mAb1

3 Strains

A312 = mAb1 Strain A Feed rate = 11 g/l/hr, 11 g/l Etoh bolus, RQ = 1.09 – 1.15
A326 = mAb1 Strain B Feed rate = 11 g/l/hr, 11 g/l Etoh bolus, RQ = 1.09 – 1.15
A343 = mAb1 Strain C Feed rate = 11 g/l/hr, 11 g/l Etoh bolus, RQ = 1.09 – 1.15

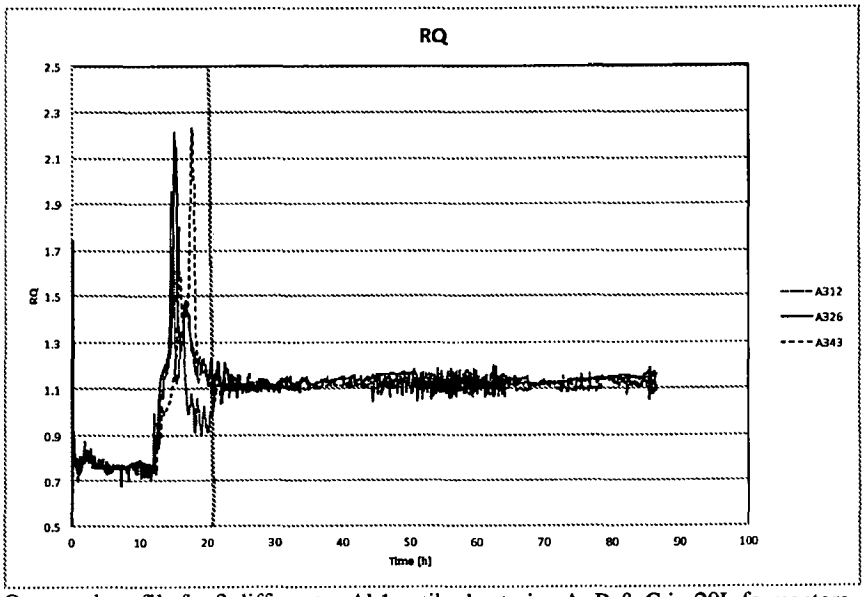


Figure 1: RQ control profile for 3 different mAb1 antibody strains A, B & C in 20L fermenters.

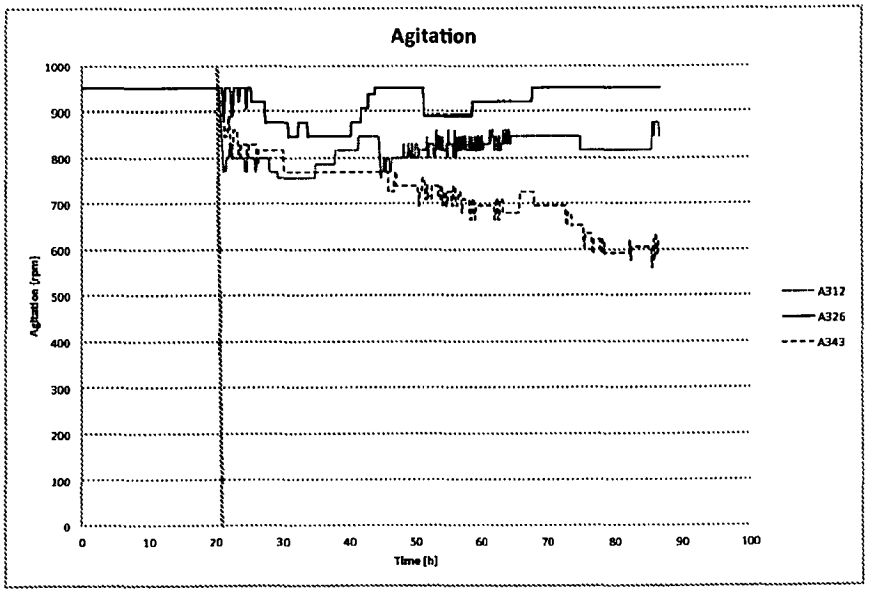


Figure 2: Agitation profile indicating agitation response to maintain desired RQ for 3 different mAb1 antibody strains A, B & C in 20L fermentors

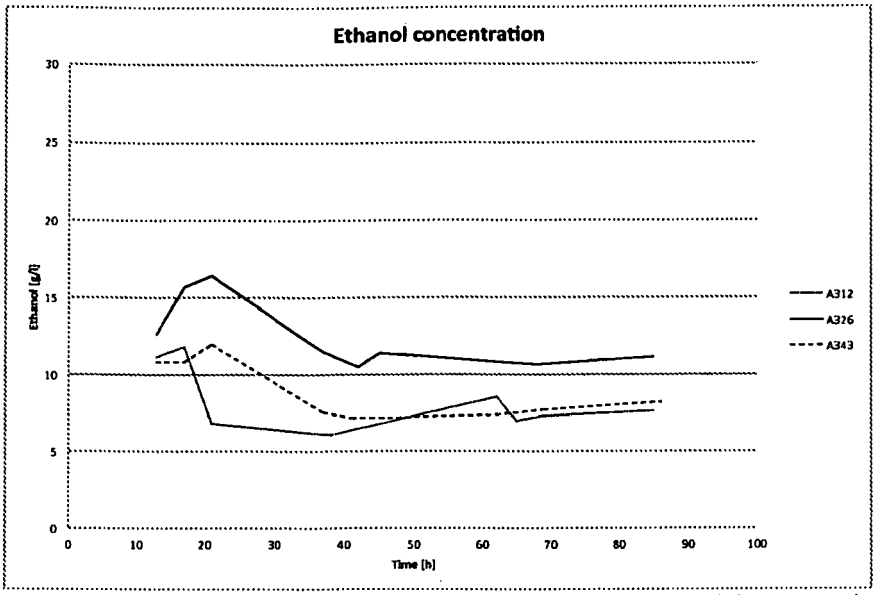


Figure 3: Ethanol concentration profile indicating effect of RQ control on maintaining non toxic ethanol levels for 3 different mAb1 antibody strains A, B & C in 20L fermentors

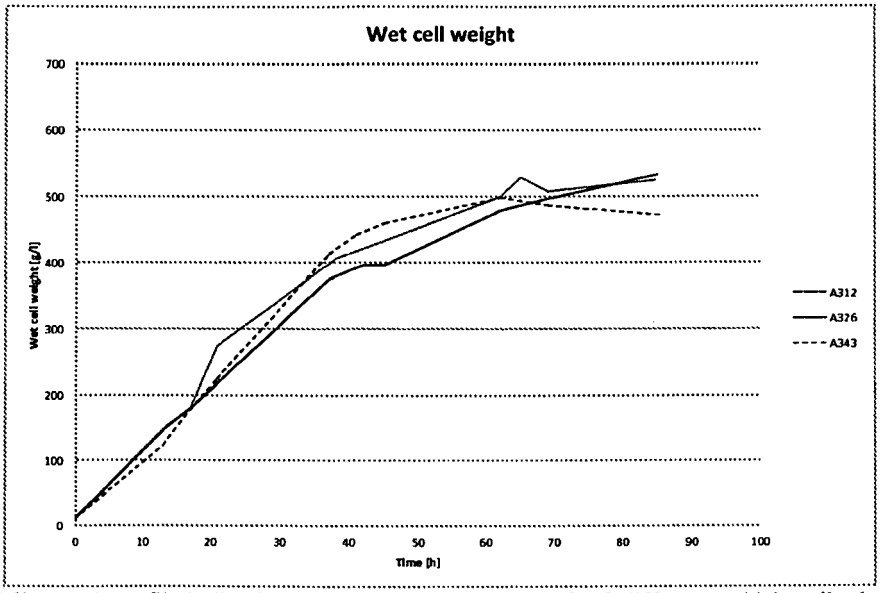


Figure 4: Cell growth profile indicating cellular growth similarity for 3 different mAb1 antibody strains A, B & C in 20L fermentors.

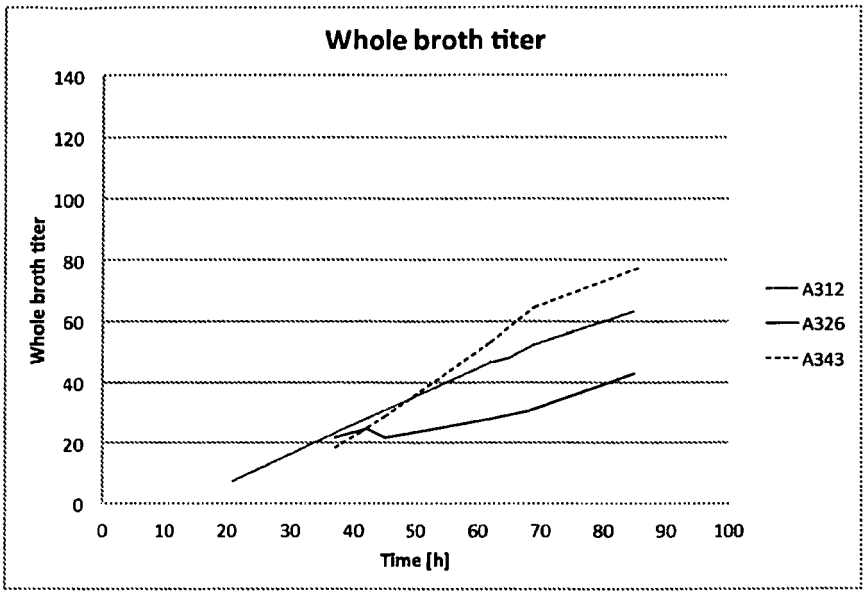


Figure 5: Whole Broth Titer profile indicating product expression for 3 different anti mAb1 antibody strains A, B & C in 20L fermentors

3 RQ Setpoints

A277 = mAb1 Strain A Feed rate = 11 g/l/hr, 11 g/l Etoh bolus, RQ = 1.19 – 1.25
A278 = mAb1 Strain A Feed rate = 11 g/l/hr, 11 g/l Etoh bolus, RQ = 1.29 – 1.35
A296 = mAb1 Strain A Feed rate = 11 g/l/hr, 11 g/l Etoh bolus, RQ = 1.09 – 1.15

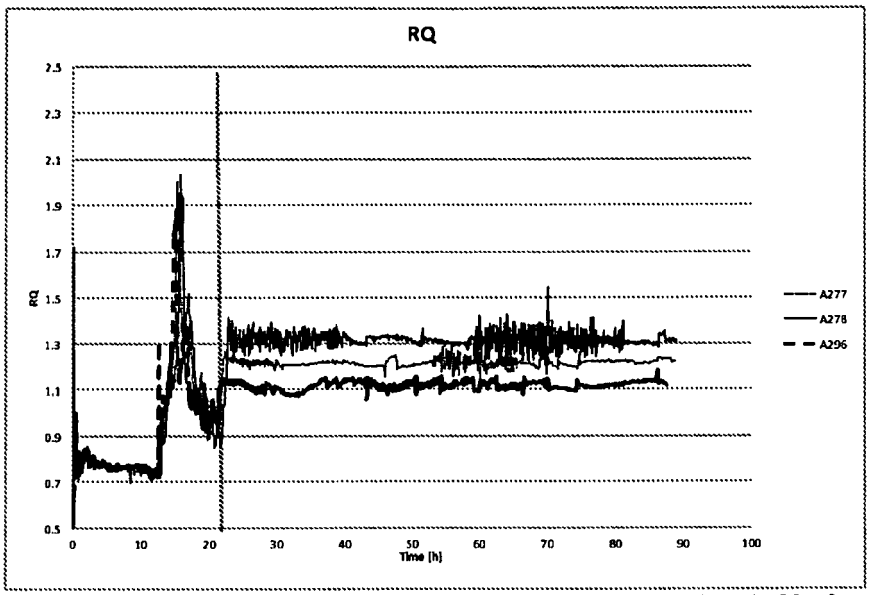


Figure 6: RQ control profile for 3 RQ control set points for mAb1 antibody Strain A in 20L fermentors

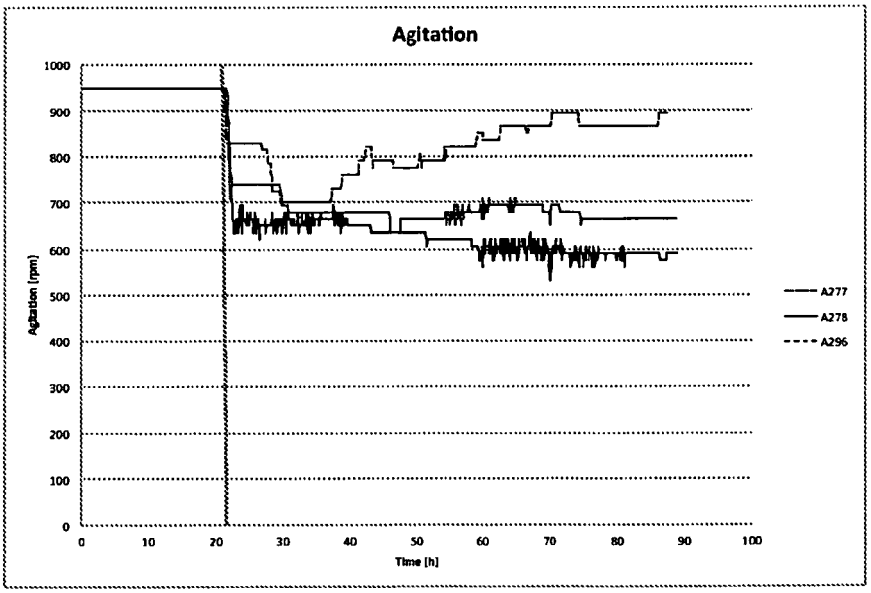


Figure 7: Agitation profile for 3 RQ control set points indicating agitation response to maintain desired RQ for mAb1 antibody Strain A 20L fermentors

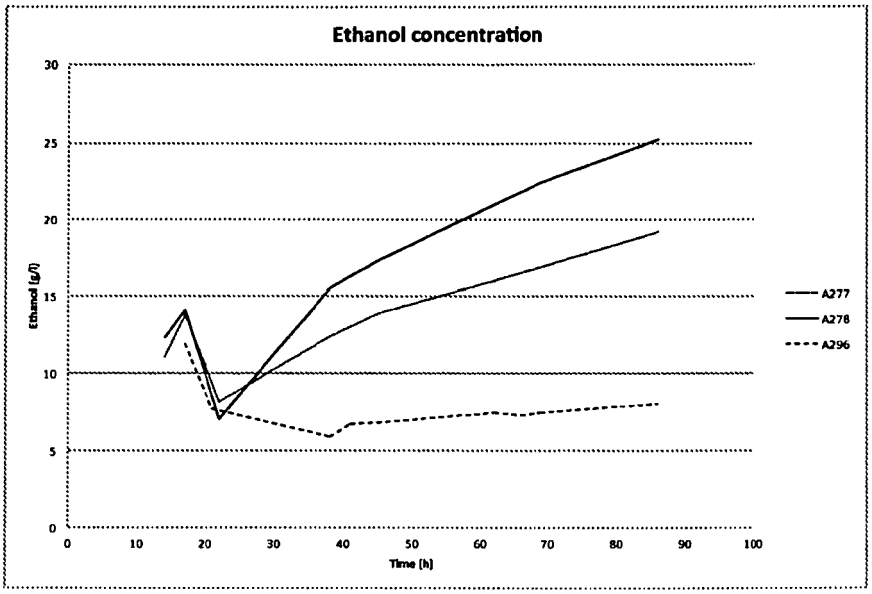


Figure 8: Ethanol concentration profile for 3 RQ control set points indicating effect of RQ control on maintaining non toxic ethanol levels for mAb1 antibody Strain A in 20L fermentors

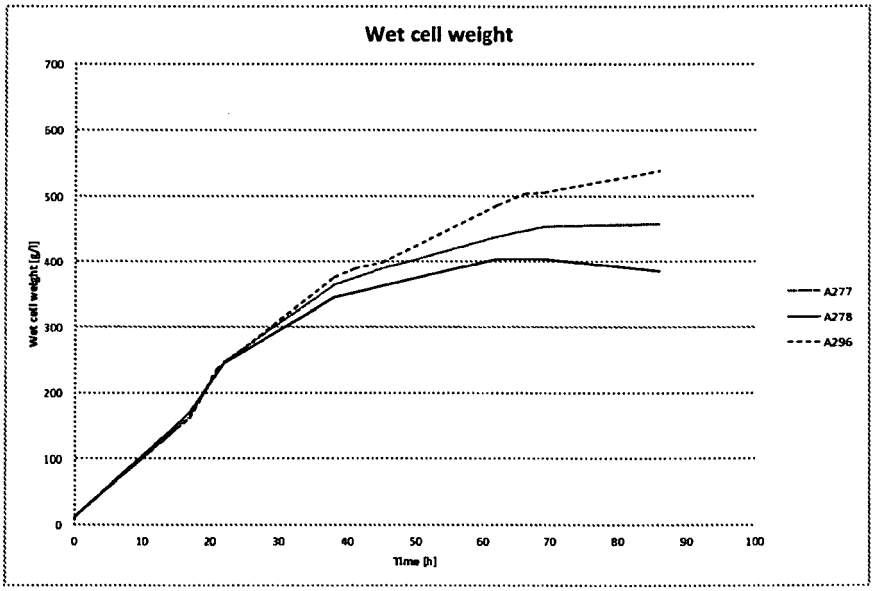


Figure 9: Cell growth profile for 3 RQ control set points indicating cellular growth for mAb1 antibody Strain A in 20L fermentors

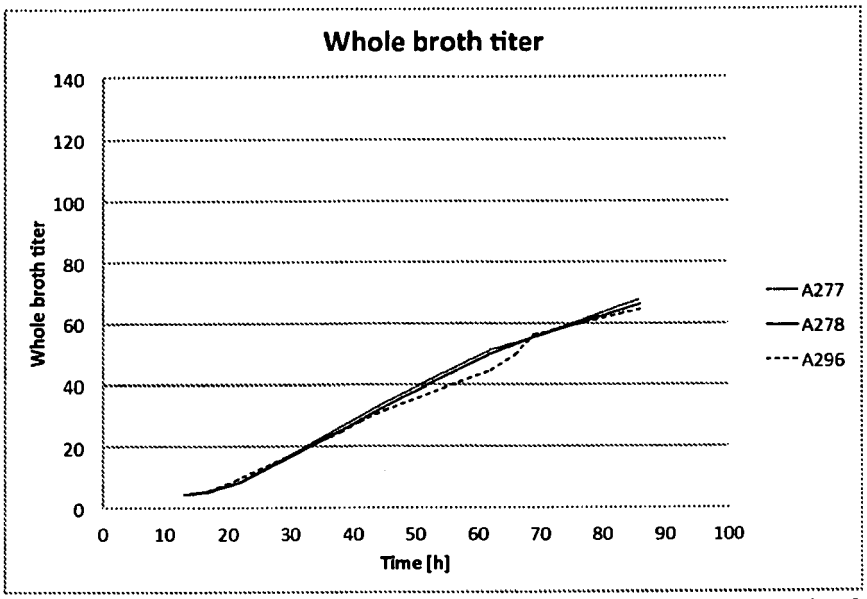


Figure 10: Whole Broth Titer profile for 3 RQ control set points indicating product expression for mAb1 antibody Strain A in 20L fermentors

Aerobic vs Hypoxic
A291 = mAb1 Strain A Aerobic
A296 = mAb1 Strain A Hypoxic,
Feed rate = 11 g/l/hr, 11 g/l Etoh bolus,
RQ = 1.09 – 1.15

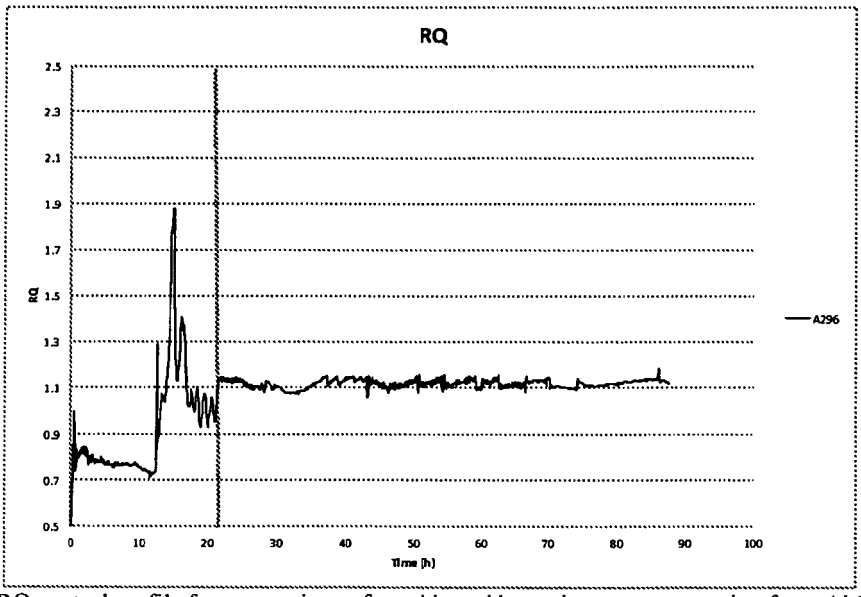


Figure 11: RQ control profile for comparison of aerobic and hypoxic state oxygenation for mAb1 antibody Strain A in 20L fermentors. Both fermentations were run at the same feed rates. RQ profile is not shown for the aerobic state.

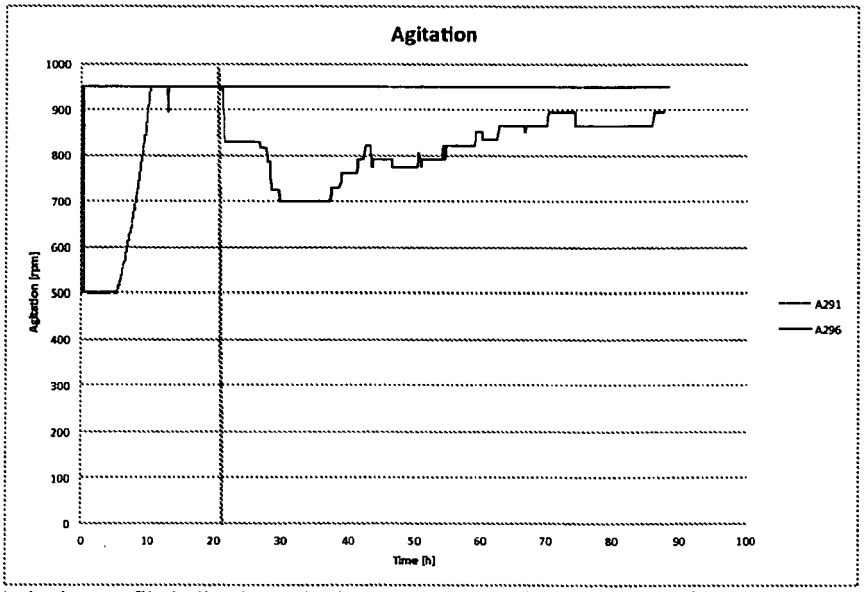


Figure 12: Agitation profile indicating agitation response to maintain desired RQ for hypoxic fermentation and constant agitation for aerobic fermentation for mAb1 antibody Strain A in 20L fermentors

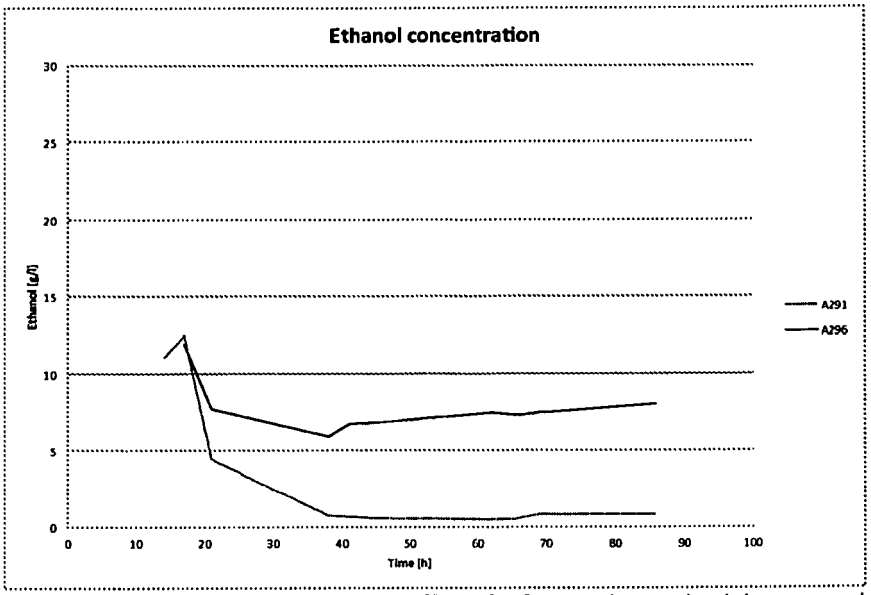


Figure 13: Ethanol concentration profile indicating effect of RQ control on maintaining non toxic ethanol levels for hypoxic fermentation and non fermentative state for aerobic fermentation for mAb1 antibody Strain A in 20L fermentors

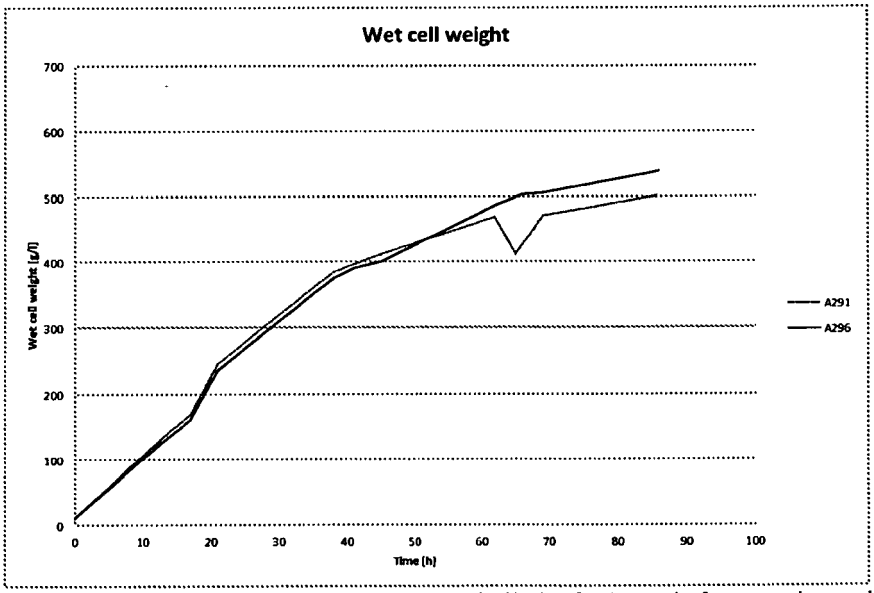


Figure 14: Cell growth profile indicating cellular growth similarity for hypoxic fermentation and aerobic fermentation for mAb1 antibody Strain A in 20L fermentors

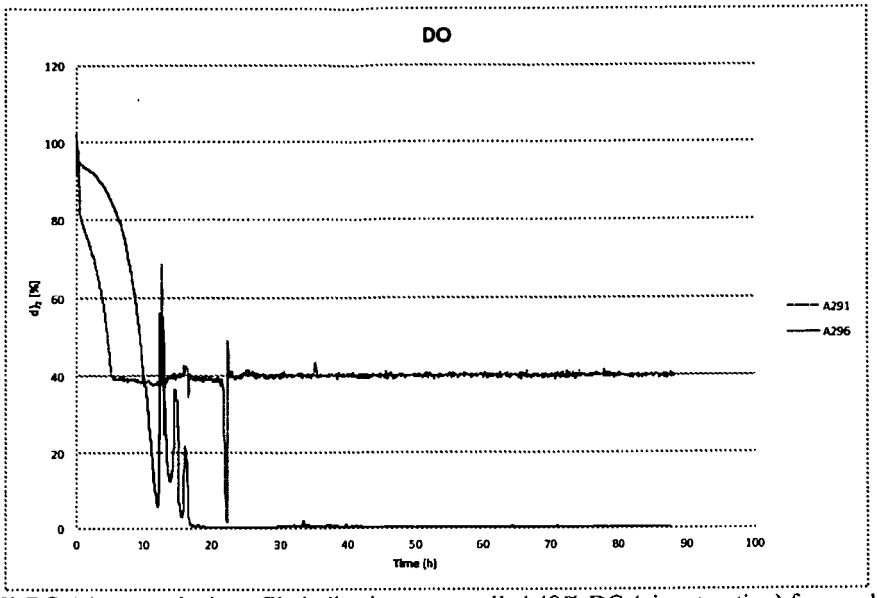


Figure 15: % DO (air saturation) profile indicating a controlled 40% DO (air saturation) for aerobic fermentation and a non measurable %DO (air saturation) after 17hrs for the hypoxic fermentation for mAb1 antibody Strain A in 20L fermentors

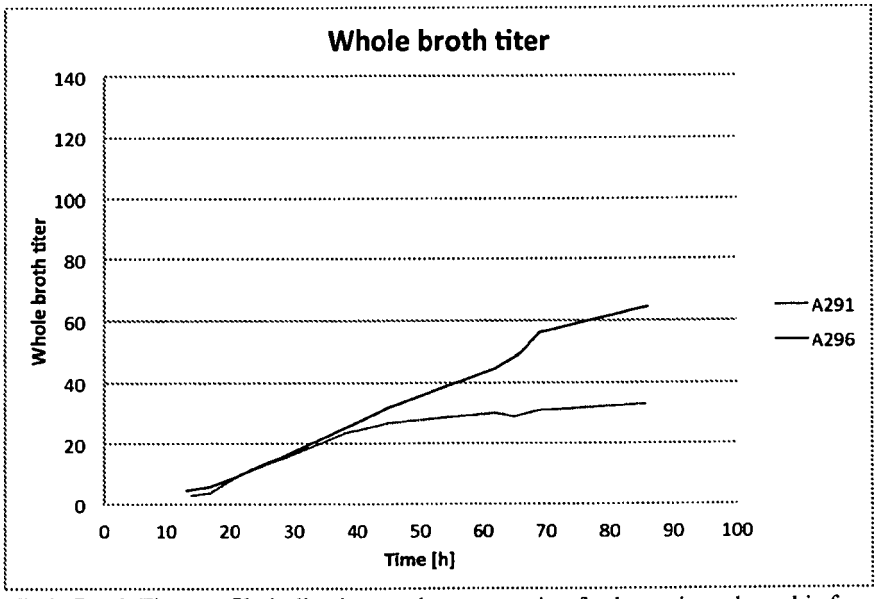


Figure 16: Whole Broth Titer profile indicating product expression for hypoxic and aerobic fermentations for mAb1 antibody Strain A in 20L fermentors

mAb2

4 Strains

A158 = mAb2 Strain A Feed rate = 18/15 step, RQ = 1.09-1.15, No Etoh bolus
A162 = mAb2 Strain B Feed rate = 18/15 step, RQ = 1.09-1.15, No Etoh bolus
A163 = mAb2 Strain C Feed rate = 18/15 step, RQ = 1.09-1.15, No Etoh bolus
A173 = mAb2 Strain D Feed rate = 18/15 step, RQ = 1.09-1.15, No Etoh bolus

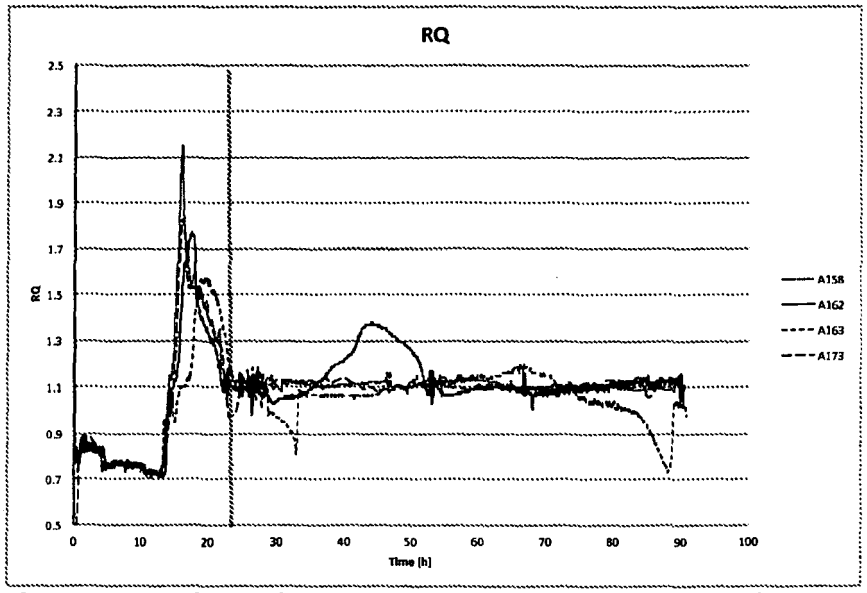


Figure 17: RQ control profile for 4 different mAb2 antibody Strains A, B, C & D in 20L fermentors

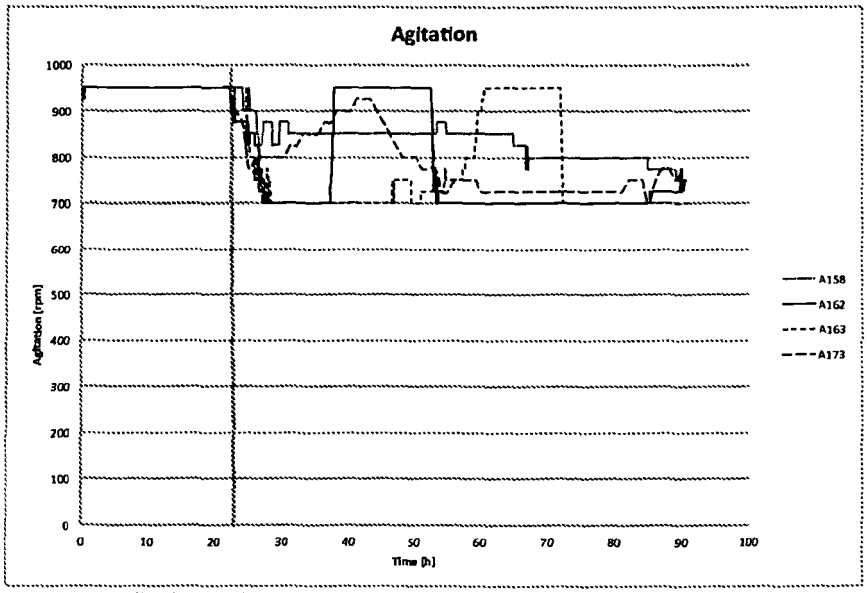


Figure 18: Agitation profile indicating agitation response to maintain desired RQ for 4 different mAb2 antibody Strains A, B, C & D in 20L fermentors

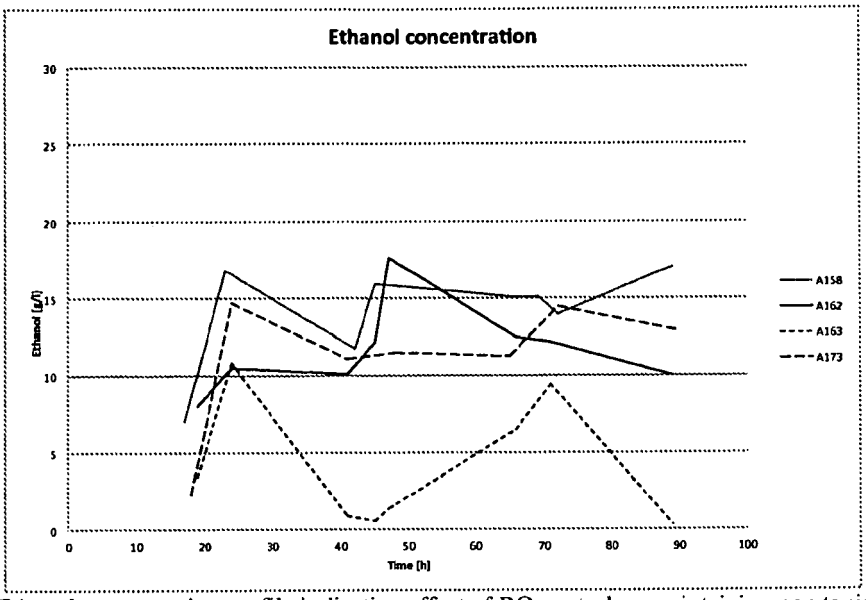


Figure 19: Ethanol concentration profile indicating effect of RQ control on maintaining non toxic ethanol levels for 4 different mAb2 antibody Strains A, B, C & D in 20L fermentors

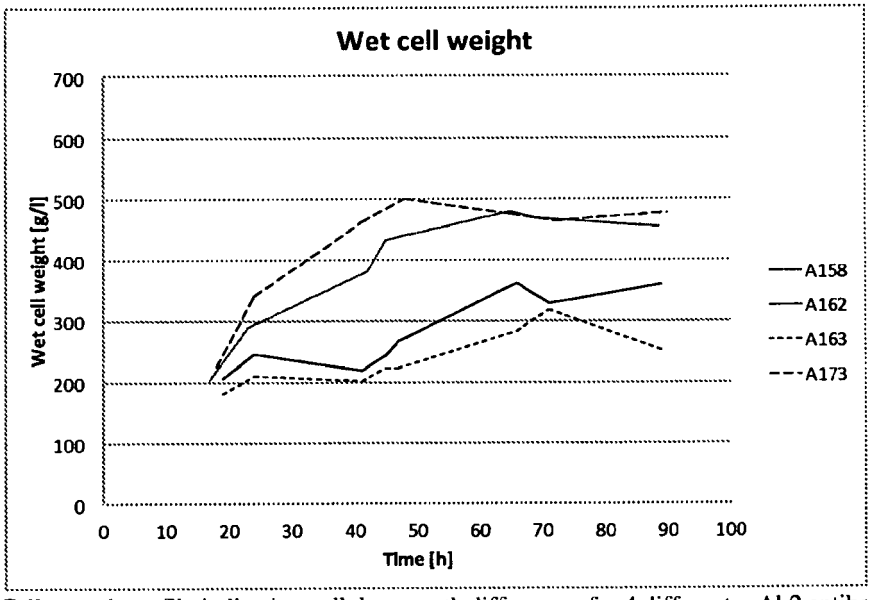


Figure 20: Cell growth profile indicating cellular growth differences for 4 different mAb2 antibody Strains A, B, C & D in 20L fermentors

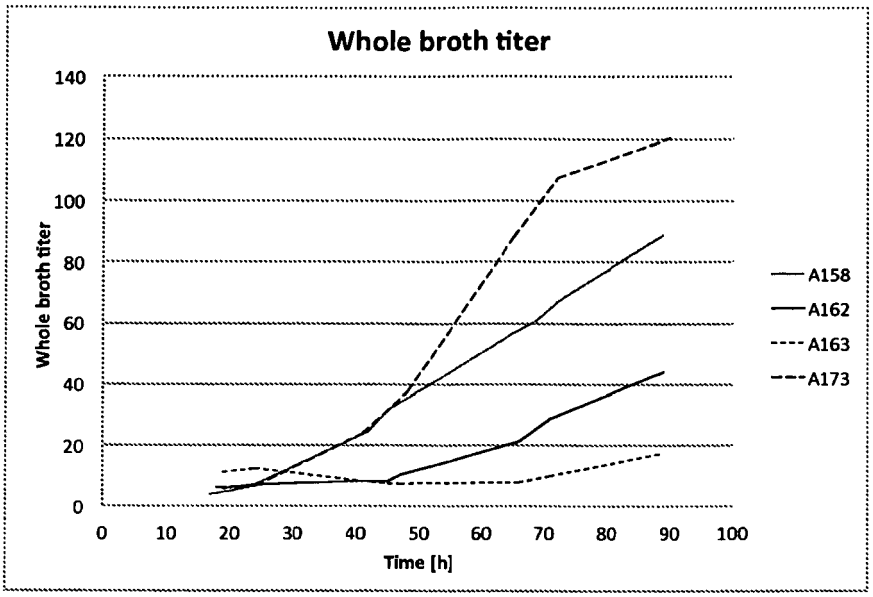


Figure 21: Whole Broth Titer profile indicating product expression for 4 different mAb2 antibody Strains A, B, C & D in 20L fermentors

mAb3

3 Strains

A225 = mAb3 Strain A Feed rate = 9g/l/hr, 11g/l Etoh Bolus, RQ = 1.09 – 1.15
A227 = mAb3 Strain B Feed rate = 9g/l/hr, 11g/l Etoh Bolus, RQ = 1.09 – 1.15
A234 = mAb3 Strain C Feed rate = 9g/l/hr, 11g/l Etoh Bolus, RQ = 1.09 – 1.15

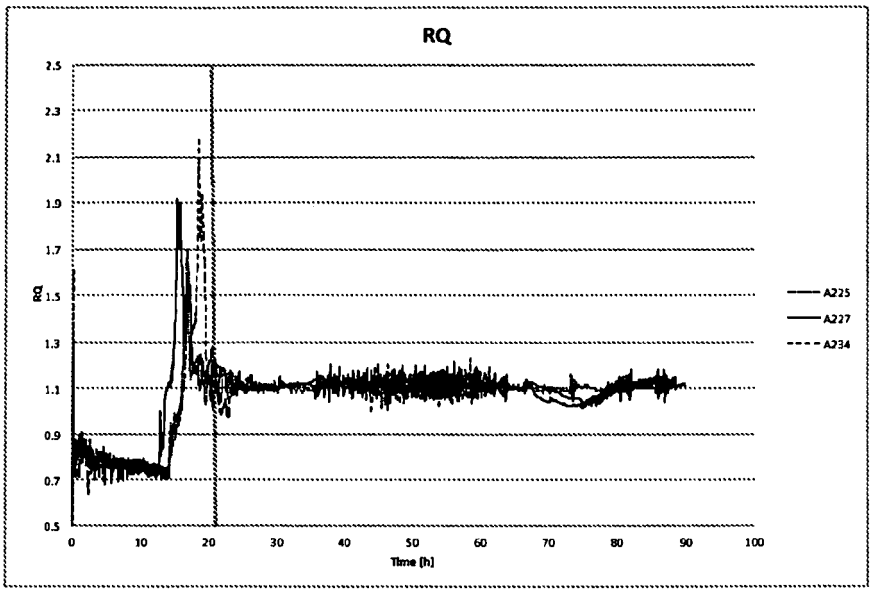


Figure 22: RQ control profile for 3 different mAb3 antibody Strains A, B & C in 20L fermentors

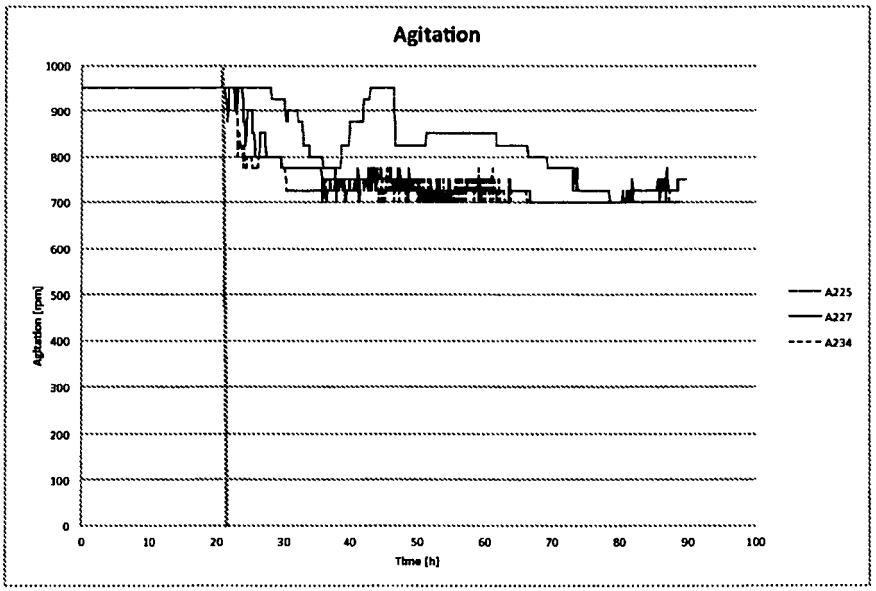


Figure 23: Agitation profile indicating agitation response to maintain desired RQ for 3 different mAb3 antibody Strains A, B & C in 20L fermentors

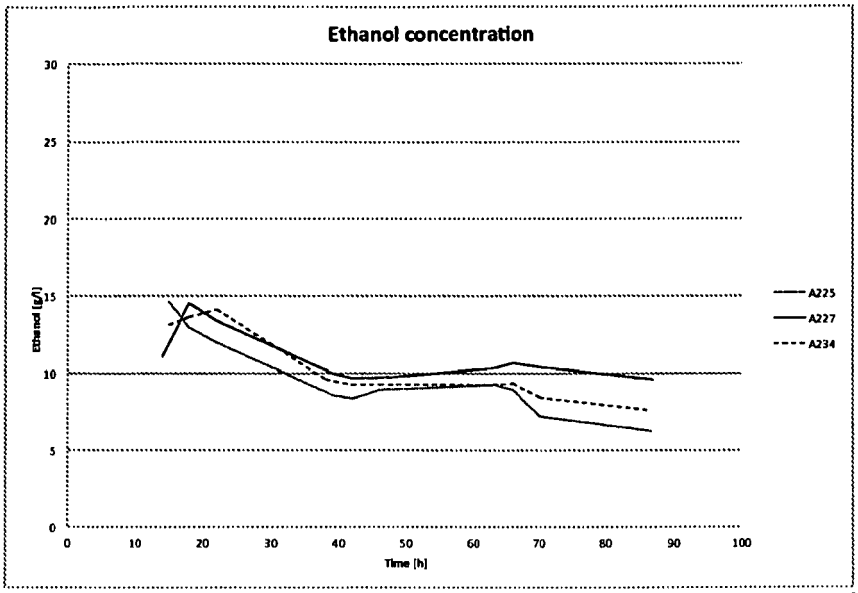


Figure 24: Ethanol concentration profile indicating effect of RQ for 3 different mAb3 antibody Strains A, B & C in 20L fermentors

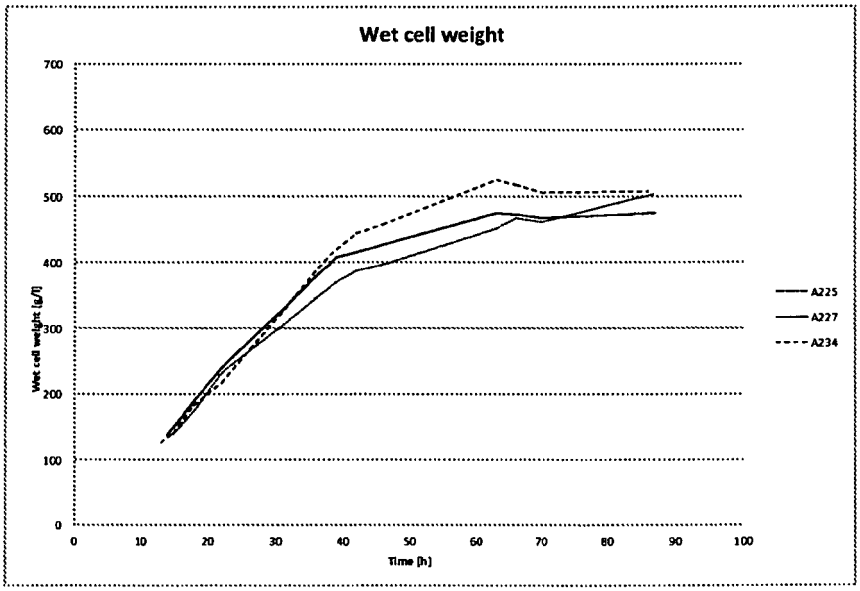


Figure 25: Cell growth profile indicating cellular growth for 3 different mAb3 antibody Strains A, B & C in 20L fermentors

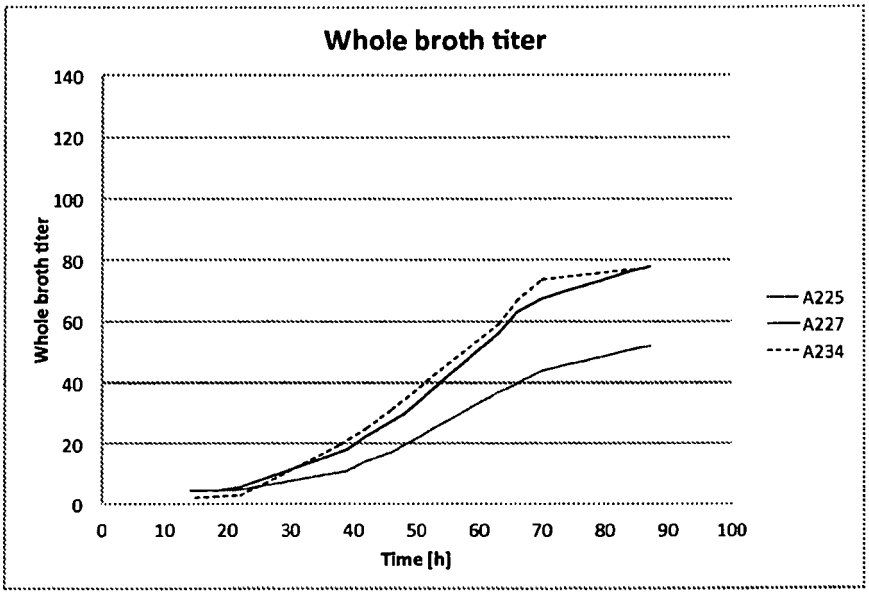


Figure 26: Whole Broth Titer profile indicating product expression for 3 different mAb3 antibody Strains A, B & C in 20L fermentors

mAb3

4 Feed Rates

A226 = mAb3 Strain B Feed Rate = 9 g/l/hr, 11g/l Etoh bolus, RQ = 1.09 – 1.15
A230 = mAb3 Strain B Feed Rate = 11g/l/hr, 11g/l Etoh bolus, RQ = 1.09 – 1.15
A235 = mAb3 Strain B Feed Rate = 6 g/l/hr, 11g/l Etoh bolus, RQ = 1.09 – 1.15
A238 = mAb3 Strain B Feed Rate = 7 g/l/hr, 11g/l Etoh bolus, RQ = 1.09 – 1.15

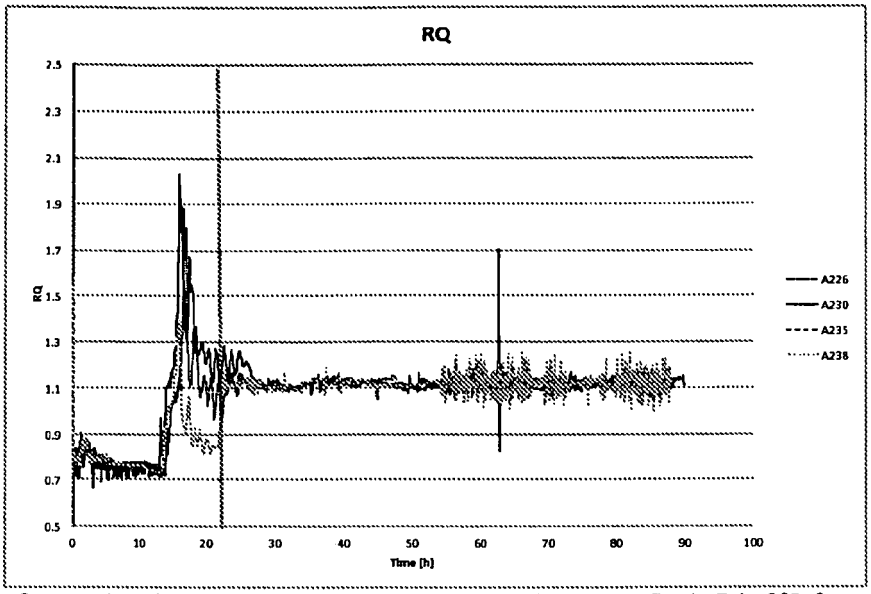


Figure 27: RQ control profile for 4 different feed rates for mAb3 antibody Strain B in 20L fermentors

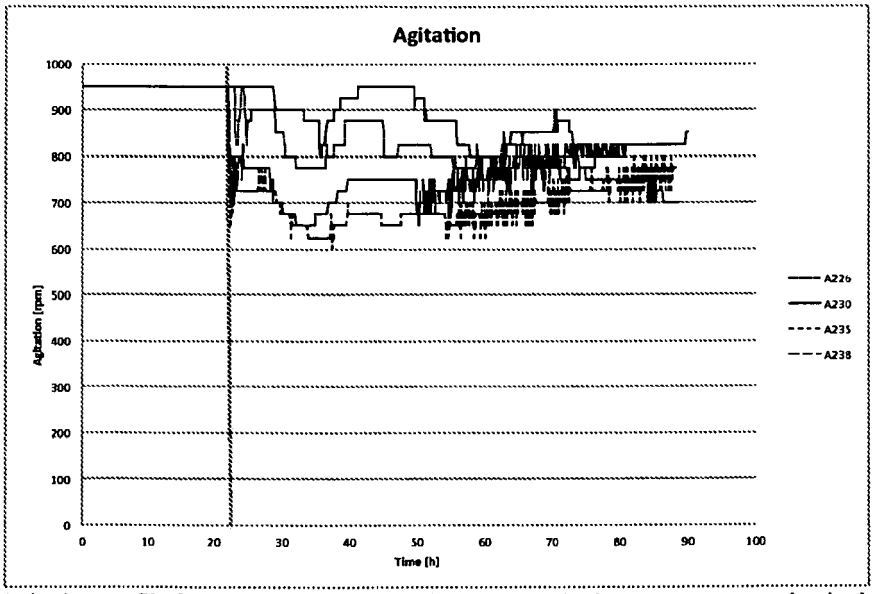


Figure 28: Agitation profile for 4 different feed rates indicating agitation response to maintain desired RQ for mAb3 antibody Strain B in 20L fermentors

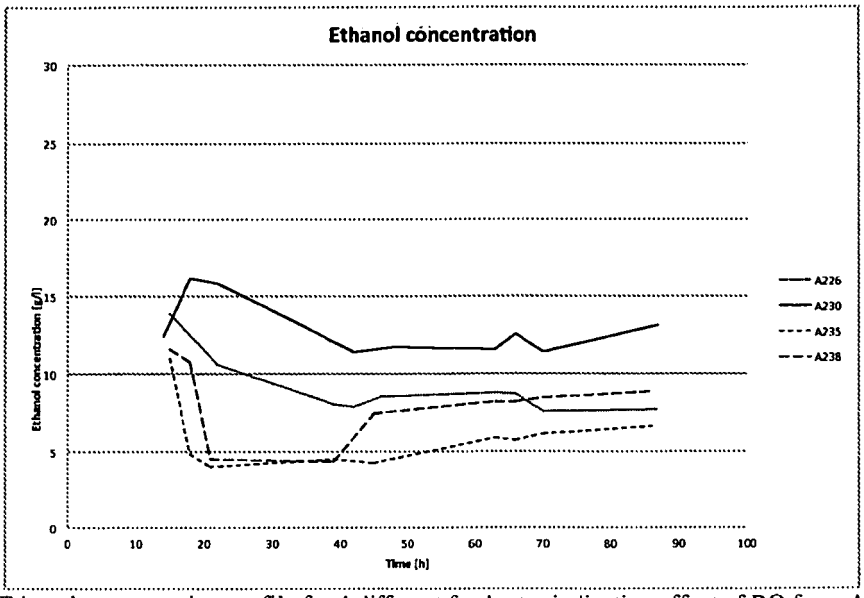


Figure 29: Ethanol concentration profile for 4 different feed rates indicating effect of RQ for mAb3 antibody Strain B in 20L fermentors

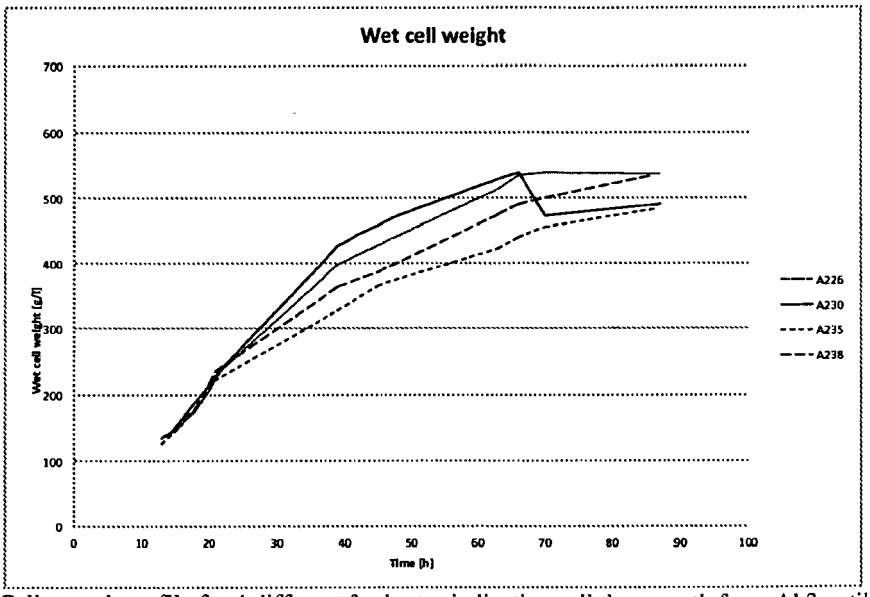


Figure 30: Cell growth profile for 4 different feed rates indicating cellular growth for mAb3 antibody Strain B in 20L fermentors

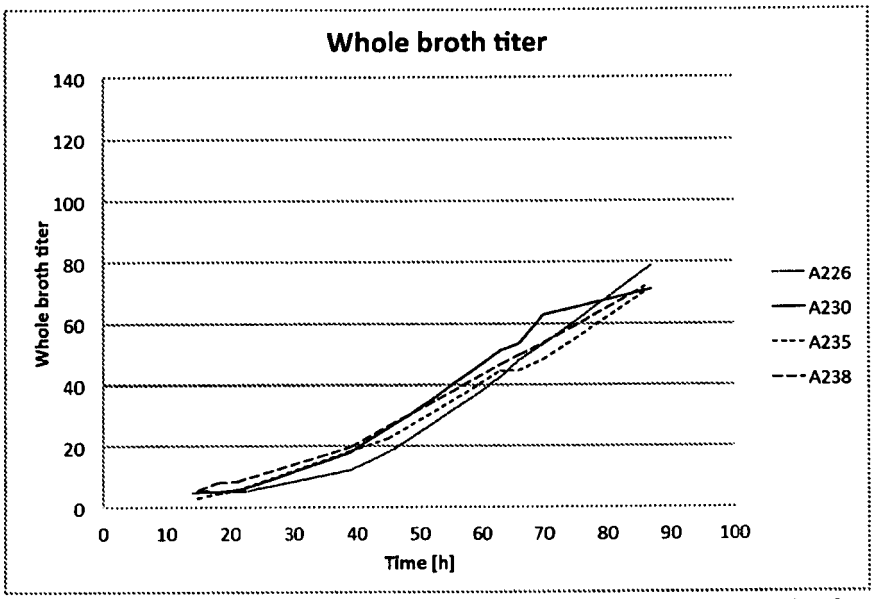


Figure 31: Whole Broth Titer profile for 4 different feed rates indicating product expression for mAb3 antibody Strain B in 20L fermentors

mAb4

2 Feed Rates

A197 = mAb4 Strain A Feed rate = 13 g/l/hr, RQ = 1.09 – 1.15, No Etoh bolus
A198 = mAb4 Strain A Feed rate = 11 g/l/hr, RQ = 1.09 – 1.15, No Etoh bolus

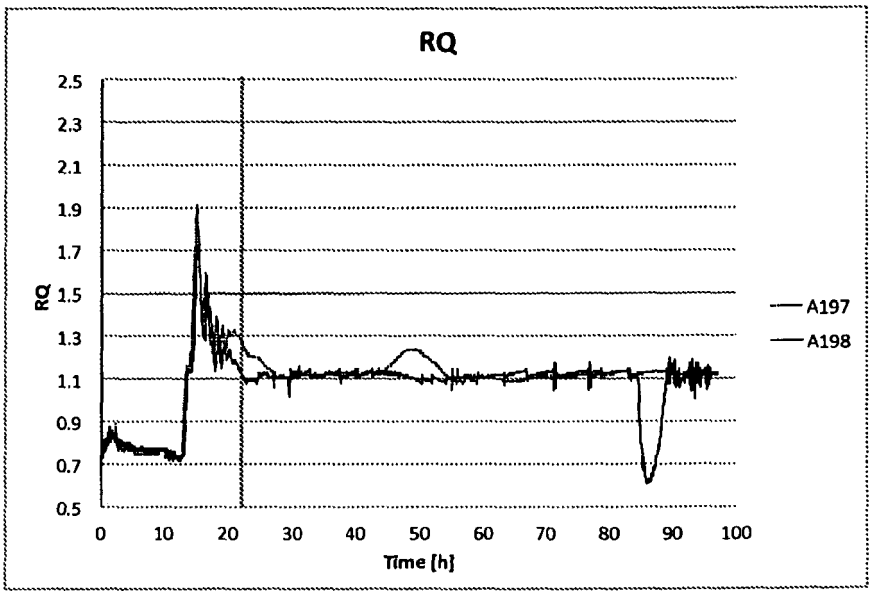


Figure 32: RQ control profile at different feed rates for mAb4 antibody Strain A with a different sequence in the CDR region in 20L fermentors

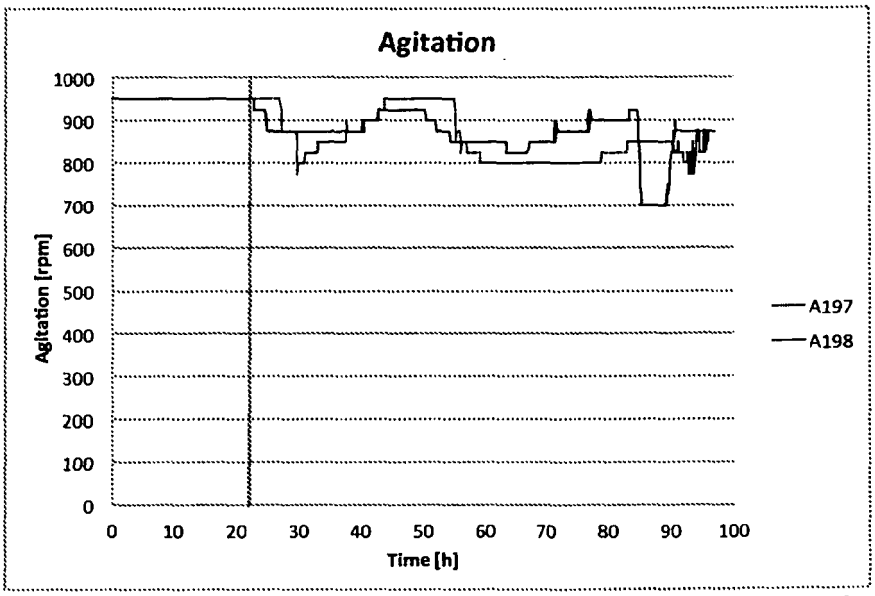


Figure 33: Agitation profile indicating agitation response to maintain desired RQ at different feed rates for mAb4 antibody Strain A with a different sequence in the CDR region in 20L fermentors

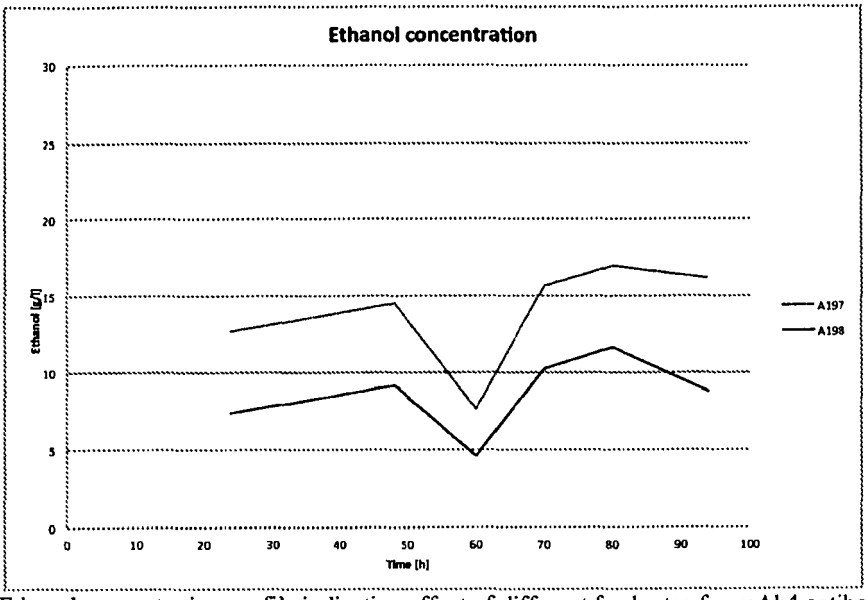


Figure 34: Ethanol concentration profile indicating effect of different feed rates for mAb4 antibody Strain A with a different sequence in the CDR region in 20L fermenters

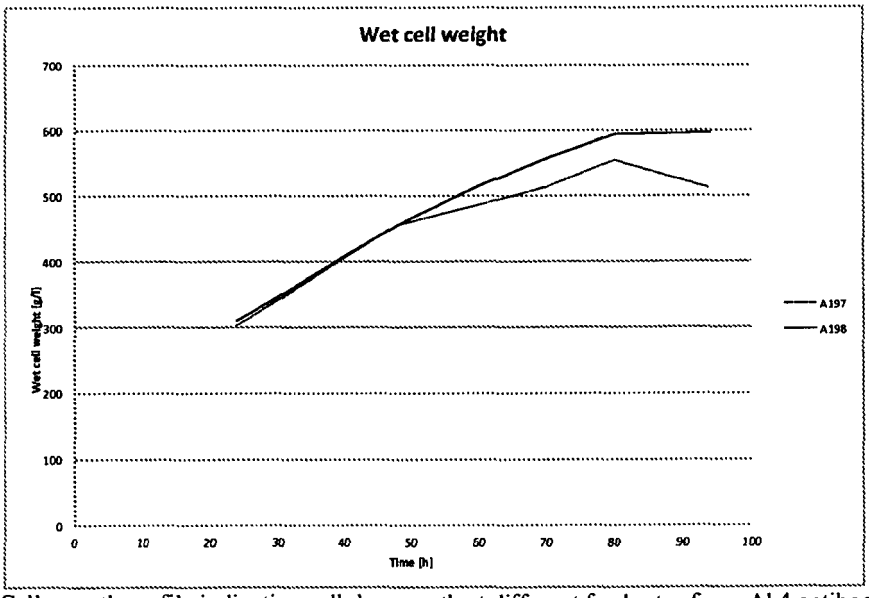


Figure 35: Cell growth profile indicating cellular growth at different feed rates for mAb4 antibody Strain A with a different sequence in the CDR region in 20L fermentors

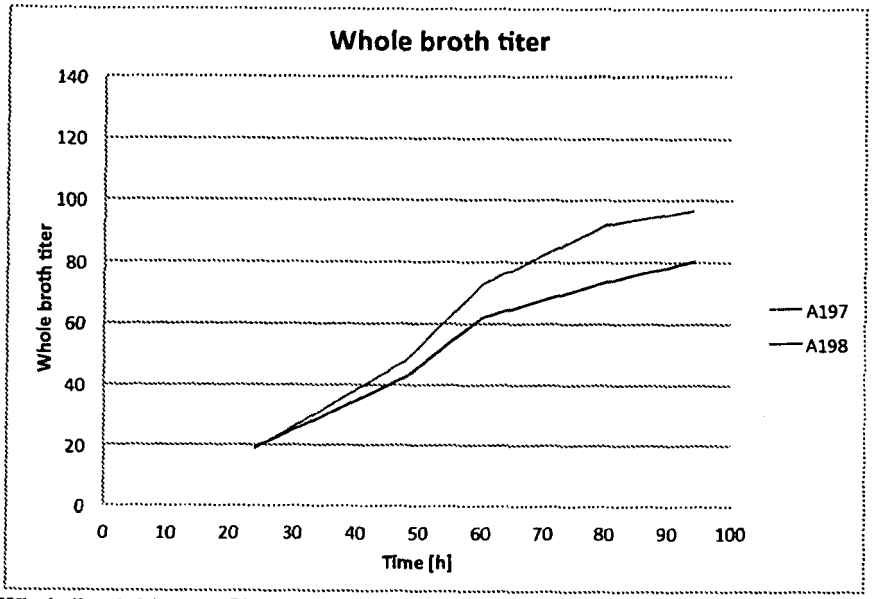


Figure 36: Whole Broth Titer profile indicating product expression at different feed rates for mAb4 antibody Strain A with a different sequence in the CDR region in 20L fermentors

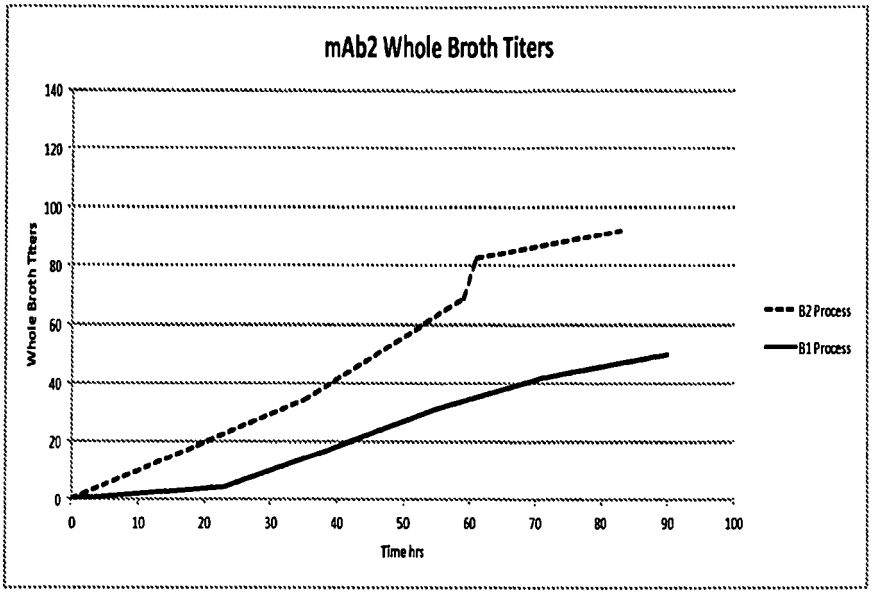


Figure 37: Whole Broth Titer profile indicating product expression for aerobic fermentation for mAb2 antibody Strain A in large scale fermentors

mAb1 Strain A A291 & A296 SDS – PAGE
Non - Reduced and Reduced at EFT 62,70,86 hours
Aerobic vs Hypoxic

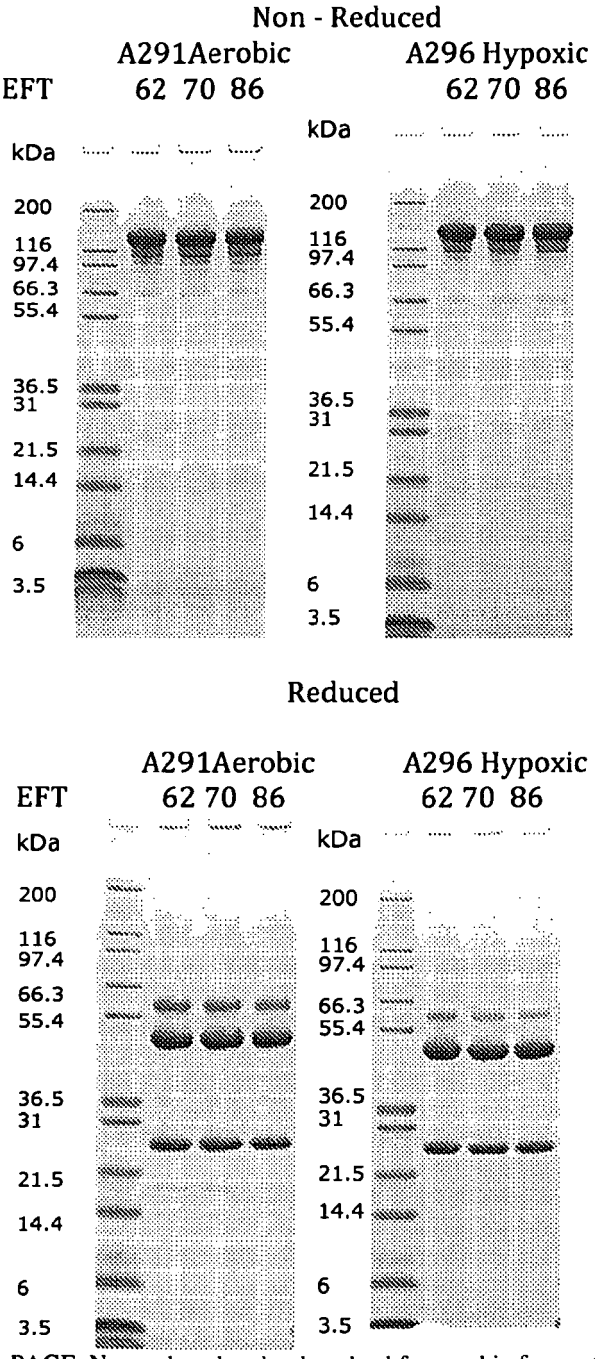


Figure 38: shows SDS – PAGE Non-reduced and reduced gel for aerobic fermentation and a hypoxic fermentation for mAb1 antibody Strain A at 3 different fermentation times in 20L fermentors

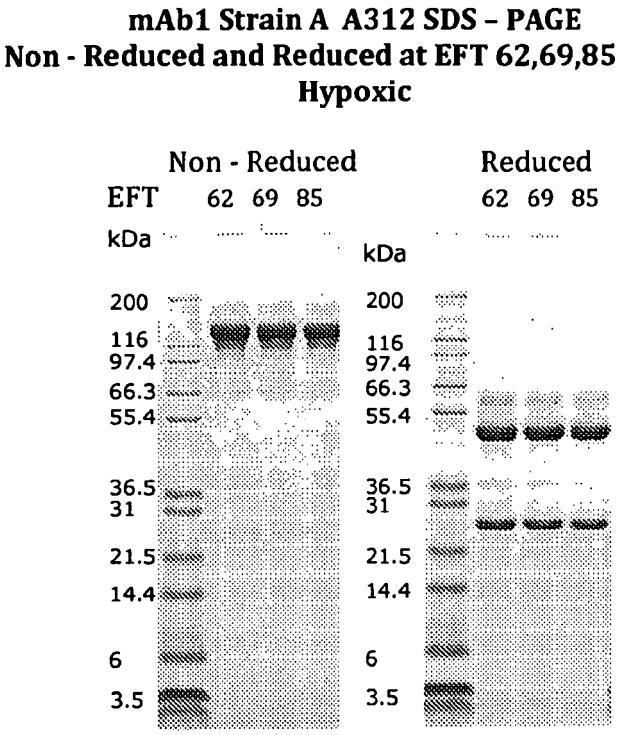


Figure 39: shows SDS-PAGE Non-reduced and reduced gel for hypoxic fermentation for mAb1 Strain A at 3 different fermentation times in 20L fermentors

FIG. 40. Ab Titer Is Increased By Temperature Shift

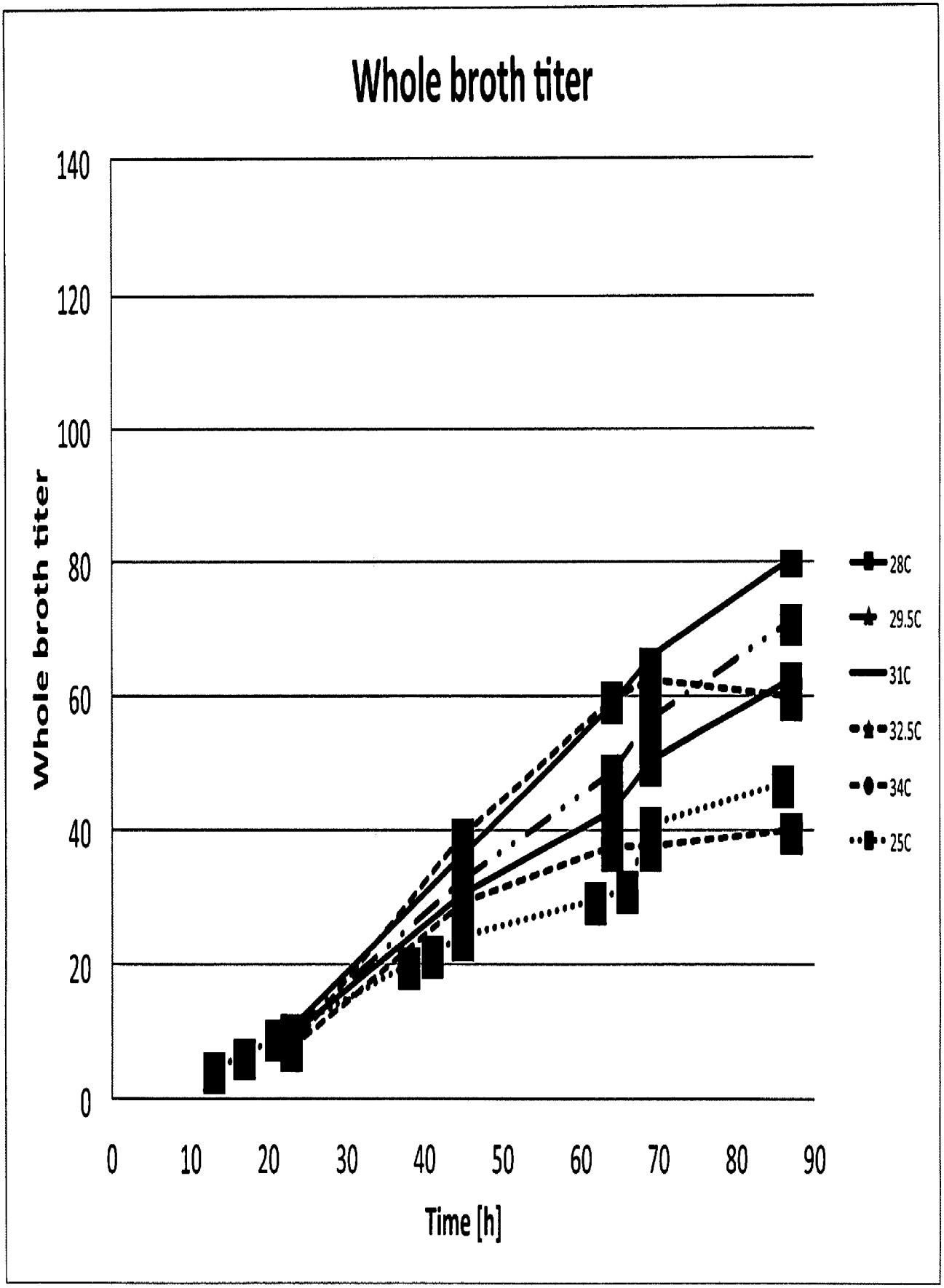


FIG. 41. Antibody Purity (Ab-A)

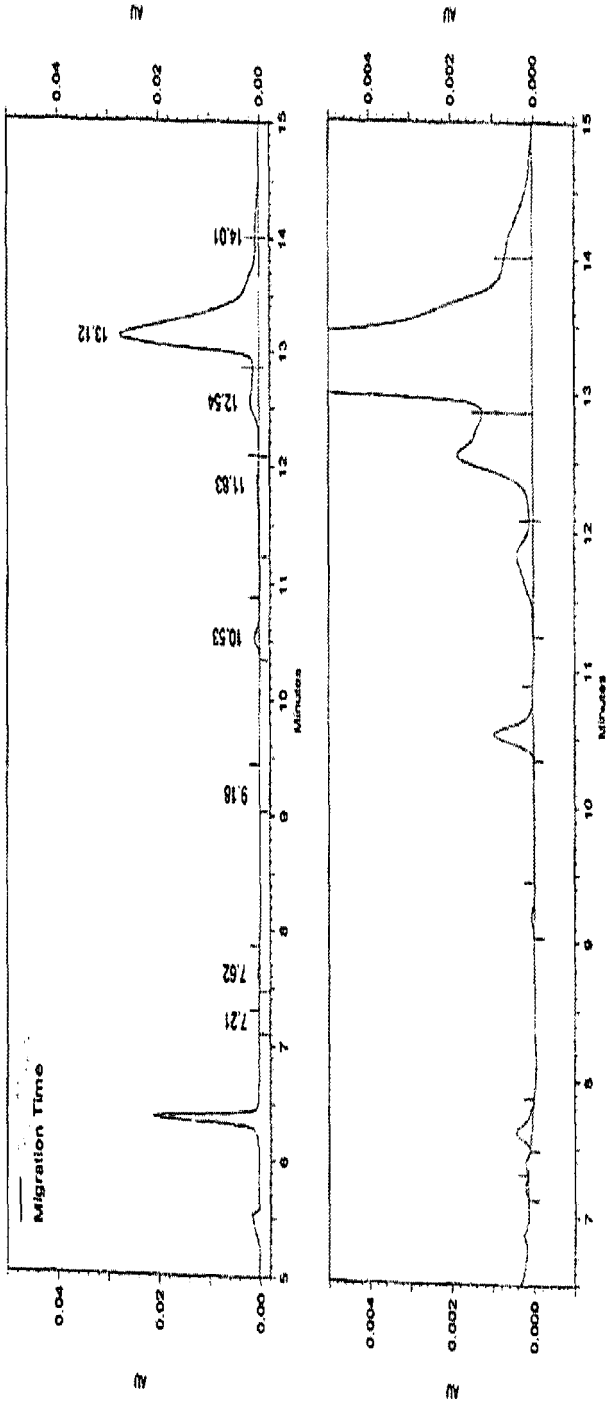
A. Non Reduced

Temp C	Main peak IgG	Prepeak HHL	75kD HL	Total
25	86.3	7.1	1.7	95.1
28	89.0	6.8	1.7	97.5
29.5	88.7	6.8	1.8	97.3
31	88.0	7.5	1.6	97.1
32.5	70.4	7.3	16.3	94.0
34	73.8	7.1	15.0	95.9

B. Reduced

Temp C	HC	LC	RT 9.80	RT 10.16	RT 10.80	Total HC+LC
25	58.2	31.4	1.22	1.08	6.82	89.6
28	59.1	33.0	1.33	1.62	4.30	92.1
29.5	58.2	33.6	1.41	1.45	4.12	91.8
31	62.5	33.3	0.92	0.72	1.95	95.8
32.5	64.0	31.6	0.50	0.23	2.15	95.6
34	64.3	31.8	0.42	0.11	2.07	96.1

Fig. 42A. Ab-A temperature shift to 25° C



PDA - 220nm Results					
Migration Time	Height	Corr. Area	MW	Corr Area %	
7.21	75.00	17.11	13.8690	0.11	
7.62	388.00	140.70	22.8300	0.87	
9.18	63.000	22.42	57.0290	0.14	
10.53	962.000	272.18	86.6560	1.69	
11.83	387.000	244.51	115.3690	1.52	
12.54	1831.000	1142.67	130.9140	7.09	
13.12	27504.000	13912.96	143.5330	86.32	
14.01	677.000	364.54	163.1010	2.26	
Totals		16117.09		100.00	

Fig. 42B. Ab-A temperature shift to 25° C

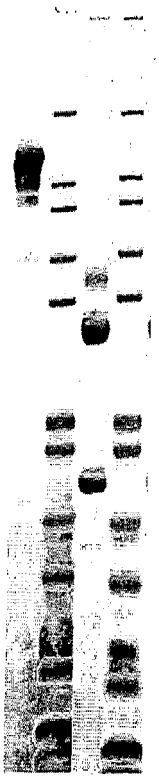
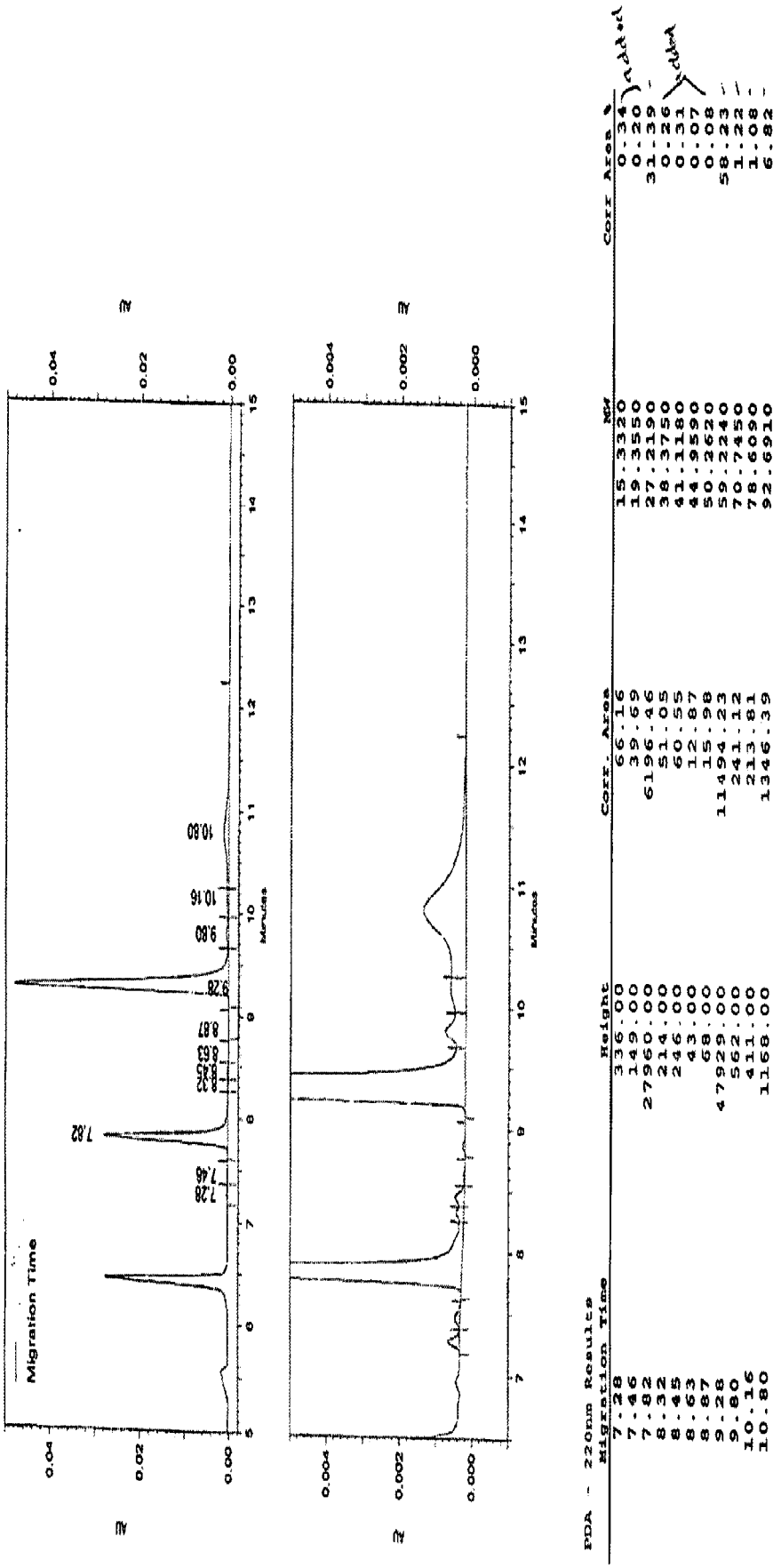


Fig. 42C. Ab-A temperature shift to 25° C



PDA - 220nm Results					
Migration Time	Height	Corr. Area	MW	Corr Area %	
7.28	336.00	66.16	15.3320	0.34	
7.46	149.00	39.69	19.3550	0.20	
7.82	27960.000	6196.46	27.2190	31.39	
8.32	214.000	51.05	38.3750	0.26	
8.45	246.000	60.55	41.1180	0.31	
8.63	43.000	12.87	44.9590	0.07	
8.87	68.000	15.98	50.2620	0.08	
9.28	47929.000	11494.23	59.2240	58.23	
9.80	562.000	241.12	70.7450	1.22	
10.16	411.000	213.81	78.6090	1.08	
10.80	1168.000	1346.39	92.6910	6.82	

Fig. 43A. Ab-A maintained at 28° C (no shift)

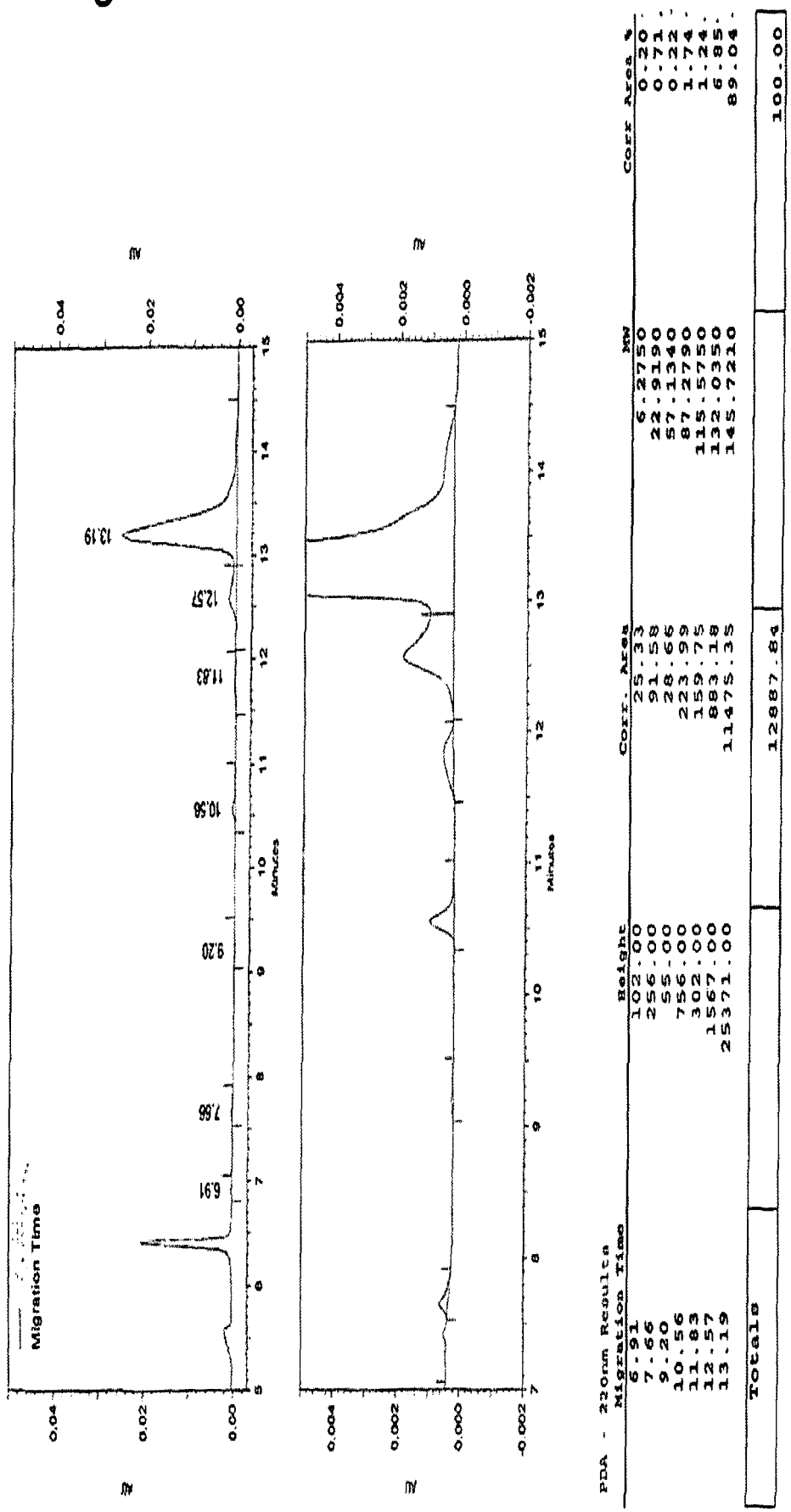


Fig. 43B. Ab-A maintained at 28° C (no shift)



Fig. 43C. Ab-A maintained at 28° C (no shift)

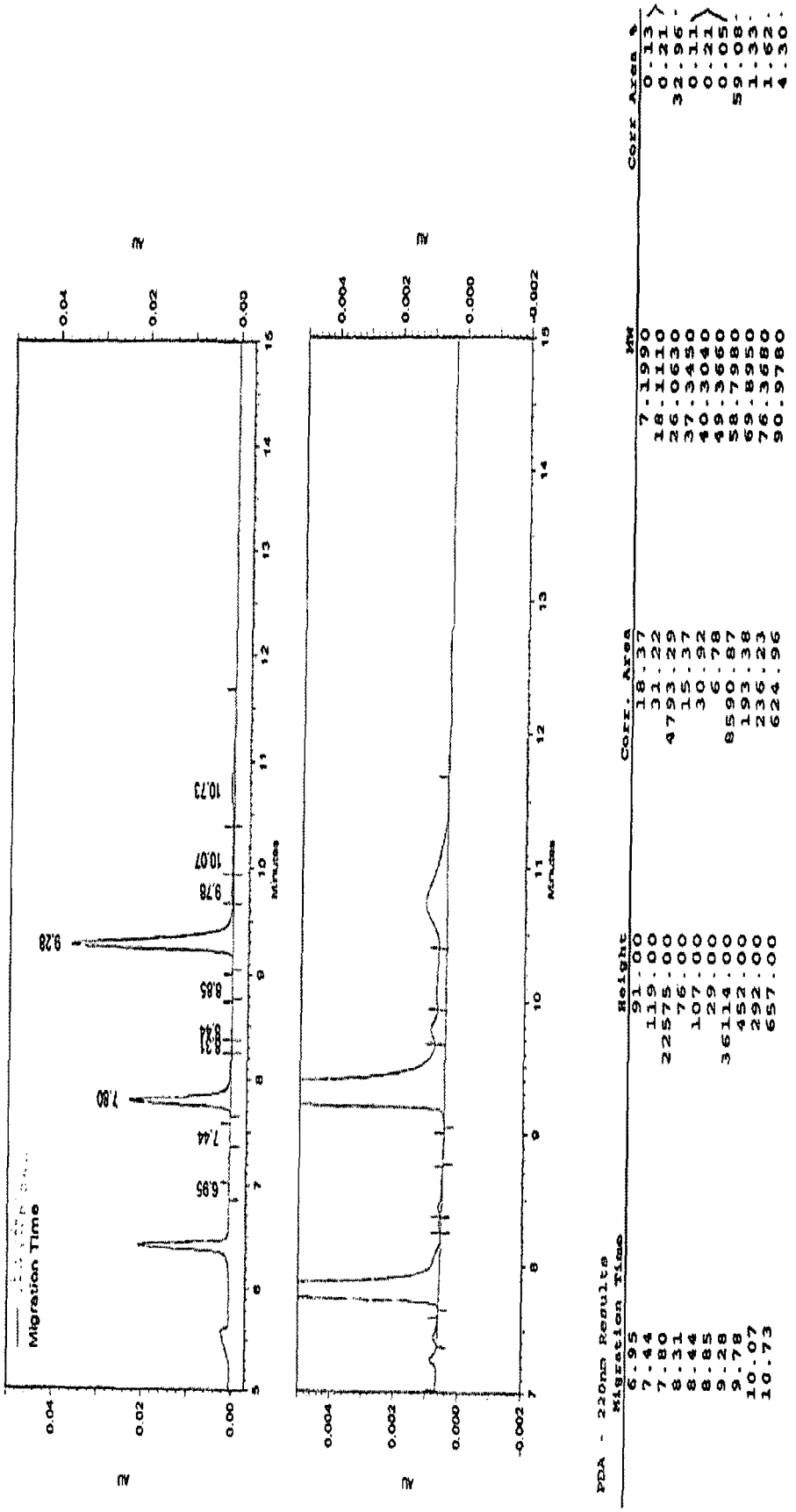


FIG. 44A. Ab-A temperature shift to 29.5° C

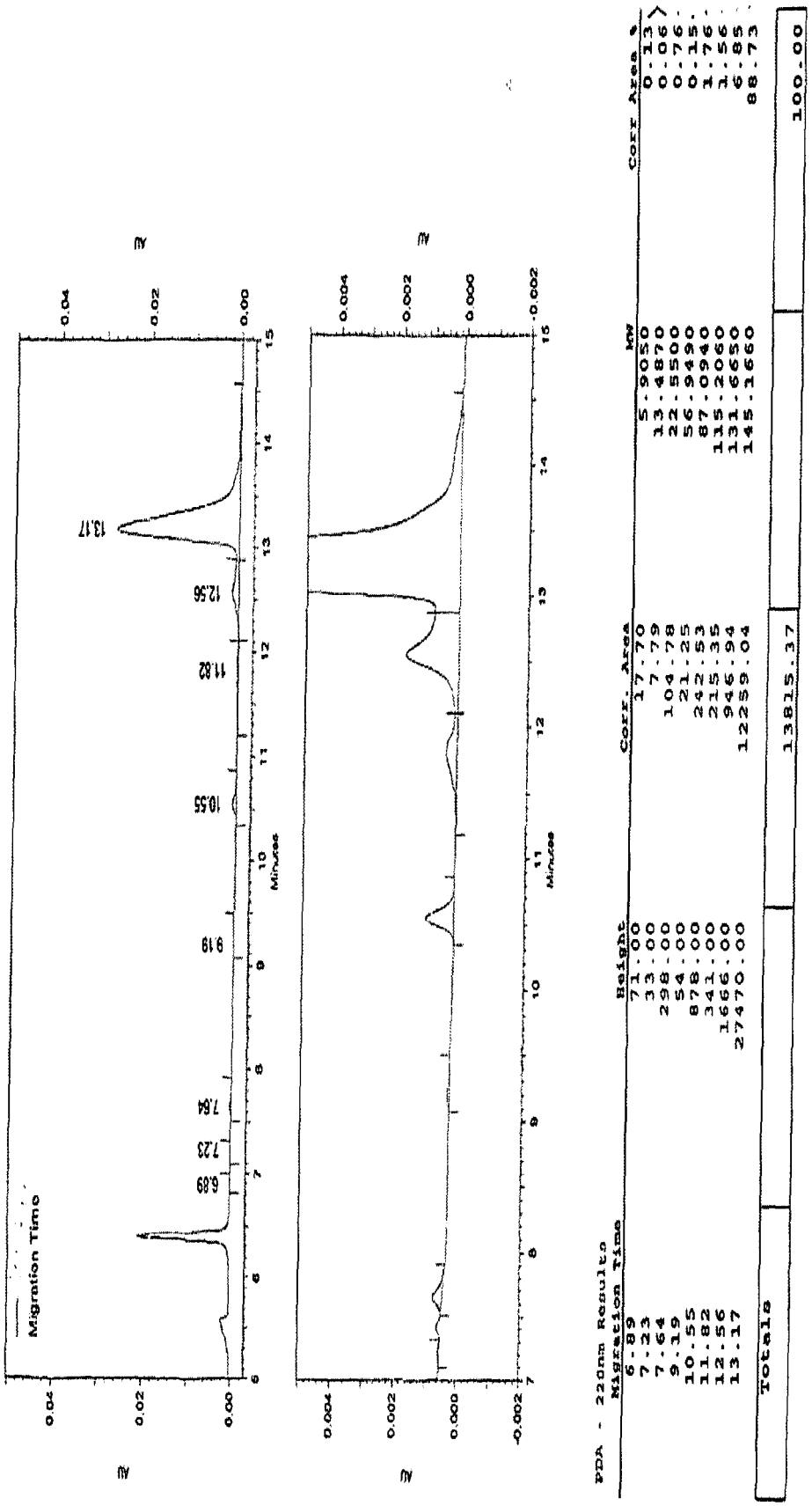
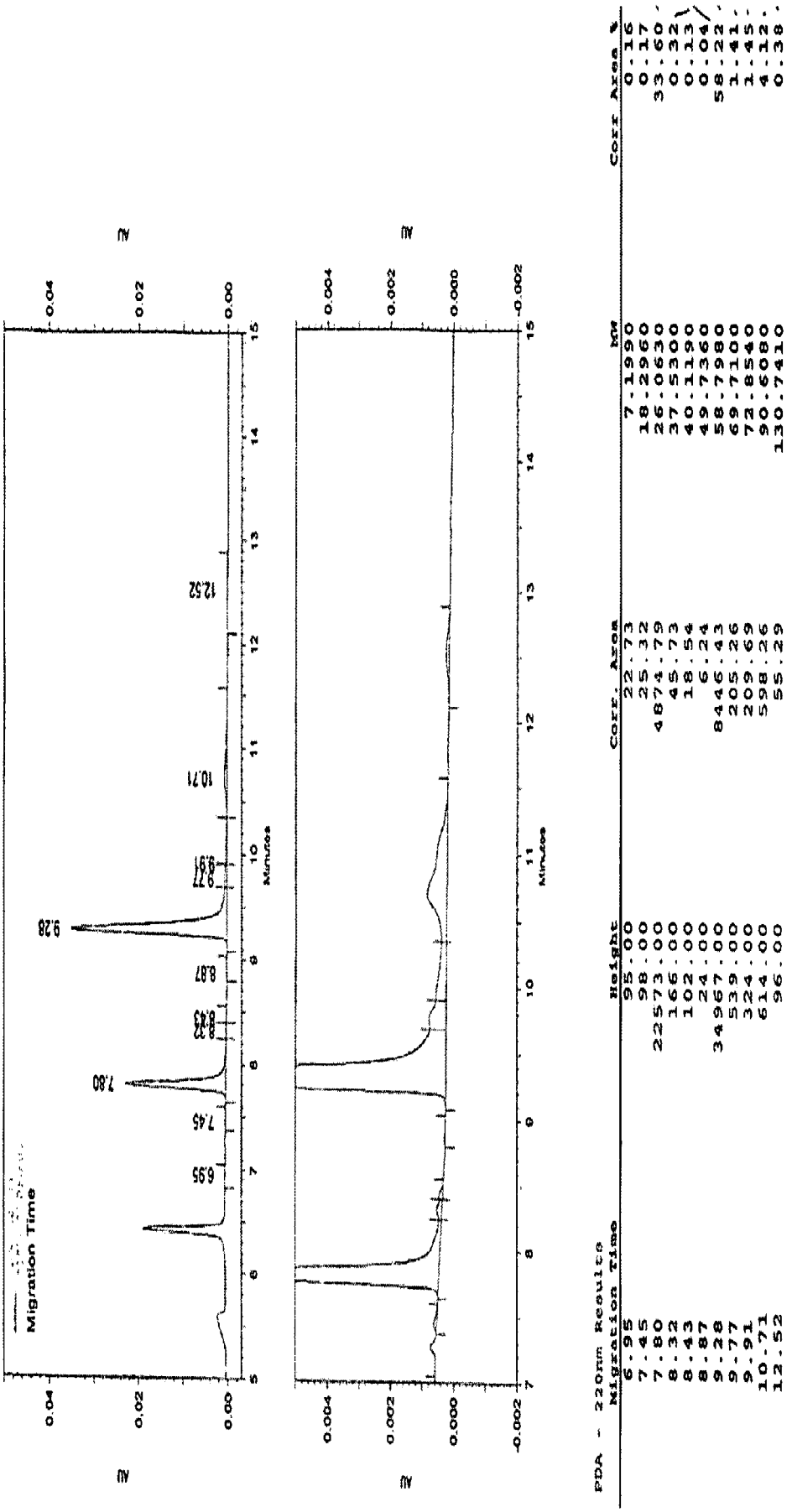


FIG. 44B. Ab-A temperature shift to 29.5° C



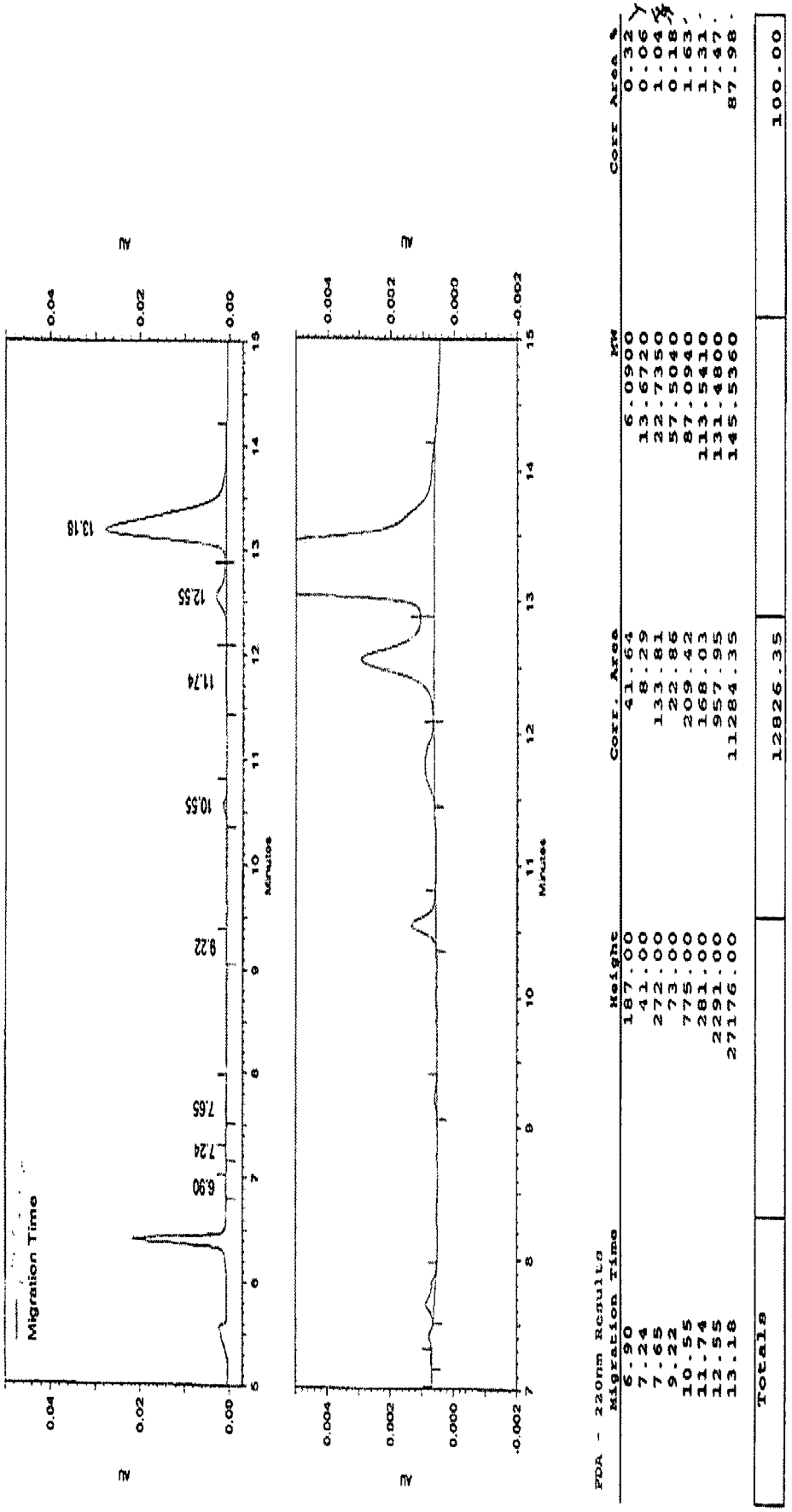
FIG. 44C. Ab-A temperature shift to 29.5° C



PDA - 220nm Results

Migration Time	Height	Corr. Area	MW	Corr Area %
6.95	95.00	22.73	7.1990	0.16
7.45	98.000	25.32	18.2960	0.17
7.80	22573.000	4874.79	26.0630	33.60
8.32	166.000	45.73	37.5300	0.32
8.43	102.000	18.54	40.1190	0.13
8.87	24.000	16.24	49.7360	0.04
9.28	34967.000	8446.43	58.7980	58.22
9.77	539.000	205.26	69.7100	1.41
9.91	324.000	209.69	73.8540	1.45
10.71	614.000	598.26	90.6080	4.12
12.52	96.000	55.29	130.7410	0.38

FIG. 45A. Ab-A temperature shift to 31° C



PDA - 220nm Results					
Migration Time	Height	Corr. Area	MW	Corr Area %	
6.90	187.000	41.64	6.0900	0.32	
7.24	41.000	8.29	13.6720	0.06	
7.65	272.000	133.81	22.7350	1.04	
8.22	73.000	22.86	57.5040	0.18	
10.55	775.000	209.42	87.0940	1.63	
11.74	281.000	168.03	113.5410	1.31	
12.55	2291.000	957.95	131.4800	7.47	
13.18	27176.000	11284.35	145.5360	87.98	
Totals		12826.35		100.00	

FIG. 45B. Ab-A temperature shift to 31° C

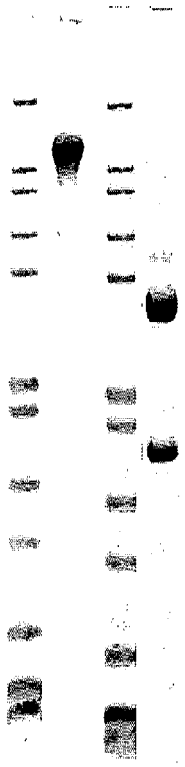


FIG. 45C. Ab-A temperature shift to 31° C

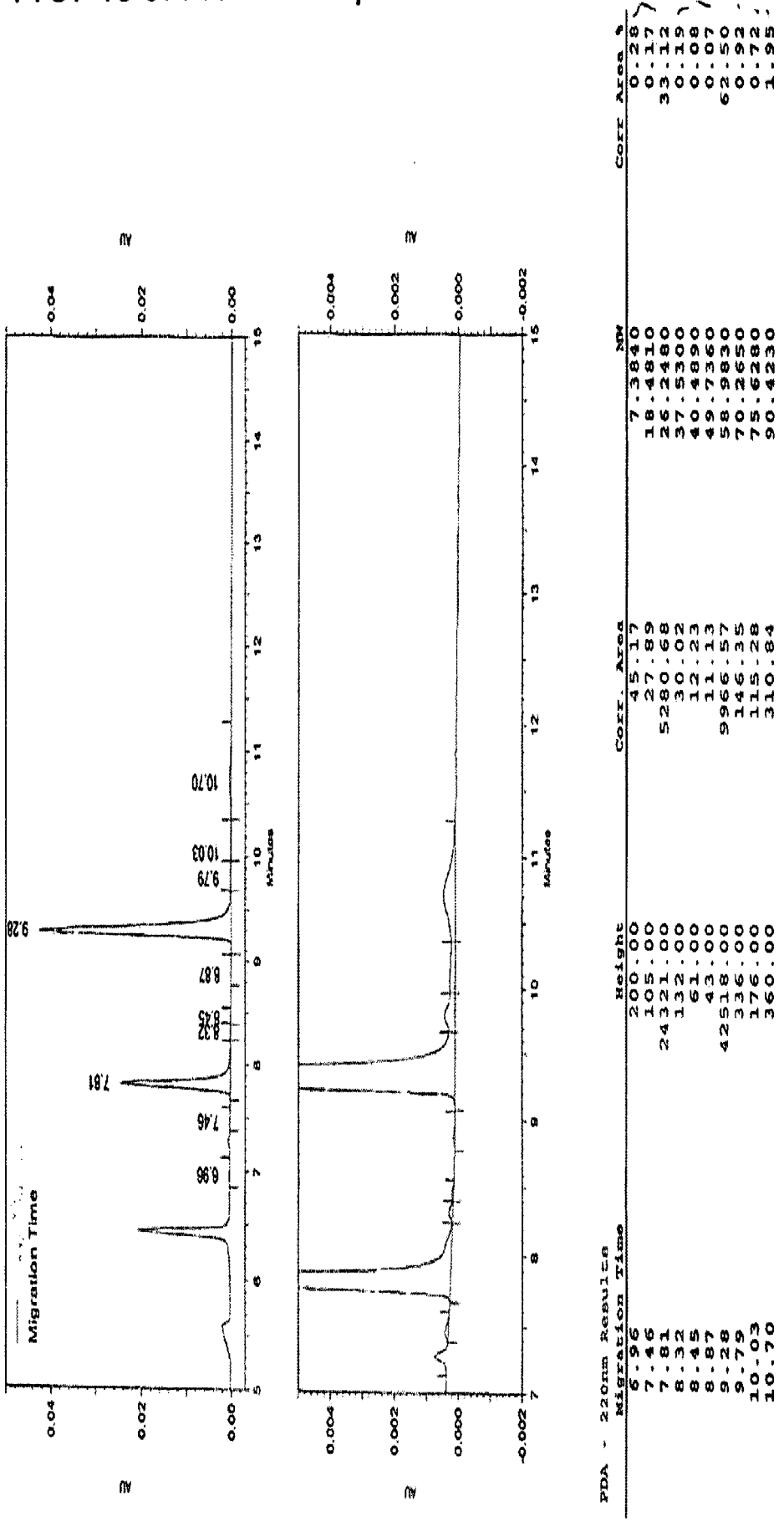


FIG. 46A. Ab-A temperature shift to 32.5° C

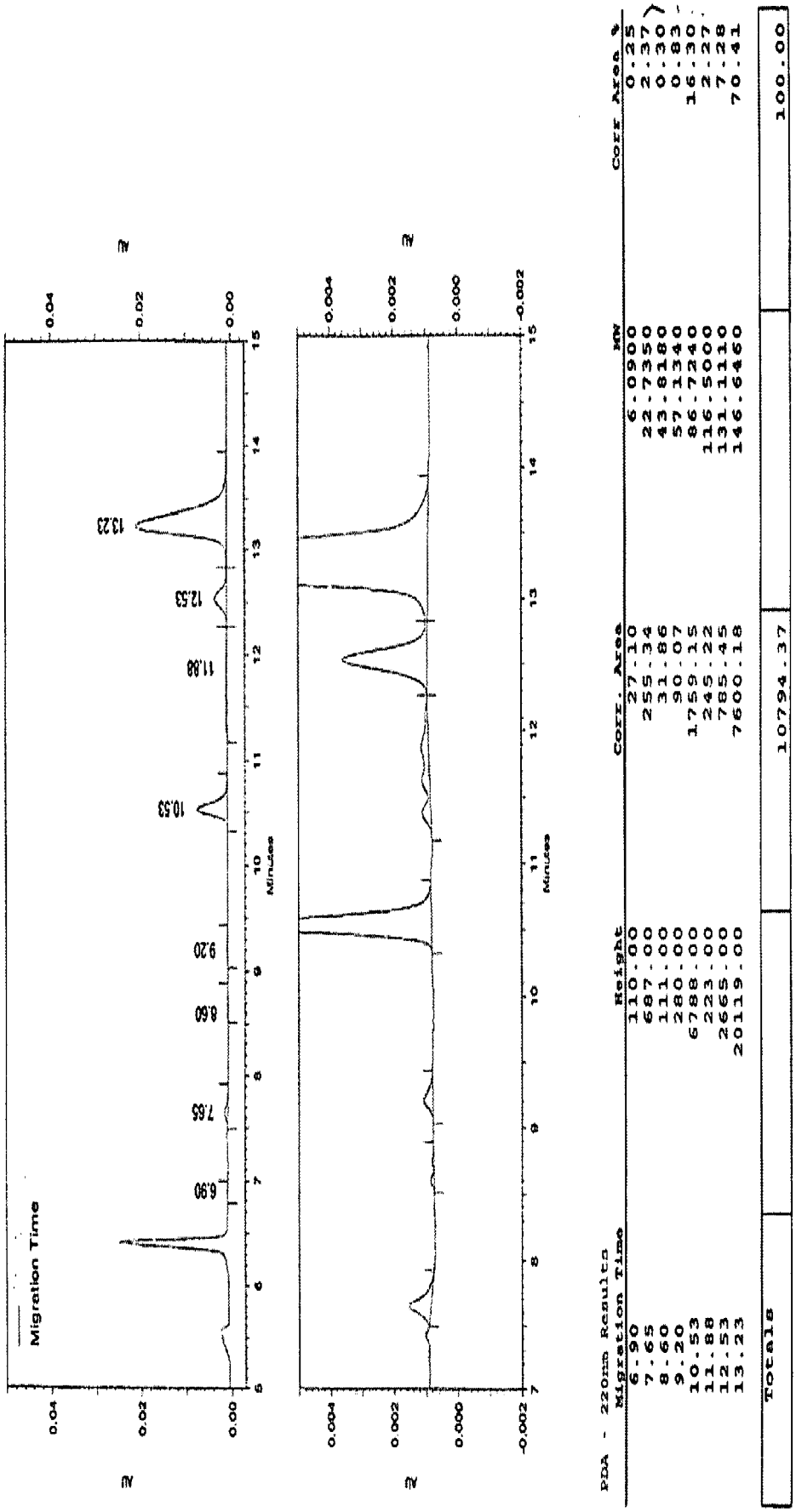


FIG. 46B. Ab-A temperature shift to 32.5° C

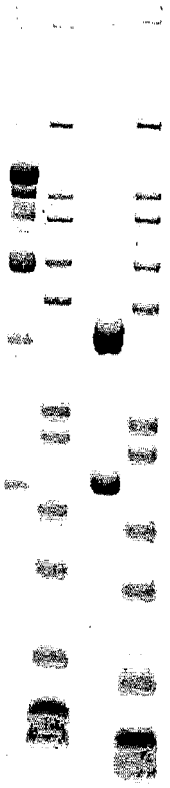


FIG. 46C. Ab-A temperature shift to 32.5° C

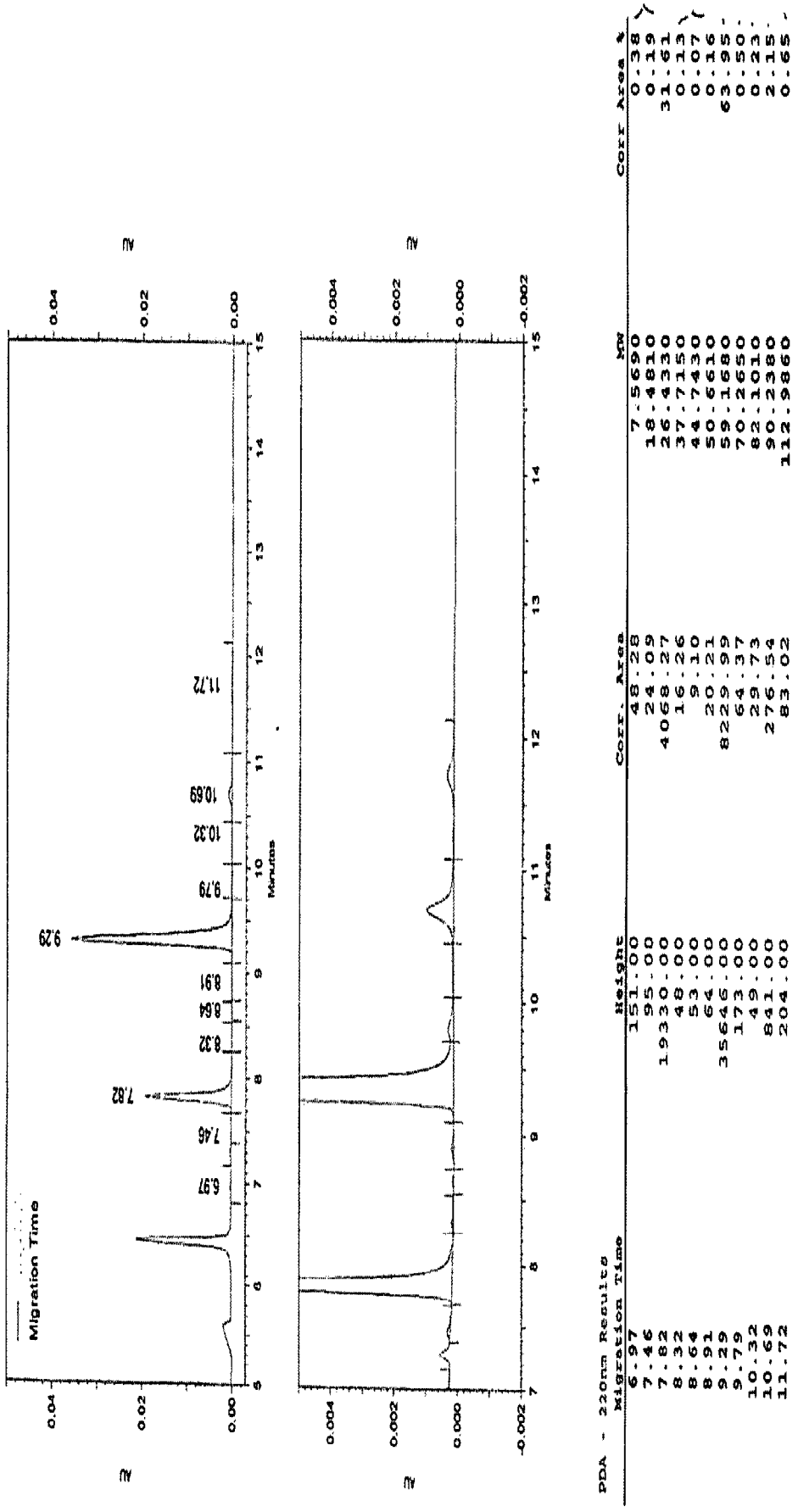


FIG. 47A. Ab-A temperature shift to 34° C

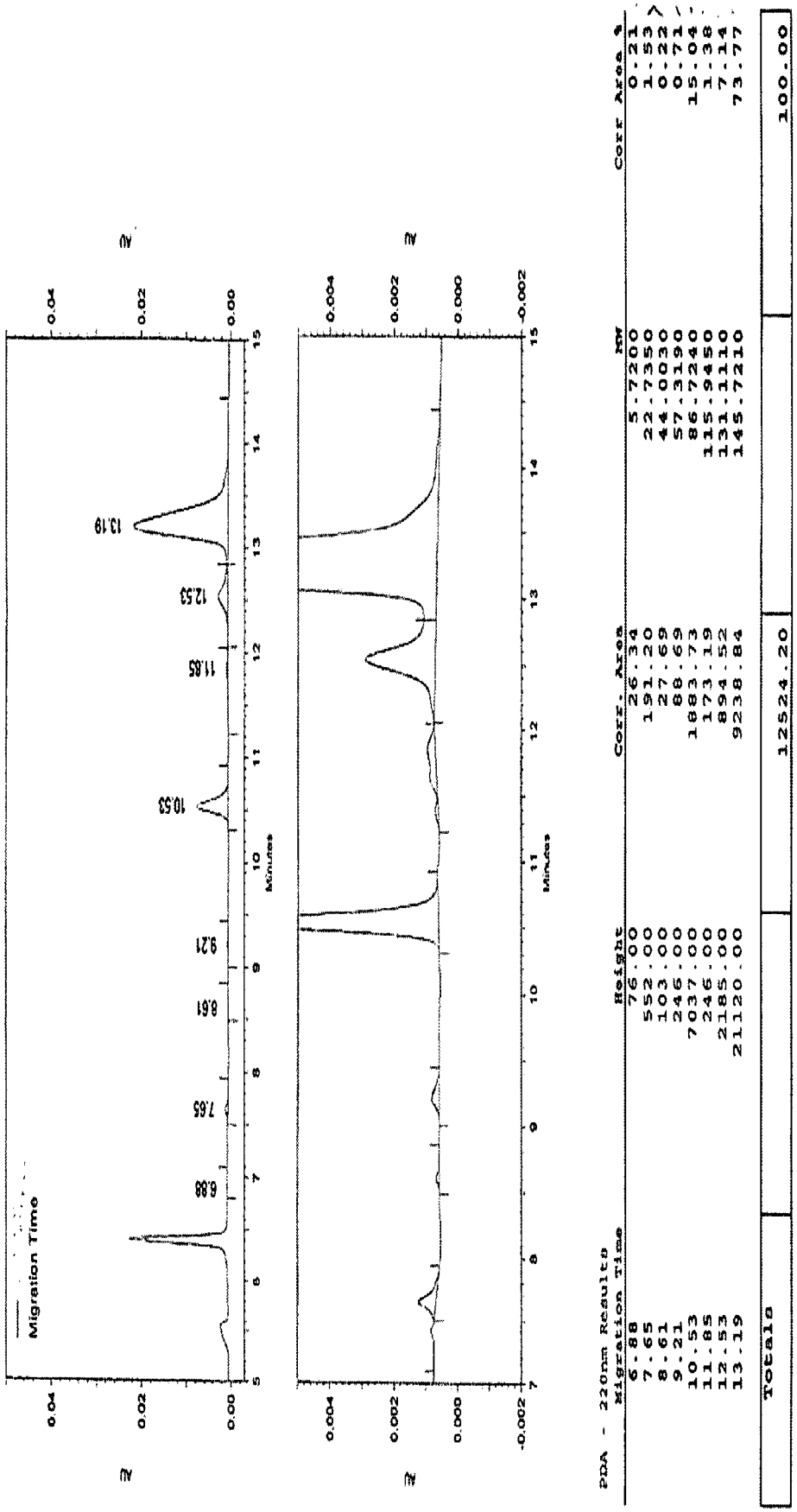


FIG. 47B. Ab-A temperature shift to 34° C

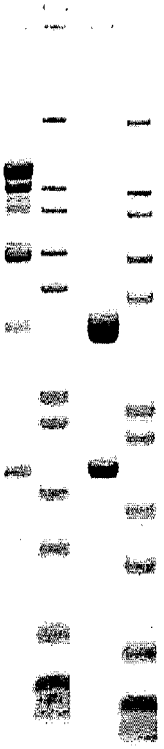


FIG. 47C. Ab-A temperature shift to 34° C

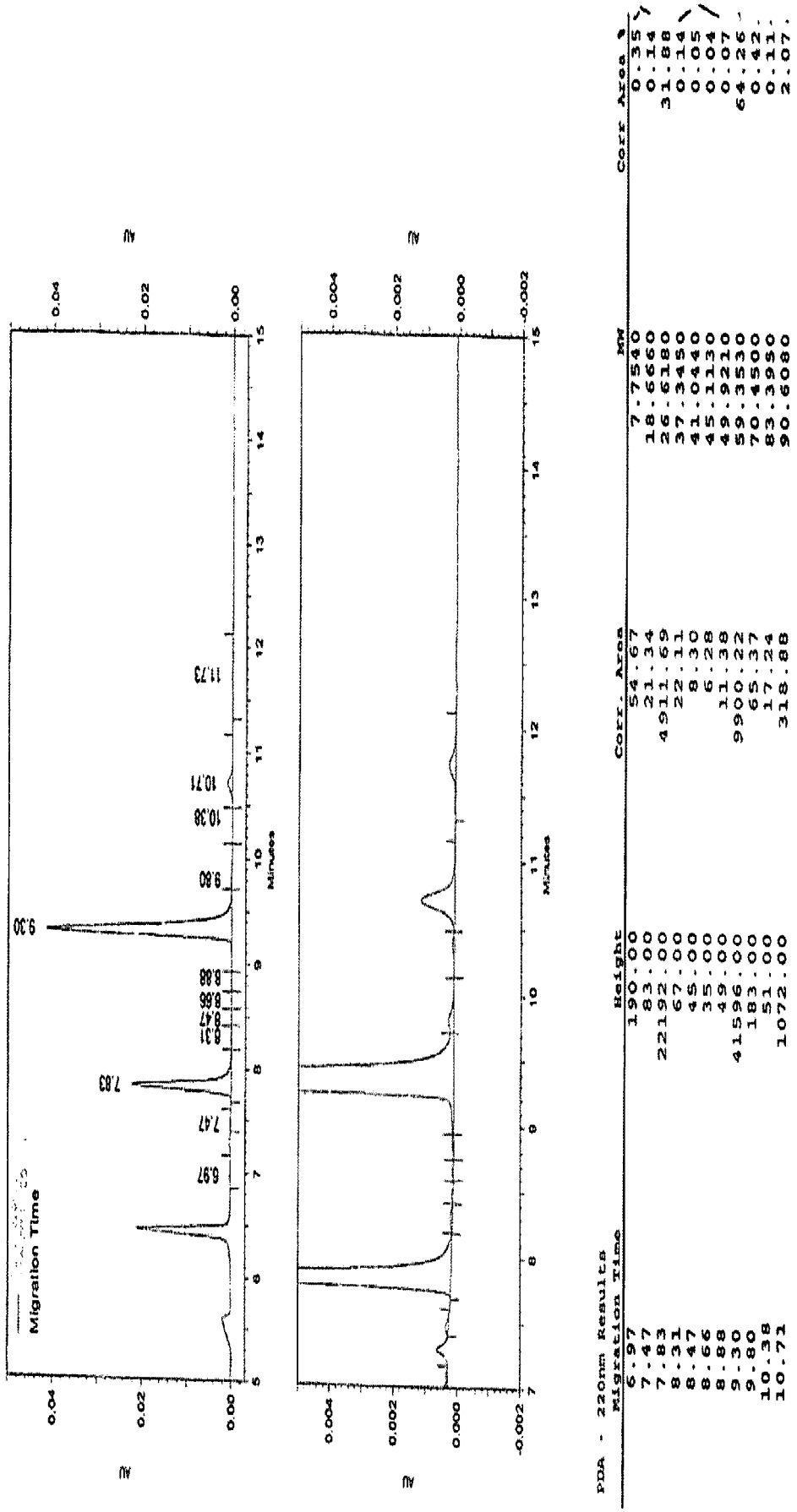


FIG. 48. Ab Titer Is Increased By Temperature Shift

A. Higher-expressing strain (H4/L3)

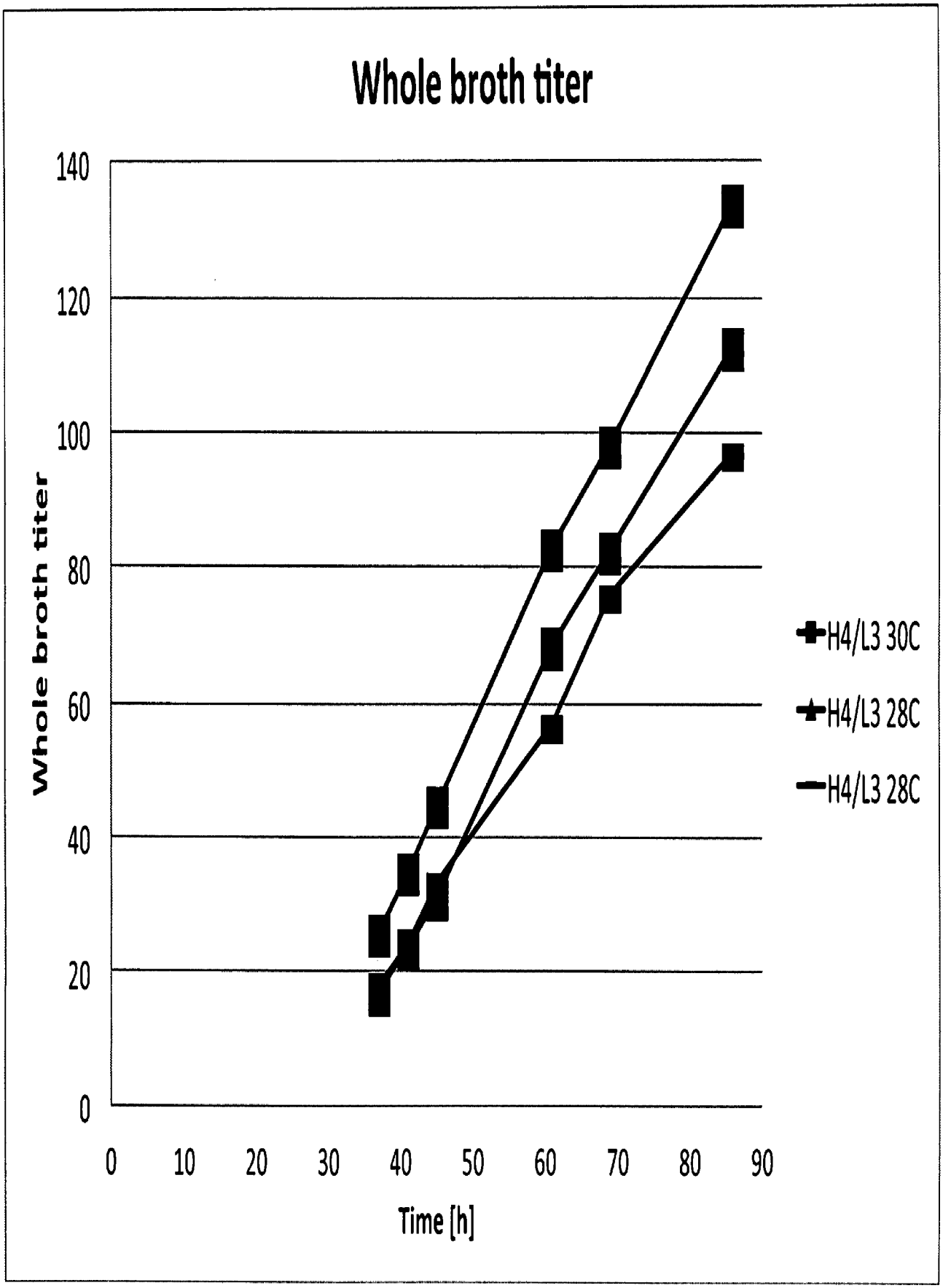


FIG. 48. Ab Titer Is Increased By Temperature Shift

B. Lower-expressing strain (H3/L3)

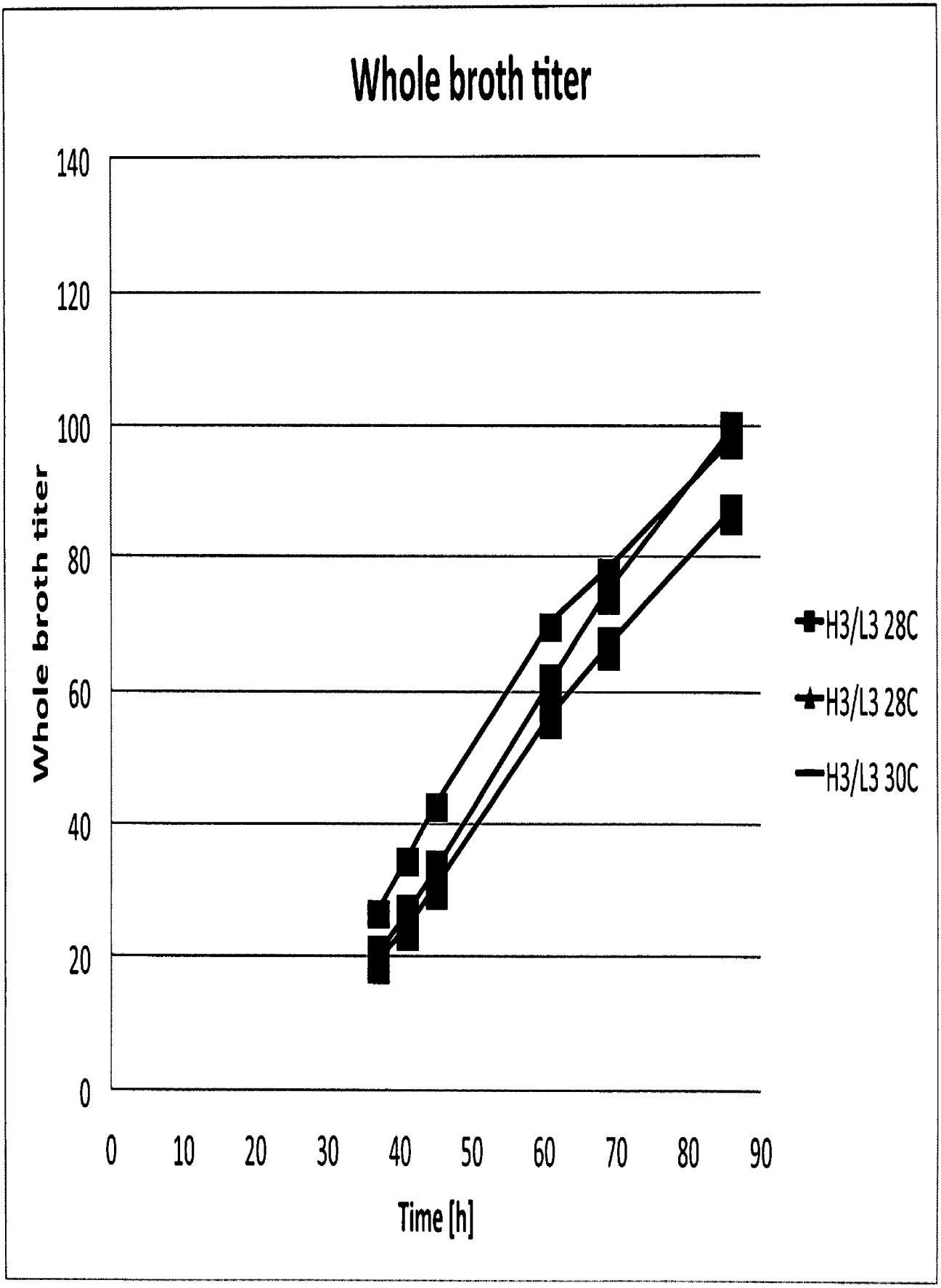


FIG. 49. Antibody Purity (Ab-B)

Non Reduced

Temp C	Main peak IgG	Prepeak HHL	75kD HL	Total
28	89.34	3.27	1.52	94.13
28	76.25	7.35	8.00	91.60
30	91.63	2.94	1.44	96.01
30	83.44	4.75	6.15	94.34
28	87.72	3.60	2.15	93.47
28	61.05	4.92	23.14	89.11

Reduced

Temp C	HC	LC	RT 10.15	RT 10.60	RT 11.08	Total HC/LC
28	67.67	29.58	0.90	0.00	0.55	97.25
28	63.75	30.62	0.69	0.00	0.53	94.37
30	67.91	29.83	0.82	0.00	0.42	97.74
30	67.20	29.55	1.04	0.00	0.57	96.75
28	67.66	29.50	0.85	0.00	0.71	97.16
28	65.42	29.64	0.89	0.00	0.89	95.06

FIG. 50. Ab Titer Is Increased By Temperature Shift

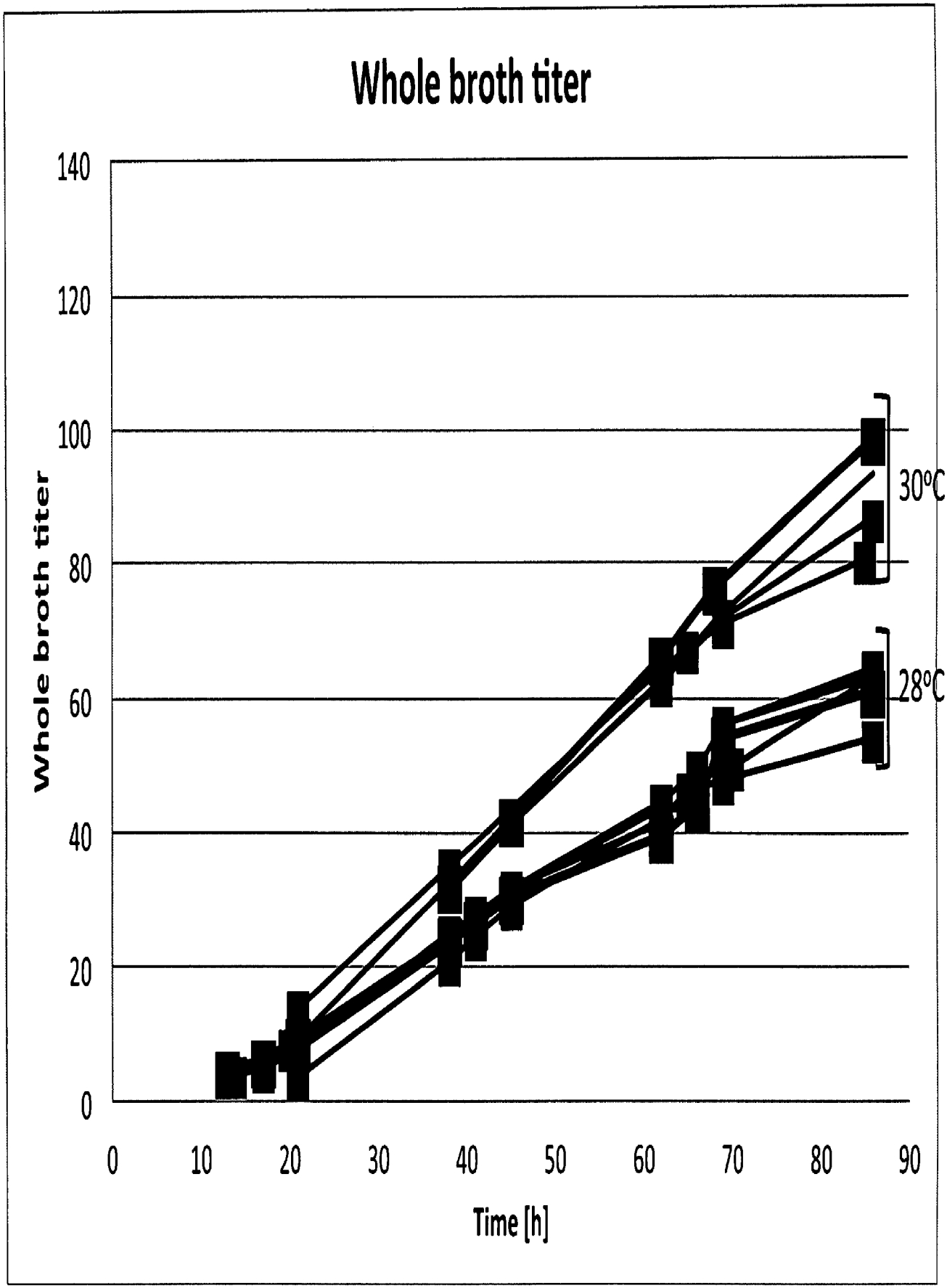
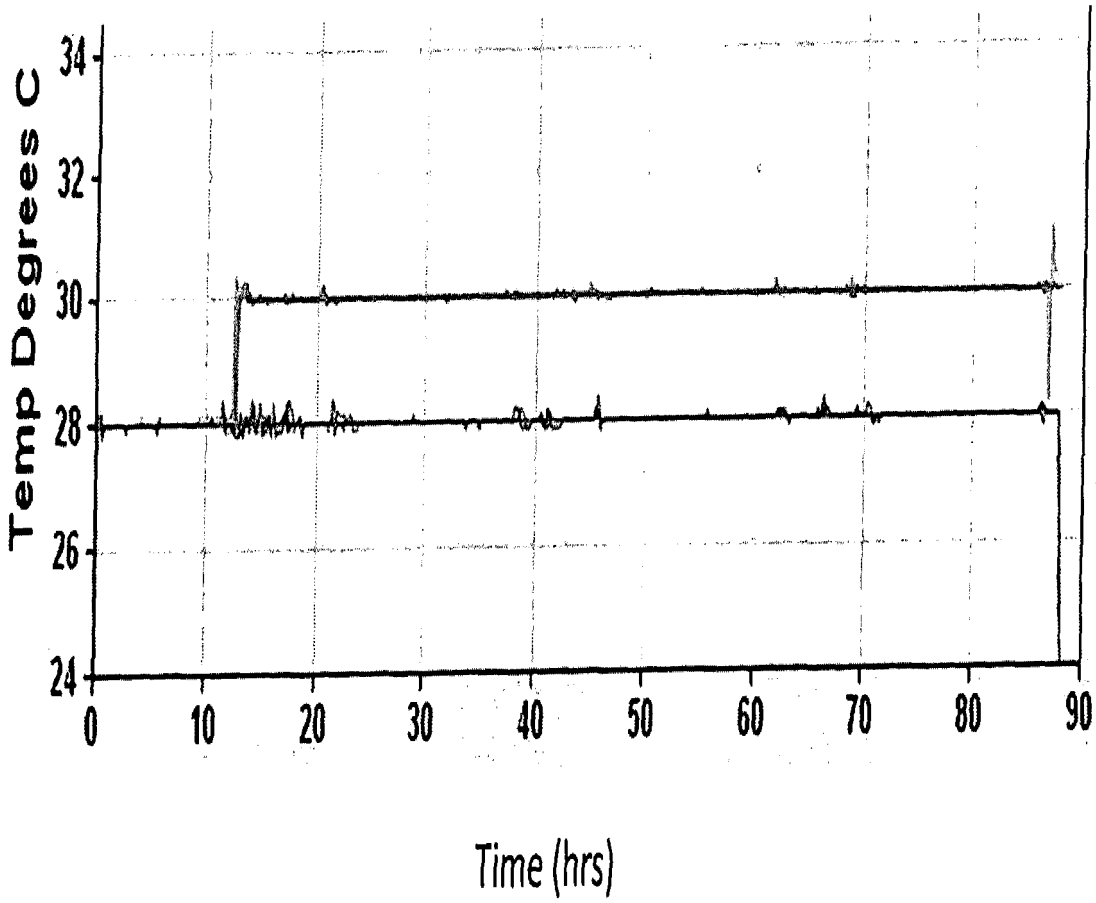


FIG. 51. Ab-A Purity

Non Reduced				
Temp C	Main peak IgG	Prepeak HHL	75kD HL	Total
28	85.64	8.23	1.54	95.41
28	84.97	7.9	1.55	94.42
28	85.87	7.63	1.63	95.13
28	85.75	7.96	1.5	95.21
28	85.96	7.52	1.84	95.32
30	87.88	6.71	1.69	96.28
30	86.64	7.07	1.79	95.5
30	89.54	5.12	2.55	97.21
30	86.56	6.02	4.1	96.68

Reduced						
Temp C	HC	LC	RT 9.80	RT 10.16	RT 10.80	Total HC/LC
28	60.88	32.23	0.96	1.02	2.69	93.11
28	60.31	32.21	1.06	1.26	3.78	92.52
28	59.24	31.53	1.21	1.57	5.18	90.77
28	59.47	32.05	1.11	1.37	4.79	91.52
28	60.05	31.69	1.16	1.27	4.5	91.74
30	63.8	31.75	0.84	0.76	1.97	95.55
30	64.47	32.04	0.8	0.61	0.89	96.51
30	66.52	31.26	0.79	0	0.97	97.78
30	66.33	31.09	0.83	0	1.24	97.42

FIG. 52


Temp Degrees C
34
32
30
28
26
24
0
10
20
30
40
50
60
70
80
90
Time (hrs)