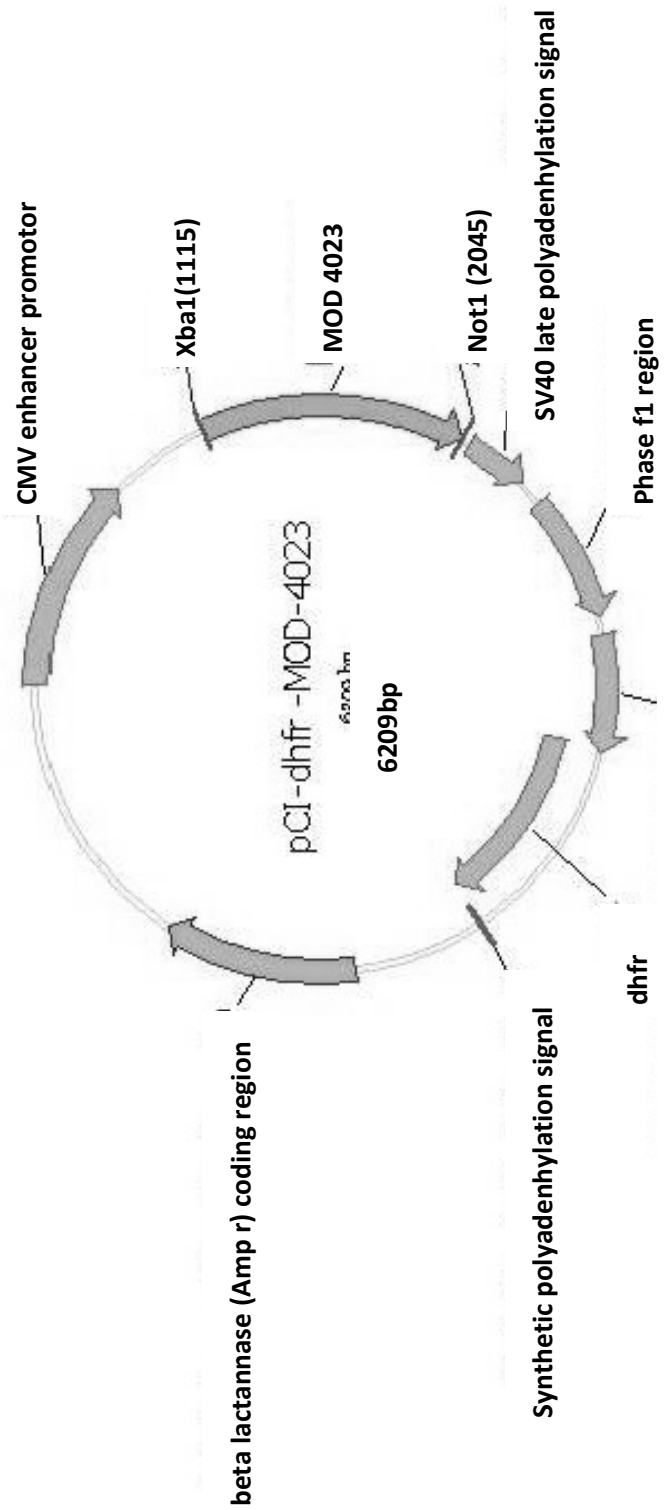

CMV enhancer promotor
Xba1(1115)
MOD 4023
Not1 (2045)
SV40 late polyadenhylation signal
Phase f1 region
pCI-dhfr -MOD-4023
6209bp
beta lactannase (Amp r) coding region
Synthetic polyadenhylation signal
dhfr
SV40 enhancer and early promotor

Figure 2

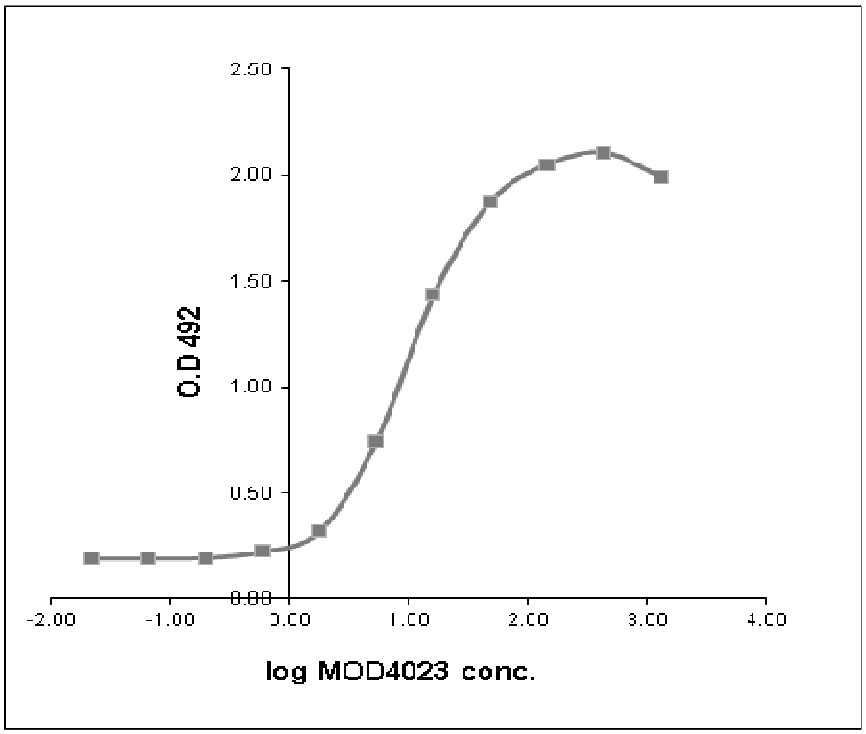
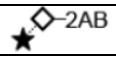


Figure 13

Column/Operation	Log10 Reduction of Model Virus			
	MuLV	**PRV**	**Reo-3**	**MVM**
Inactivation with Triton X-100	≥ 4.74	≥ 5.16	N/A	N/A
DEAE- Sepharose	> 4.02	> 3.01	N/A	1.22
CHT	2.59	4.11	N/A	1.32
SP Sepharose	≥ 4.95	5.72	2.07	3.87
Nano filtration	≥ 6.88	≥ 7.76	≥ 7.46	7.20
Overall reduction	≥ 23.18	≥ 25.76	≥ 9.53	13.61
Theoretical maximal viral load per dose	< 6.9	N/A	N/A	N/A
Safety margin	≥ 16.28	N/A	N/A	N/A

Figure 14

O-link Glycan Profile of MOD-4023

Structure	Glycan Units	% Peak Area	
		MOD-4023 sample#1	MOD-4023 sample#2
Galb1,3-GalN 	1.76	5.4	6.4
SAa2,3-Gal (peeling) 	2.25	11.2	14.2
Unknown	2.97	5.6	6.1
SAa2,3-Galb 1,2-GalN 	3.02	71.0	69.8
SAa2,3-Galb 1,3-(SAa2,6)-GalN 	4.72	6.8	3.5

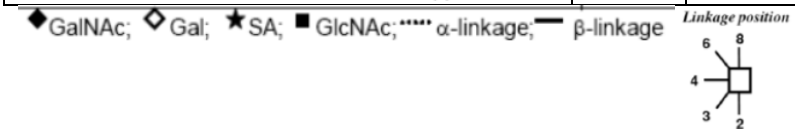


Figure 15A

Sialic Acid Type in MOD-4023 Samples

Sialic Acid Type	MOD-4023 sample#1	MOD-4023 sample#2
	% abundance (average)	% abundance (average)
Neu5Gc[1]	n.d	0.45
Neu5Ac[2]	98.65	95.95
Unknown	n.d.	0.45
Neu5,9Ac2[3]	1.35	3.1

[1]Neu5Gc-NGNA
[2]Neu5Ac- NANA
[3]Neu5,9Ac2 -di acetylated NANA
n.d –not detected

Figure 15B

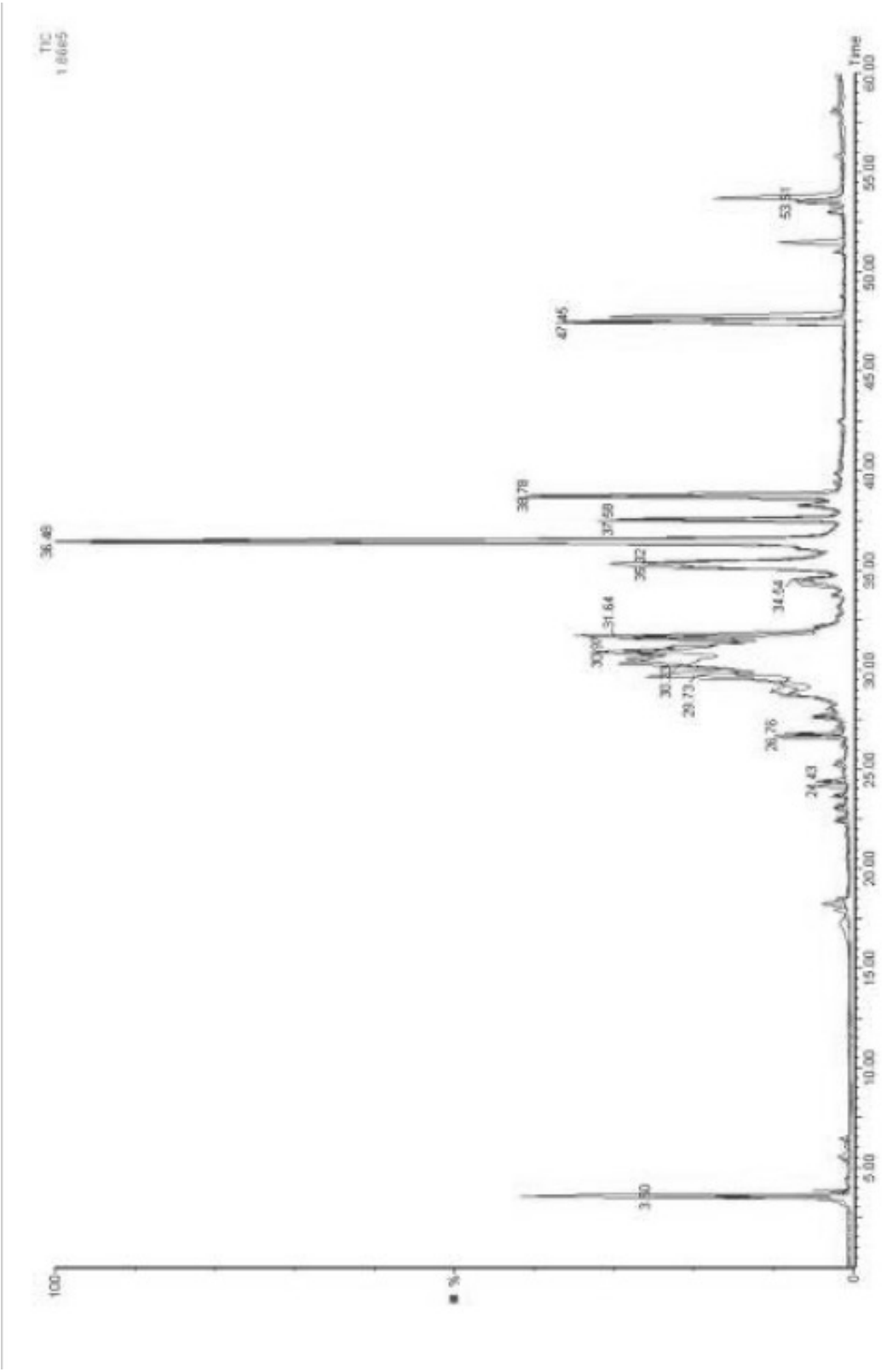

TIC
Time
100
%
0
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
3.50
24.43
26.76
29.73
30.23
30.90
31.64
34.54
35.32
36.48
37.58
38.78
47.45
53.51

Figure 16

m/z observed	z	M observed	Possible assignment		Calcualted expected mass	Detected at t_R [min]		
						648-01-10-014A	648-01-10-019A	648-01-11-003A
1384.4	4	5533.6	1-36	**SSSSKAPPPSLPSPSRLPGPSDTPILPQ**FPTIPLSR [+ 5 HexNAc-Hex] (SEQ ID NO: 15)	5533.7	35.4	35.4	35.4
1475.8	4	5899.2	1-36	**SSSSKAPPPSLPSPSRLPGPSDTPILPQ**FPTIPLSR [+ 6 HexNAc-Hex] (SEQ ID NO: 15)	5898.8	34.5	34.5	34.6
1174.1	4	4692.4	6-36	**APPPSLPSPSRLPGPSDTPILPQ**FPTIPLSR [+ 4 HexNAc-Hex] (SEQ ID NO: 11)	4692.3	35.6	35.6	35.6
837.8	3	2510.4	17-36	**LPGPSDTPILPQ**FPTIPLSR [+ 1 HexNAc-Hex] (SEQ ID NO: 10)	2510.3	38.1	38.1	38.1
1025.4	2	2048.8	212-224	SVEGSCGF**SSSSK** [+ 2 HexNAc-Hex] (SEQ ID NO: 13)	2048.8	22.4	22.6	22.4
1337.7	6	8020.2	225-275	**APPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ** [+ 8 HexNAc-Hex] (SEQ ID NO: 14)	8019.8	31.2	31.2	31.3
1798.8 (averaged)	6	10787	212-275	SVEGSCGF**SSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ** [+ 12 HexNAc-Hex] (SEQ ID NO: 17)	10787 (average)	30.5	30.3	30.4
1139.6	3	3415.8	253-275	**APPPSLPSPSRLPGPSDTPILPQ** [+ 3 HexNAc-Hex] (SEQ ID NO: 16)	3415.6	31.4	31.4	31.5
800.4	2	1598.8	264-275	**LPGPSDTPILPQ** [+ 1 HexNAc-Hex] (SEQ ID NO: 12)	1598.8	31.1	31.1	31.1

Figure 17

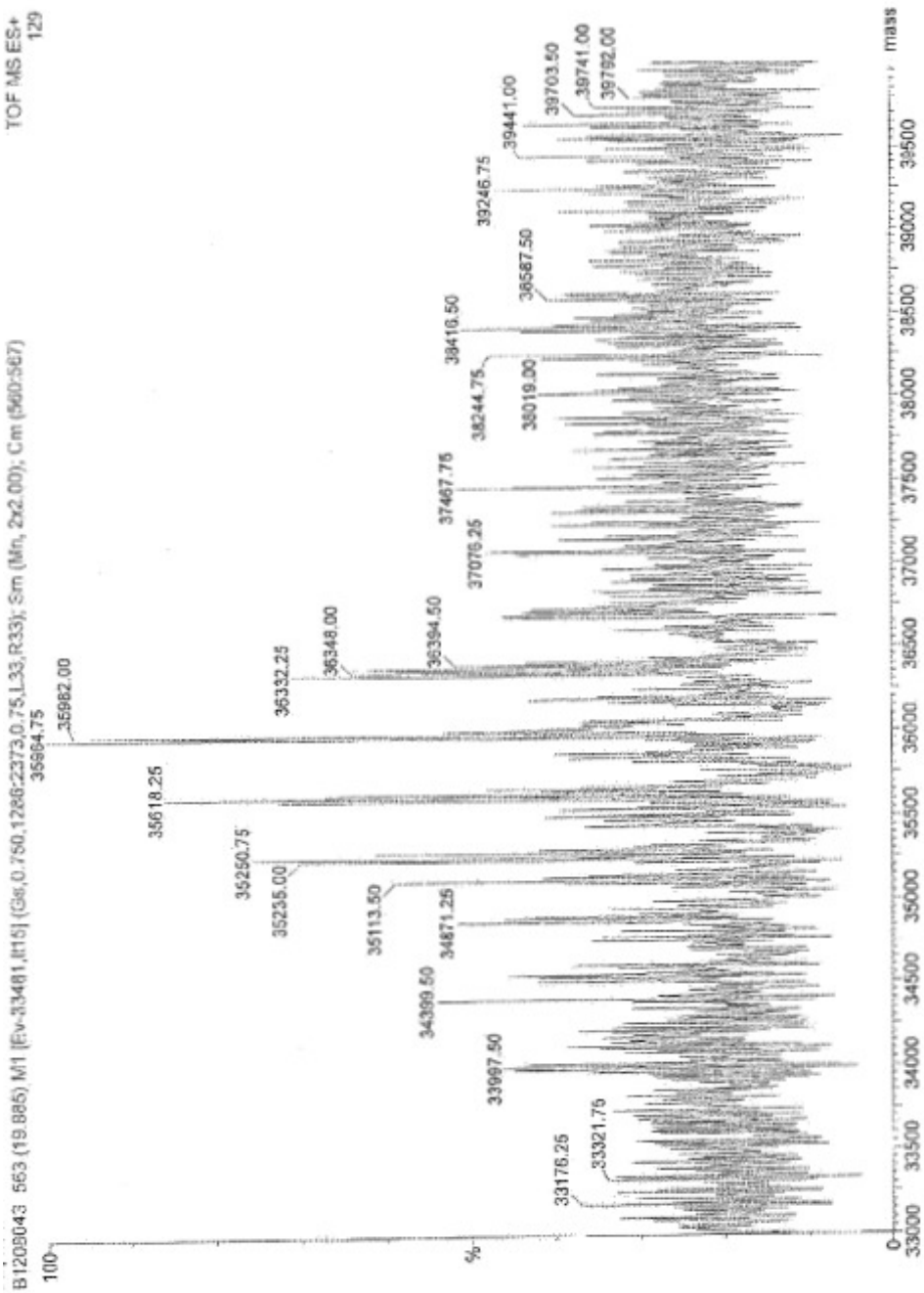

B1209043 563 (19.885) M1 [Ev-33481,It15] (Gs,0.750,1266:2373,0.75,L33,R33); Sm (Mn, 2x2.00); Cm (560:567)
TOF MS ES+
129
100
%
0
35984.75
35982.00
35618.25
35260.75
35235.00
36332.25
36348.00
36394.50
35113.50
34871.25
34399.50
33997.50
33321.75
33176.25
37467.75
37076.25
38244.75
38019.00
38416.50
38587.50
39246.75
39441.00
39703.50
39741.00
39792.00
33000
33500
34000
34500
35000
35500
36000
36500
37000
37500
38000
38500
39000
39500
mass

Figure 20

Potency	Method	DS GMP1 1008137	DS GMP2 1009976	DS GMP3 1016455	DS GMP4 1016456	DS GMP5 1019182	DS GMP6 1025279	DS GMP7 1025281	DS GMP8 1025282	Range
Bioidentity Test	Weight Gain Assay (USP) is performed according to the Bioidentity Test, described in USP edition 35 somatropin monograph. Hypophysectomized rats (rats from which the pituitary gland has been removed) lose their ability to gain weight. Injections of MOD-4023 to these rats result in weight gain. Based on the measured weight gain after a defined period of time and the amount of MOD-4023 injected, the specific activity of MOD-4023 is determined.	6 USP Somatropin units per mg	4.77 USP Somatropin units per mg	4.5 USP Somatropin units per mg	3.5 USP Somatropin units per mg	3.2 USP Somatropin units per mg	2.3 USP Somatropin units per mg	3.4 USP Somatropin units per mg	3.0 USP Somatropin units per mg	Not Less than 2 USP Somatropin units per mg
Cell based assay	Pro-B cell line Baf/BO3 was used for the assessment of hGH potency, demonstrating an hGH dose-dependent proliferative response, as measured by the stimulation of cellular division. MOD-4023, can also induces a dose-dependent cell proliferation response by binding to the human growth hormone receptor (hGHR) on the Baf/HGHR/B2B2 surface.	0.8	0.9	1.1	1.1	0.9	1.2	1.4	1.1	The relative potency of the sample versus the Reference Standard should be within 0.7-1.4.

Figure 23

Impurity	Method	DS GMP1 1008137	DS GMP2 1009976	DS GMP3 1016455	DS GMP4 1016456	DS GMP5 1019182	DS GMP6 1025279	DS GMP7 1025281	DS GMP8 1025282	Range
HCP	CHO host cell proteins (HCP) in MOD-4023 is determined using a validated ELISA kit F550 from Cygnus Technologies, which is a complete kit for the quantitative determination of Chinese Hamster Ovary (CHO) host cell proteins.	23 ng/mg (ppm)	14 ng/mg (ppm)	12 ng/mg (ppm)	< 12 ng/mg (ppm)	7 ng/mg (ppm)	13 ng/mg (ppm)	5 ng/mg (ppm)	5 ng/mg (ppm)	≤ 100 ng/mg (ppm)
DNA	Residual DNA content is determined using quantitative real-time PCR (qPCR).	< 1 pg/mg (ppb)	< 1 pg/mg (ppb)	< 1 pg/mg (ppb)	< 1 pg/mg (ppb)	< 1 pg/mg (ppb)	< 1 pg/mg (ppb)	< 1 pg/mg (ppb)	< 1 pg/mg (ppb)	≤10 pg/mg (ppb)
MTX	Quantitation of residual methotrexate in bulk Drug Substance is performed using a liquid chromatography-tandem mass spectrometry limit test	< 50 ng/mL	< 50 ng/mL	< 50 ng/mL	< 50 ng/mL	< 50 ng/mL	< 50 ng/mL	< 50 ng/mL	< 50 ng/mL	< 50 ng/mL
PG	Determination of residual propylene glycol in DS is performed by a headspace gas chromatographic method using an RTX-35 30 meter megabore column and FID detector	< 60 µg/mL	< 60 µg/mL	≤ 60 µg/mL	< 60 µg/mL	≤ 60 µg/mL	≤ 60 µg/mL	≤ 60 µg/mL	≤ 60 µg/mL	≤ 60 µg/mL
Triton	Triton is detected using a validated RP-HPLC method with UV detection at 230nm.	≤ 2.5 µg/mL	≤ 2.5 µg/mL	≤ 2.5 µg/mL	≤ 2.5 µg/mL	≤ 2.5 µg/mL	≤ 2.5 µg/mL	≤ 2.5 µg/mL	≤ 2.5 µg/mL	≤ 2.5 µg/mL
Insulin	Quantitation of residual insulin in bulk Drug Substance is performed using a commercial ELISA kit (Mercodia)	<115 pg/ml	<115 pg/ml	<115 pg/ml	<115 pg/ml	<115 pg/ml	<115 pg/ml	<115 pg/ml	<115 pg/ml	≤ 115 pg/mL
DMSO	Quantitation of DMSO is performed using a gas chromatography method employing a DB-624 30 meter Megabore column	<250 µg/mL	<250 µg/mL	<250 µg/mL	<250 µg/mL	<250 µg/mL	<250 µg/mL	<250 µg/mL	<250 µg/mL	<250 µg/mL
Bioburden	Bioburden test is based on USP <61> and EP 2.6.12	0 cfu /10 mL	0 cfu /10 mL	0 cfu /10 mL	0 cfu /10 mL	0 cfu /10 mL	0 cfu /10 mL	0 cfu /10 mL	0 cfu /10 mL	≤ 10 cfu /10 mL

Figure 24