$1.3x10^{10}$ IU/mL (3)	$6.7x10^{9}$ IU/mL (3)
Subvisible Particles	SOP-262	≤ 6000 particles/cont ≥ 10 µm ≤ 600 particles/cont ≥ 25 µm	22 particles/cont ≥ 10 µm 4 particles/cont ≥ 25 µm	119 particles/cont ≥ 10µm 4 particles/cont ≥ 25µm	9 particles/cont ≥ 10µm 2 particles/cont ≥ 25µm
pH	SOP-057	7.5 – 8.5	7.9	7.9	8.0
Osmolality	SOP-128	384 – 448 mOsm/kg	410 mOsm/kg	415 mOsm/kg	410 mOsm/kg
Appearance by Visual Inspection	SOP-164	Clear to slightly opaque, colorless to faint white solution, free of visible particulates	Clear and colorless solution, free of visible particles	Faint white, slightly opaque, free of visible particles (4)	Colorless, slightly opaque, free of visible particles
Total Protein by BCA	SOP-184	128 – 320 µg/mL per $1.0x10^{13}$ vg/mL	167 µg/mL (2) (177 µg/mL) (3)	179 µg/mL (2) (661 µg/mL) (3)	176 µg/mL (2) (702 µg/mL) (3)
Residual hcDNA by qPCR	SOP-180	≤ $1.2x10^{6}$ pg/mL per $1.0x10^{13}$ vg/mL	$3.7×10^{5}$ pg/mL (2) ($3.9×10^{6}$ pg/mL) (3)	$0.76×10^{5}$ pg/mL (2) ($2.8×10^{5}$ pg/mL) (3)	$0.68×10^{5}$ pg/mL (2) ($2.72×10^{5}$ pg/mL) (3)
% Empty Capsid by AUC	SOP-263	≤ 7%	7%	2%	4%
Vector ID by SDSPAGE	SOP-180	Main Bands of VP1, VP2, VP3 co-migrate with AVXS-101 control	Main Bands of VP1, VP2, VP3 co-migrate with AVXS-101 control Main Band MWs: VP1: 84.9–89.5 kDa VP2: 65.9–69.1 kDa VP3: 57.1–59.0 kDa	Main Bands of VP1, VP2, VP3 co-migrate with AVXS-101 control Main Band MWs: VP1: 83.6-84.4 VP2: 64.6-65.5 VP3: 56.2-57.2	Main Bands of VP1, VP2, VP3 co-migrate with AVXS-101 control Main Band MWs: VP1: 82.9-84.0 VP2: 64.3-65.2 VP3: 56.0-56.7
Purity by SDS-PAGE	SOP-180	% Total Purity (VP1, VP2, VP3): ≥ 95%	% Total Purity: 98% (Avg. n=4 gels)	% Total Purity: 99% (Avg. n=4 gels)	% Total Purity: 99% (Avg. n=4 gels)

Figure 17A

Test Description	Test Method	Proposed Test Limit	NCH Phase I Lot NCHAAV9SMN0613 Date Manufactured: 18-Dec-2013 Test Date: 13-Feb-2017 to 08-Aug-2017 [3]	AVXS-101 Lot 600156 Date Manufactured: 07-Nov-2017 Test Date: 08-Nov-2017 to 26-Dec-2017	AVXS-101 Lot 600307 Date Manufactured: 04-Dec-2017 Test Date: 05-Dec-2017 to 30-Jan-2017
Total Impurities by SDS-PAGE	SOP-180	% Total Impurities ≤5% No single un-named impurity >2% <u>Named Impurities: Report value</u> – Imp 1A (~71-73kDa) – Imp 1 (~61–67 kDa) – Imp 2 (~56–64 kDa) – Imp 3 (~48–58 kDa) – Imp 4 (~33–38 kDa) – Imp 5 (~30–34 kDa)	% Total Impurities: 2% (Avg n=4 gels) <u>Individual Impurities (Avg n=4 gels)</u> – Imp 1: 1.3% – Imp 3: <LOQ (1.0%)	% Total Impurities: 1% (Avg n=4 gels) <u>Individual Impurities (Avg n=4 gels)</u> – Imp 1: <LOQ (1.0%) – Imp 3: <LOQ (1.0%) – Imp 4: <LOQ (1.0%)	% Total Impurities: 1% (Avg n=4 gels) <u>Individual Impurities (Avg n=4 gels)</u> – Imp 1: 1.0% – Imp 4: <LOQ (1.0%)
Vector Identity by Western	SOP-179	Positive for AAV Capsid Protein	Positive for AAV Capsid Protein Main Band MW VP1: 79.7 kDa VP2: 64.1 kDa VP3: 55.7 kDa	Positive for AAV Capsid Protein Main Band MW VP1: 80.2 kDa VP2: 66.2 kDa VP3: 58.2 kDa	Positive for AAV Capsid Protein Main Band MW VP1: 79.1 kDa VP2: 64.8 kDa VP3: 57.5 kDa
Residual HCP by ELISA	SOP-185	≤ 40 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (8 ng/mL) [5]	< LOQ (8 ng/mL) [5]	< LOQ (8 ng/mL) [5]
Vector Genome Identity by ddPCR	SOP-137	Confirms	Confirms	Confirms	Confirms
Residual BSA by ELISA	SOP-181	≤ 3.0 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (0.50 ng/mL) [5]	< LOQ (0.50 ng/mL) [5]	< LOQ (0.50 ng/mL) [5]
Residual Benzonase by ELISA	SOP-182	≤ 1.0 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (0.20 ng/mL) [5]	< LOQ (0.20 ng/mL) [5]	< LOQ (0.20 ng/mL) [5]
In-vivo Relative Potency	SOP-285	80 – 130%	100% [6]	97%	96%

[1] NCH Phase I Lot AAV9SMN0613 was manufactured prior to the current "Proposed Test Limit" for genomic titer by ddPCR. Genomic titer value for this lot was re-established in August 2017 using improved SOP-137 (v3).

[2] Differences in Genomic Titer results between Process A and Process B lots are due to different manufacturing target concentrations. Lot NCH AAV9SMN0613 was originally formulated a lower target titer concentration now determined to be $1.1x10^{13}$ vg/mL by the currently used ddPCR assay (SOP-137), while AVXS-101 Lots 600156 and 600307 were formulated with a target titer concentration of $4.0x10^{13}$ vg/mL when measured by the same method (SOP-137).

[3] Adjusted result per $1.0x10^{13}$ vg/mL to enable appropriate specifications across range of acceptable concentrations from $2.0x10^{13}$ vg/mL to $6.0x10^{13}$ vg/mL. Actual values have been multiplied by the following factors to provide values per $1.0x10^{13}$ vg/mL: 1/1.06 (Lot NCH AAV9SMN0613), 1/3.7 (Lot 600156) and 1/4.0 (Lot 600307).

[4] Differences in appearance results between Process A and Process B are due to different vector concentrations (genomic titer). Lot NCH AAV9SMN0613 has a significantly lower vector concentration than the Process B lots. As a result, Lot NCH AAV9SMN0613 is more dilute leading to a more clear and colorless solution while the colorless to white and slightly opaque observations for Process B lots results from a close to 4 times concentration of viral particles in solution per mL.

[5] Actual result not adjusted to $1.0x10^{13}$ vg/mL because results are below LOQ of the respective methods.

[6] Lot NCHAAV9SMN0613 is designated as the initial potency Reference Standard for SOP-285 with an assigned potency value of 100%. All results generated using SOP-285 v5.

Figure 17B

Test Description	Proposed Test Limits	Pair Wise Comparison		
		Process A (Phase 1)	Process B (Phase 3)	Relative %Difference
Genomic Titer by ddPCR	$2.0x10^{13}$ – $6.0x10^{13}$ vg/mL	$1.1x10^{13}$ vg/mL	$3.7x10^{13}$ vg/mL	NA [1]
Infectious Titer	Report Results	$0.59x10^{10}$ IU/mL	$1.3x10^{10}$ IU/mL	120% [2]
Subvisible Particles ≥ 10 µm	≤ 6000 particles/cont ≥ 10 µm	22	119	441% [3]
Subvisible Particles ≥ 25 µm	≤ 600 particles/cont ≥ 25 µm	4	4	0%
pH	7.5 – 8.5	7.9	7.9	0%
Osmolality	384 – 448 mOsm/kg	410 mOsm/kg	415 mOsm/kg	1%
Appearance	Clear to slightly opaque, colorless to faint white solution, free of visible particulates	Clear and colorless solution, free of visible particles	Faint white, slightly opaque, free of visible particles	NA
Total Protein by BCA	128 – 320 µg/mL per $1.0x10^{13}$ vg/mL	167 µg/mL	179 µg/mL	7%
Residual hcDNA by qPCR	≤ $1.2x10^{6}$ pg/mL per $1.0x10^{13}$ vg/mL	$3.7×10^{5}$ pg/mL	$0.76×10^{5}$ pg/mL	80%
% Empty Capsid by AUC	≤ 7%	7%	2%	71% [4]
Purity by SDS-PAGE	≥ 95%	98%	99%	1%
Total Impurities by SDS-PAGE	≤ 5%	2%	1%	50%
Residual HCP by ELISA	≤ 40 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (8 ng/mL)	< LOQ (8 ng/mL)	0%
Residual BSA by ELISA	≤ 3.0 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (0.50 ng/mL)	< LOQ (0.50 ng/mL)	0%
Residual Benzonase by ELISA	≤ 1.0 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (0.20 ng/mL)	< LOQ (0.20 ng/mL)	0%
In-vivo Relative Potency	80 – 120%	100%	97%	3%

[1] Relative % difference not applicable as the manufacturing target for genomic titer was changed between Process A and Process B.

[2] This value should be viewed in the context of expected variability for a biological assay which can often vary between 50% to 200% of the true value. That being said, AveXis recognized the need for improvement and has recently performed additional development and qualification work to improve this assay (SOP-328 and RPT-471). It should also be noted that infectivity efficiency of the HeLa RC32 cell line by AAV9-based viral vectors remains challenging.

[3] Considerable relative % difference for subvisible particles between Process A and Process B is noted; however, the absolute value for Process B is well below the proposed testing limits.

[4] Process B using CsCl gradient is more effective in removing empty capsids when compared to Process A which used iodixanol.

Figure 18

Test Description	Proposed Test Limits	Pair Wise Comparison		
		Process B (000150)	Process B (000307)	Relative %Difference
Genomic Titer by ddPCR	$2.0x10^{13}$ – $6.0x10^{13}$ vg/mL	$3.7x10^{13}$ vg/mL	$4.0x10^{13}$ vg/mL	8%
Infectious Titer	Report Results	$1.3x10^{10}$ IU/mL	$0.67x10^{10}$ IU/mL	48%
Subvisible Particles ≥ 10 µm	≤ 6000 particles/cont ≥ 10 µm	119	9	92% [a]
Subvisible Particles ≥ 25 µm	≤ 600 particles/cont ≥ 25 µm	4	2	50%
pH	7.5 – 8.5	7.9	8.0	1%
Osmolality	384 – 448 mOsm/kg	415 mOsm/kg	410 mOsm/kg	1%
Appearance	Clear to slightly opaque, colorless to faint white solution, free of visible particulates	Faint white, slightly opaque, free of visible particles	Colorless, slightly opaque, free of visible particles	NA
Total Protein by BCA	128 – 320 µg/mL per $1.0x10^{13}$ vg/mL	179 µg/mL	176 µg/mL	2%
Residual hcDNA by qPCR	≤ $1.2x10^{6}$ pg/mL per $1.0x10^{13}$ vg/mL	$0.76×10^{5}$ pg/mL	$0.68×10^{5}$ pg/mL	11%
% Empty Capsid by AUC	≤ 7%	2%	4%	100% [a]
Purity by SDS-PAGE	≥ 95%	99%	99%	0%
Total Impurities by SDS-PAGE	≤ 5%	1%	1%	0%
Residual HCP by ELISA	≤ 40 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (8 ng/mL)	< LOQ (8 ng/mL)	0%
Residual BSA by ELISA	≤ 3.0 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (0.50 ng/mL)	< LOQ (0.50 ng/mL)	0%
Residual Benzonase by ELISA	≤ 1.0 ng/mL per $1.0x10^{13}$ vg/mL	< LOQ (0.20 ng/mL)	< LOQ (0.20 ng/mL)	0%
In-vivo Relative Potency	80 – 120%	97%	96%	1%

[a] Relative % difference for subvisible particles and % Empty Capsid is ~100% between the two Process B lots, however, all values are well below the proposed testing limits and the Process B lots are considered to be consistent with regards to these test results.

Figure 19

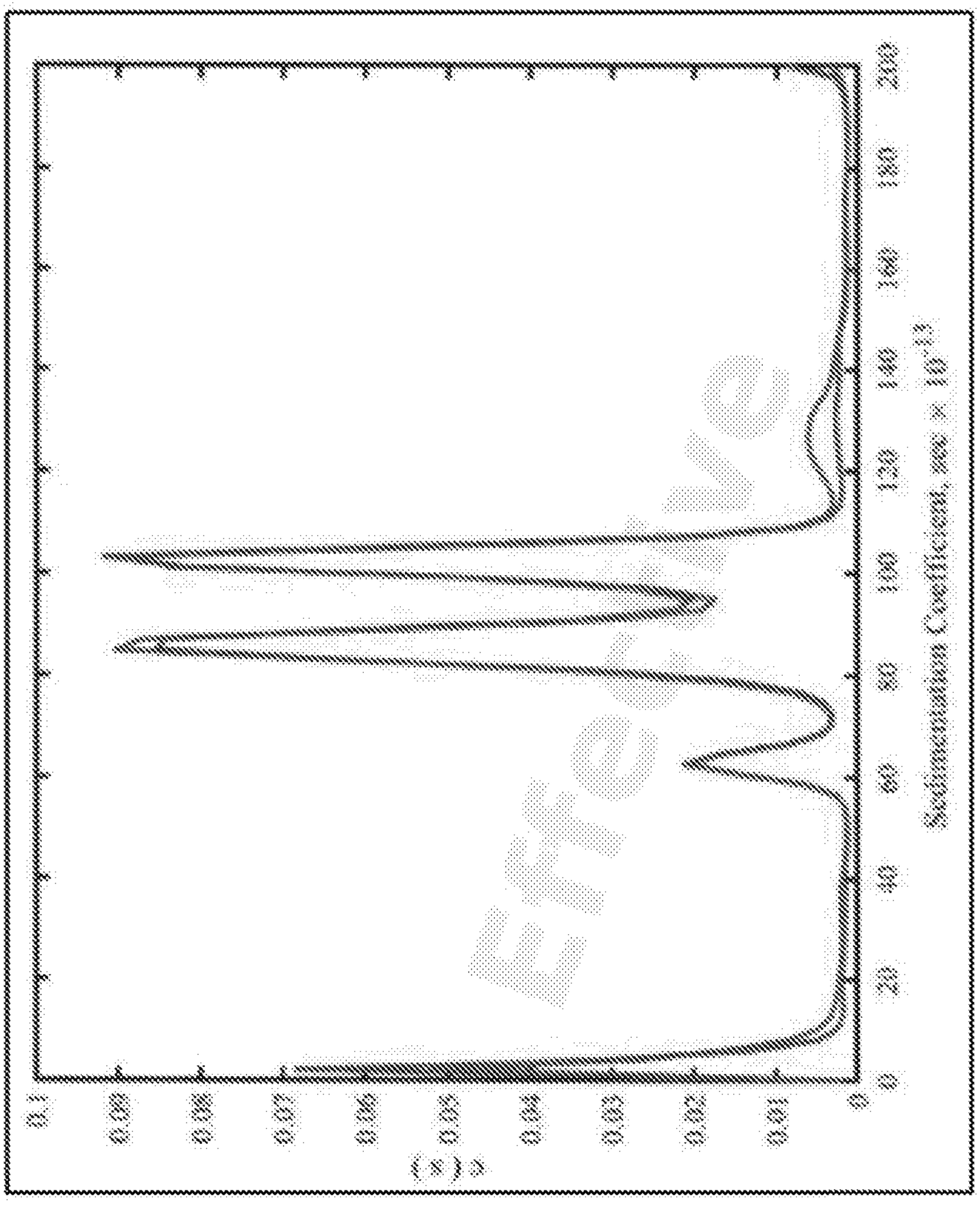
Attribute	Method	Stability Time-point (mos)				
		0	3	6	9	12
		February 2017	May 2017	August 2017	November 2017	February 2018
Genomic Titer by ddPCR	SOP-137 v1.0	$6.6x10^{12}$ vg/mL	$6.1x10^{12}$ vg/mL	$6.5x10^{12}$ vg/mL	NA	NA
	SOP-137 v3.0	N/A	N/A	$1.1x10^{13}$ vg/mL [2]	$8.7x10^{12}$ vg/mL	Target 26Feb18
In vivo Relative Potency [2]	SOP-285	100%	92%	95%	101%	Pending
Infectious Titer	SOP-102	$6.3x10^{9}$ IU/mL	$8.4x10^{9}$ IU/mL [3]	$1.1x10^{10}$ IU/mL	$6.3x10^{9}$ IU/mL	Pending
Purity by SDS-PAGE	SOP-180	%Total Purity: 98% (n=4 gels) %Total Impurities: 2% (n=4 gels) Individual Impurities: Imp 1: 1.7% Imp 3: <LOQ (1.0%)	% Total Purity: 98% (n=4 gels) %Total Impurities: 2% (n=4 gels) Individual Impurities: Imp 1: 1.7%	% Total Purity: 99% (n=4 gels) %Total Impurities: 1% (n=4 gels) Individual Impurities: Imp 1: 1.2%	% Total Purity: 99% (Avg n=4 gels) %Total Impurities: 1% (Avg n=4 gels Individual Impurities: Imp 1: 1.3%	Pending

[1] Lot was re-tested using an improved method, SOP-137 v3.0, per CAPA-19 around the 6-mo timepoint. As a result, a new titer value of $1.1x10^{13}$ vg/mL was re-assigned for NCH Lot NCHAAV9SMN0613. All subsequent stability time-points were analyzed using SOP-137 v3.0.

[2] Lot NCHAAV9SMN0613 was manufactured in December 2013 and was tested on February 2017 as T0. A 100% potency was assigned at T0 and the potencies for all subsequent time points were calculated relative to the T0 value.

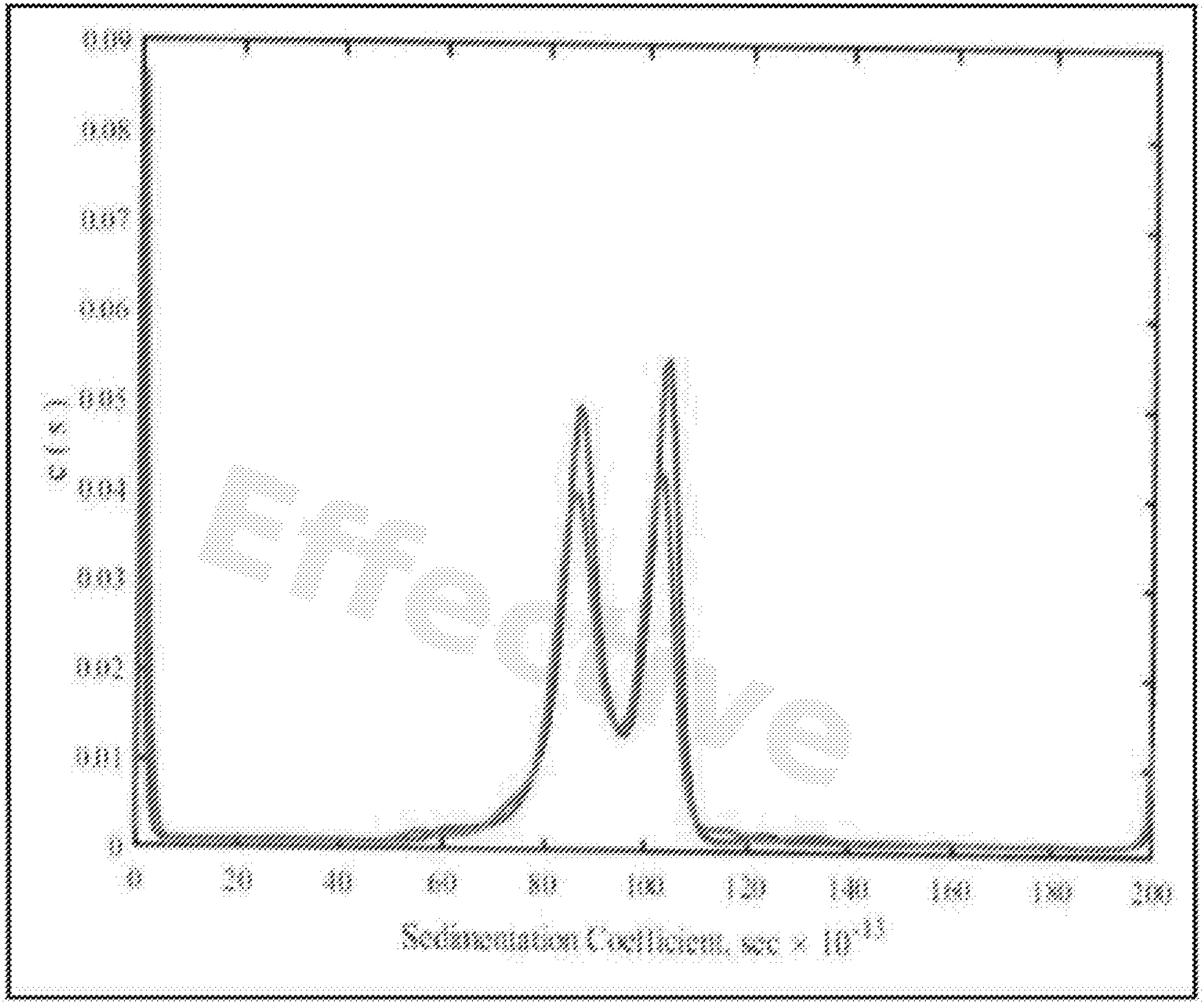
[3] Infectious Titer testing was performed approximately 4 months from T_0 as it was added to the respective stability protocol on June 2017.

Figure 20



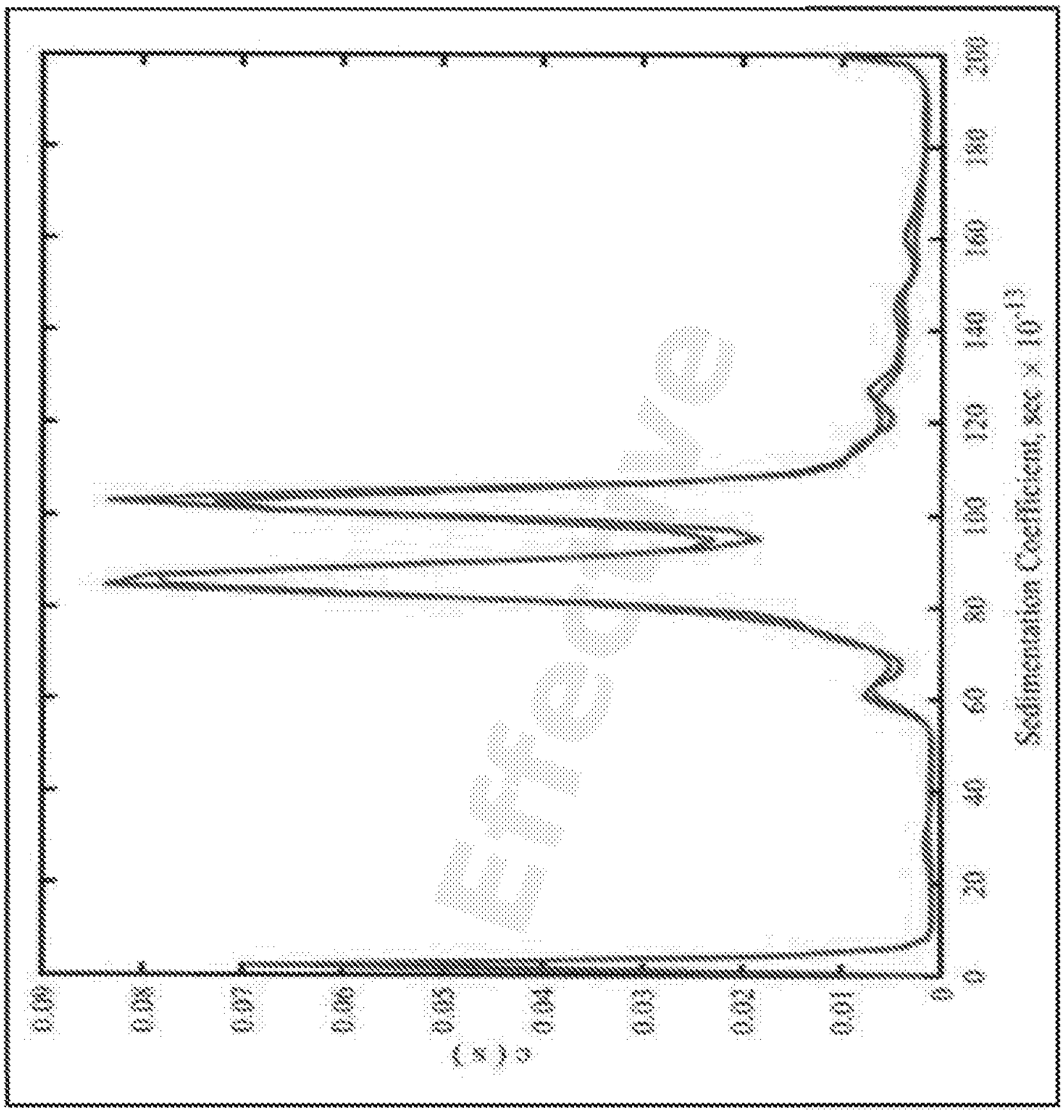
Exp. Peak			Fab Peak 1			Fab Peak 2		
XLEAP sample	Rep 1 (red)	Rep 2 (blue)	Ave	Rep 1 (red)	Rep 2 (blue)	Ave	Rep 1 (red)	Rep 2 (blue)
r_H value	62.58	62.50	62.54	85.23	85.33	85.28	101.76	101.94
MH (nm)	1.97	2.36	2.17	1.97	2.36	2.17	3.11	3.77
α_{area}	8.53	8.39	8.46	47.26	49.14	48.45	37.20	39.36
r_H value	101.85							
MEM (nm)	3.44							
α_{area}	38.28							
MEM (nm)	4.43							
α_{area}	4.85							
MEM (nm)	4.01							

Figure 21



600156	Empty Peak			Full Peak 1			Full Peak 2		
	s-value	%area	MW (MDa)	s-value	%area	MW (MDa)	s-value	%area	MW (MDa)
Rep 1 (red)	61.80	1.29	2.77	86.74	54.23	4.62	104.12	43.72	6.06
Rep 2 (blue)	55.52	1.83	2.97	85.89	54.95	5.55	102.86	41.80	7.31
Avg	**58.66**	**1.56**	**2.87**	**86.32**	**54.59**	**5.09**	**103.49**	**42.76**	**6.69**

Figure 22



	Empty Peak		Full Peak 1		Full Peak 2	
	s-value	%area	s-value	%area	s-value	%area
Rep 1 (red)	62.12	3.68	85.51	53.18	101.91	5.30
Rep 2 (blue)	60.83	3.78	85.14	53.43	102.33	4.93
Ave	61.48	3.73	85.33	53.31	102.13	4.97

Figure 23