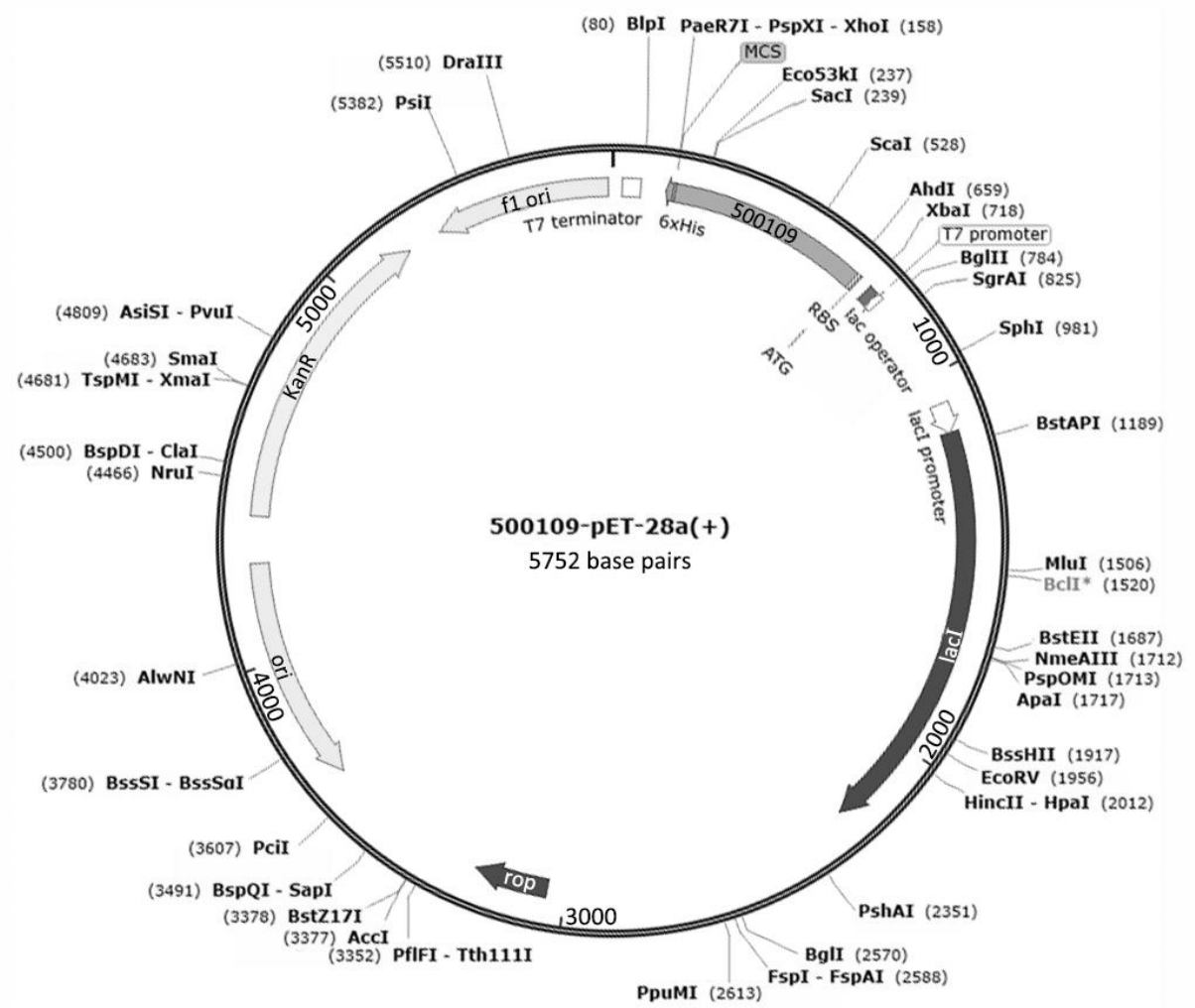

500109-pET-28a(+)
5752 base pairs
(80) BlpI
PaeR7I - PspXI - XhoI (158)
MCS
Eco53kI (237)
SacI (239)
ScaI (528)
AhdI (659)
XbaI (718)
T7 promoter
BglII (784)
SgrAI (825)
SphI (981)
BstAPI (1189)
MluI (1506)
BclI* (1520)
BstEII (1687)
NmeAIII (1712)
PspOMI (1713)
ApaI (1717)
BssHII (1917)
EcoRV (1956)
HincII - HpaI (2012)
PshAI (2351)
BglI (2570)
FspI - FspAI (2588)
PpuMI (2613)
(3352) PflFI - Tth111I
(3377) AccI
(3378) BstZ17I
(3491) BspQI - SapI
(3607) PciI
(3780) BssSI - BssSαI
(4023) AlwNI
(4466) NruI
(4500) BspDI - ClaI
(4681) TspMI - XmaI
(4683) SmaI
(4809) AsiSI - PvuI
(5382) PsiI
(5510) DraIII
f1 ori
T7 terminator
6xHis
500109
RBS
lac operator
ATG
lacI promoter
lacI
rop
ori
KanR
1000
2000
3000
4000
5000

FIG. 1

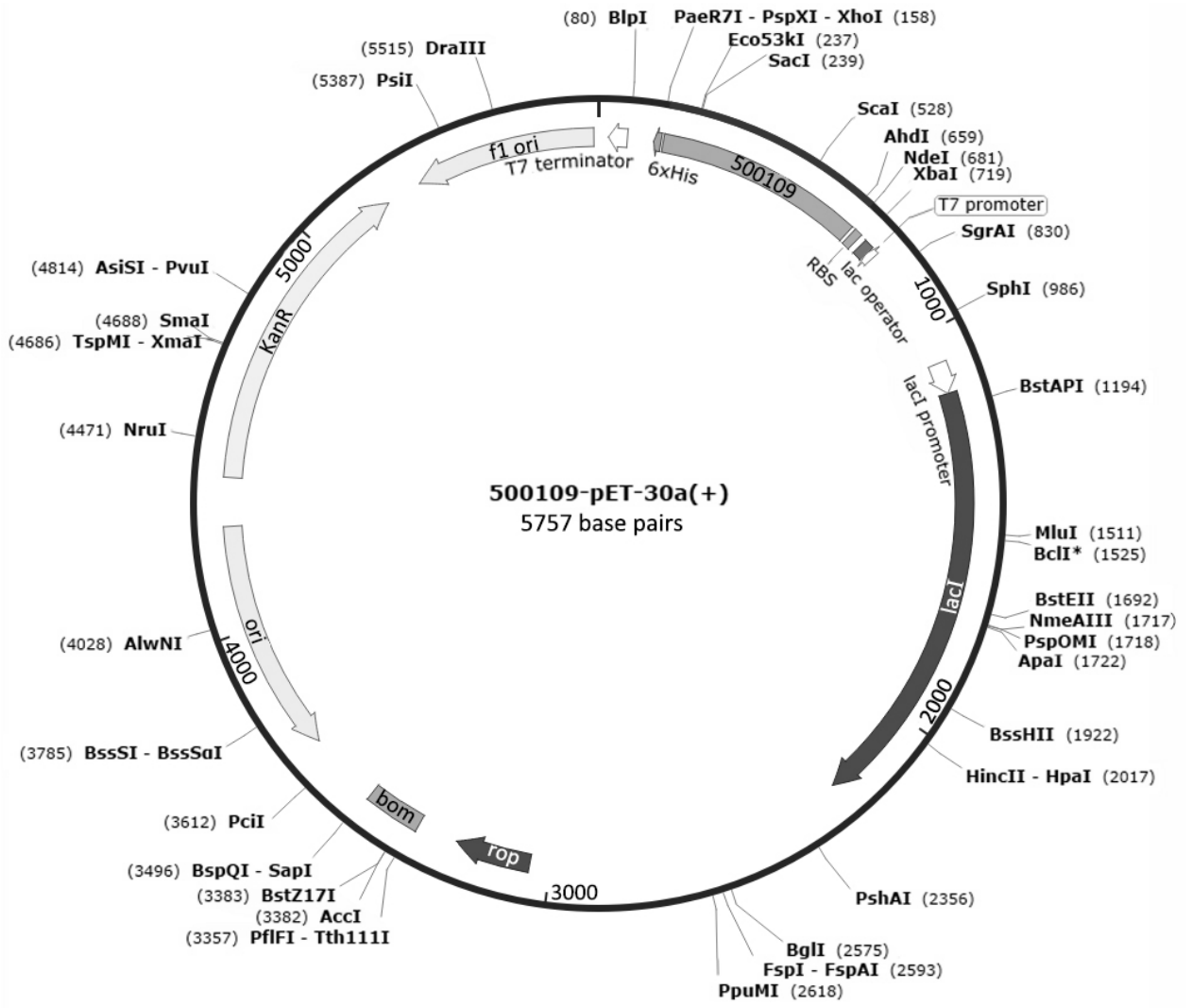

500109-pET-30a(+)
5757 base pairs
(80) BlpI
PaeR7I - PspXI - XhoI (158)
Eco53kI (237)
SacI (239)
ScaI (528)
AhdI (659)
NdeI (681)
XbaI (719)
T7 promoter
SgrAI (830)
SphI (986)
BstAPI (1194)
MluI (1511)
BclI* (1525)
BstEII (1692)
NmeAIII (1717)
PspOMI (1718)
ApaI (1722)
BssHII (1922)
HincII - HpaI (2017)
PshAI (2356)
BglI (2575)
FspI - FspAI (2593)
PpuMI (2618)
3000
rop
bom
(3612) PciI
(3496) BspQI - SapI
(3383) BstZ17I
(3382) AccI
(3357) PflFI - Tth111I
(3785) BssSI - BssSαI
(4028) AlwNI
4000
ori
(4471) NruI
(4686) TspMI - XmaI
(4688) SmaI
(4814) AsiSI - PvuI
5000
KanR
(5387) PsiI
(5515) DraIII
f1 ori
T7 terminator
6xHis
500109
RBS
lac operator
1000
lacI promoter
lacI
2000

FIG. 2

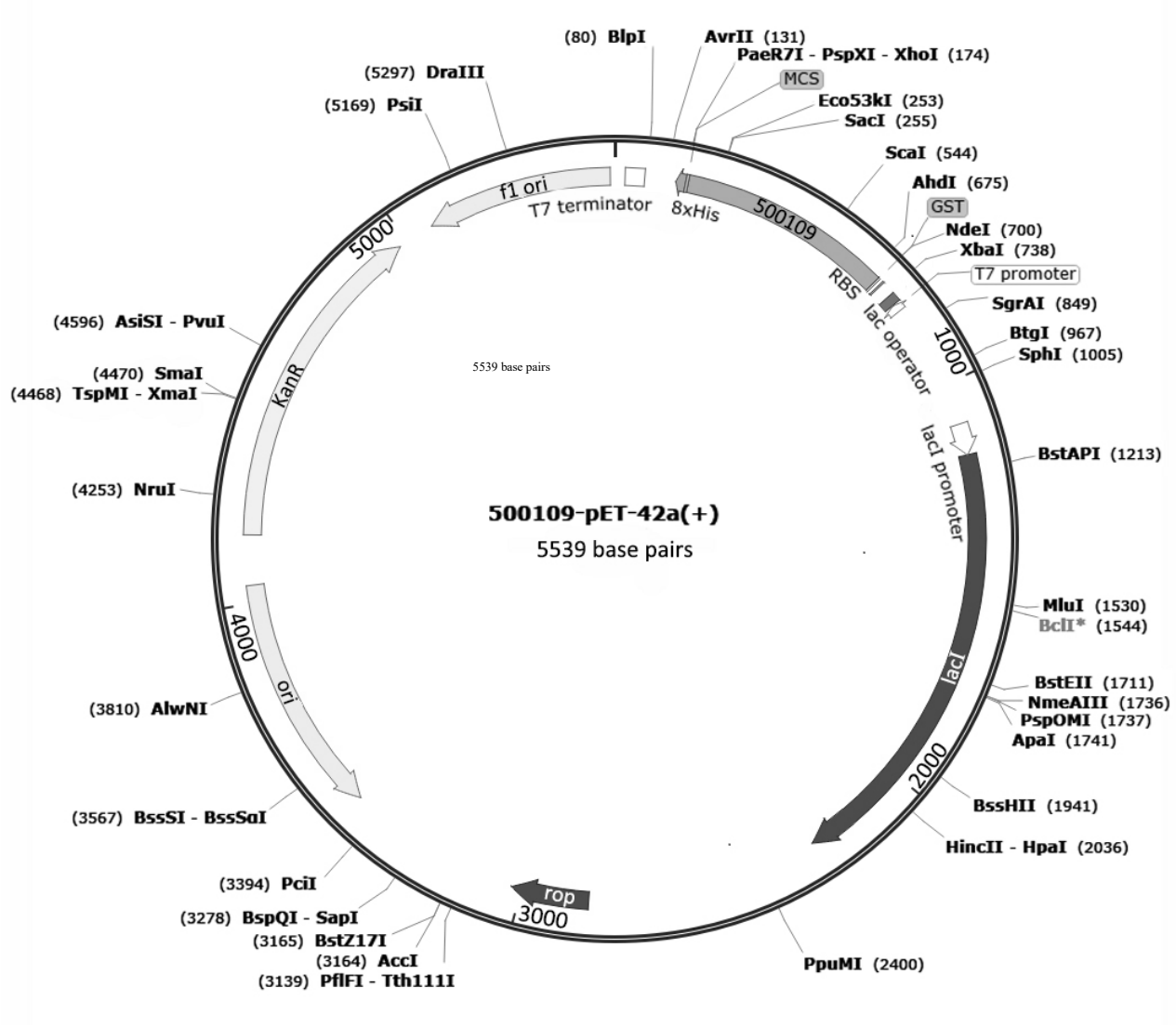

(80) BlpI
AvrII (131)
PaeR7I - PspXI - XhoI (174)
MCS
Eco53kI (253)
SacI (255)
ScaI (544)
AhdI (675)
GST
NdeI (700)
XbaI (738)
T7 promoter
SgrAI (849)
BtgI (967)
SphI (1005)
BstAPI (1213)
MluI (1530)
BclI* (1544)
BstEII (1711)
NmeAIII (1736)
PspOMI (1737)
ApaI (1741)
BssHII (1941)
HincII - HpaI (2036)
PpuMI (2400)
(3139) PflFI - Tth111I
(3164) AccI
(3165) BstZ17I
(3278) BspQI - SapI
(3394) PciI
(3567) BssSI - BssSαI
(3810) AlwNI
(4253) NruI
(4468) TspMI - XmaI
(4470) SmaI
(4596) AsiSI - PvuI
(5169) PsiI
(5297) DraIII
f1 ori
T7 terminator
8xHis
500109
RBS
lac operator
lacI promoter
lacI
ori
KanR
rop
1000
2000
3000
4000
5000
500109-pET-42a(+)
5539 base pairs

FIG. 3

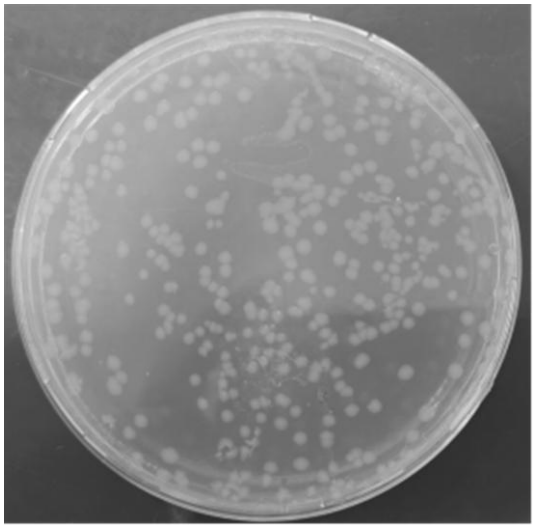


FIG. 4

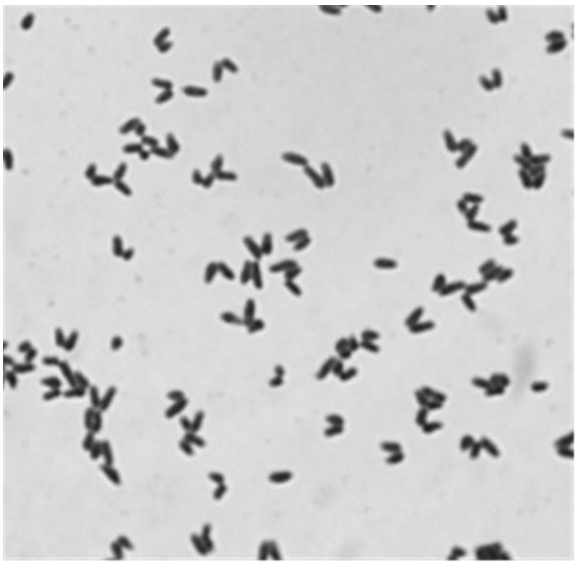


FIG. 5

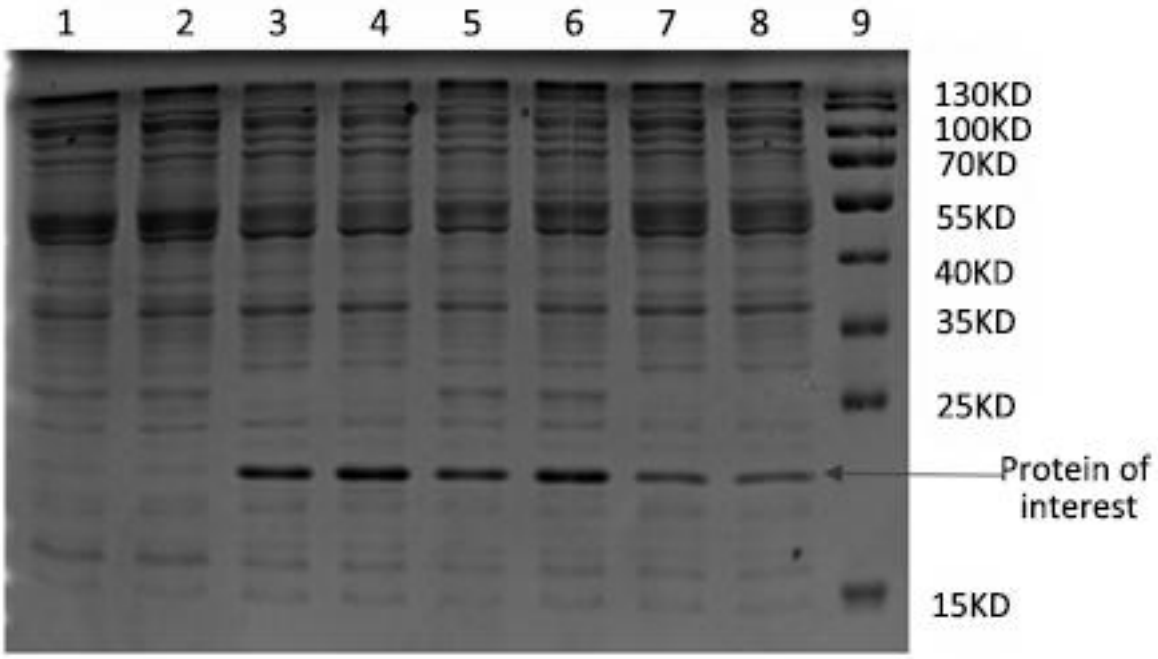

1
2
3
4
5
6
7
8
9
130KD
100KD
70KD
55KD
40KD
35KD
25KD
Protein of interest
15KD

FIG. 6

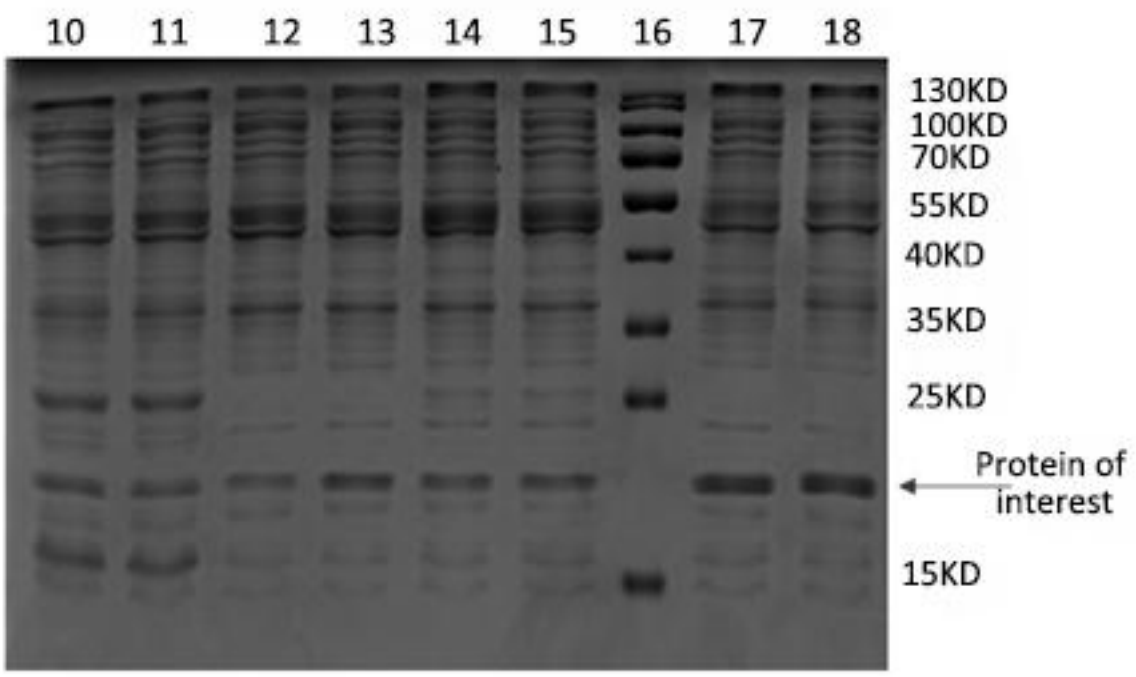

10
11
12
13
14
15
16
17
18
130KD
100KD
70KD
55KD
40KD
35KD
25KD
Protein of interest
15KD

FIG. 7

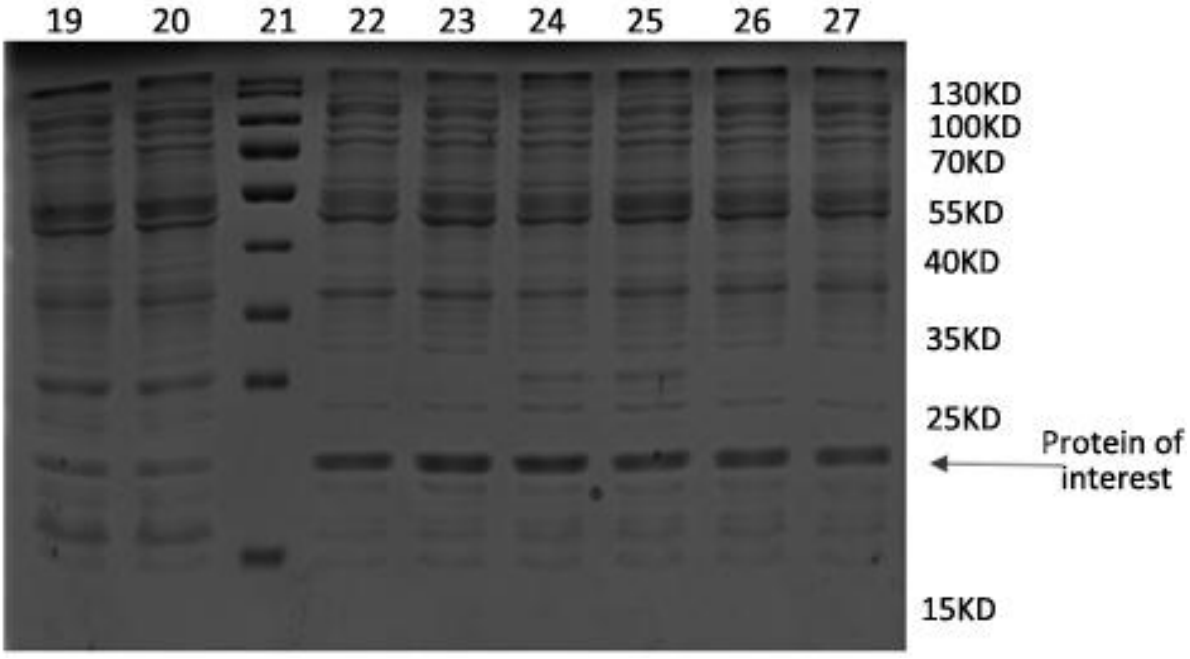

19
20
21
22
23
24
25
26
27
130KD
100KD
70KD
55KD
40KD
35KD
25KD
Protein of interest
15KD

FIG. 8

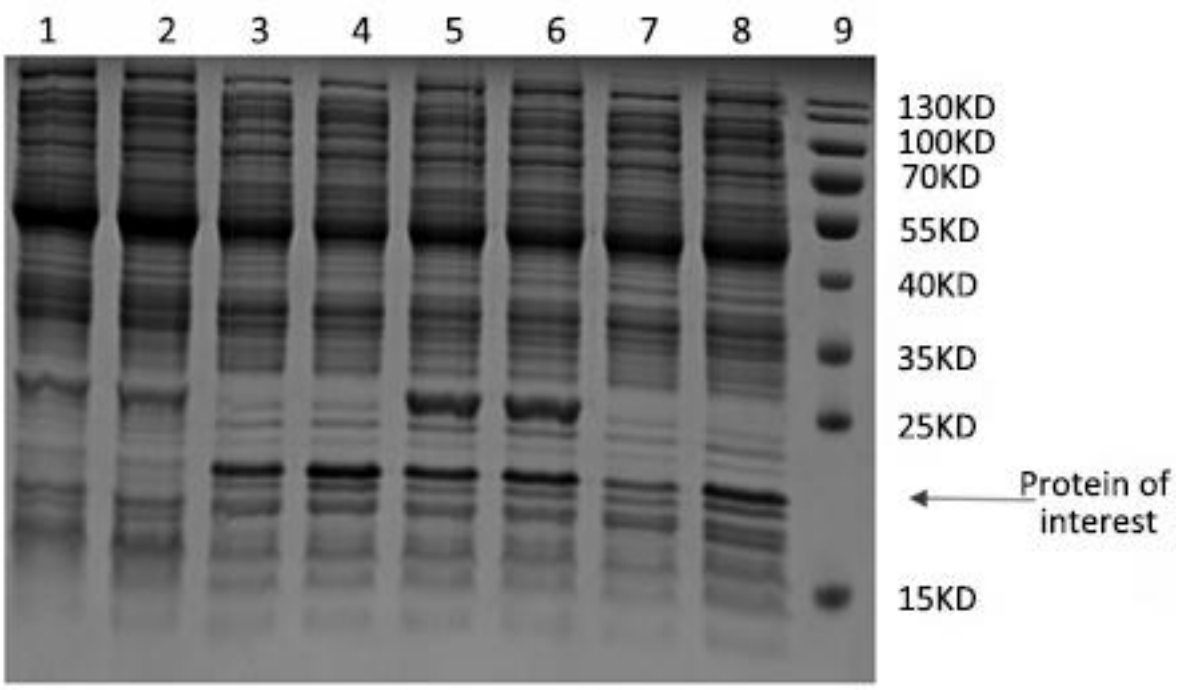

1
2
3
4
5
6
7
8
9
130KD
100KD
70KD
55KD
40KD
35KD
25KD
Protein of interest
15KD

FIG. 9

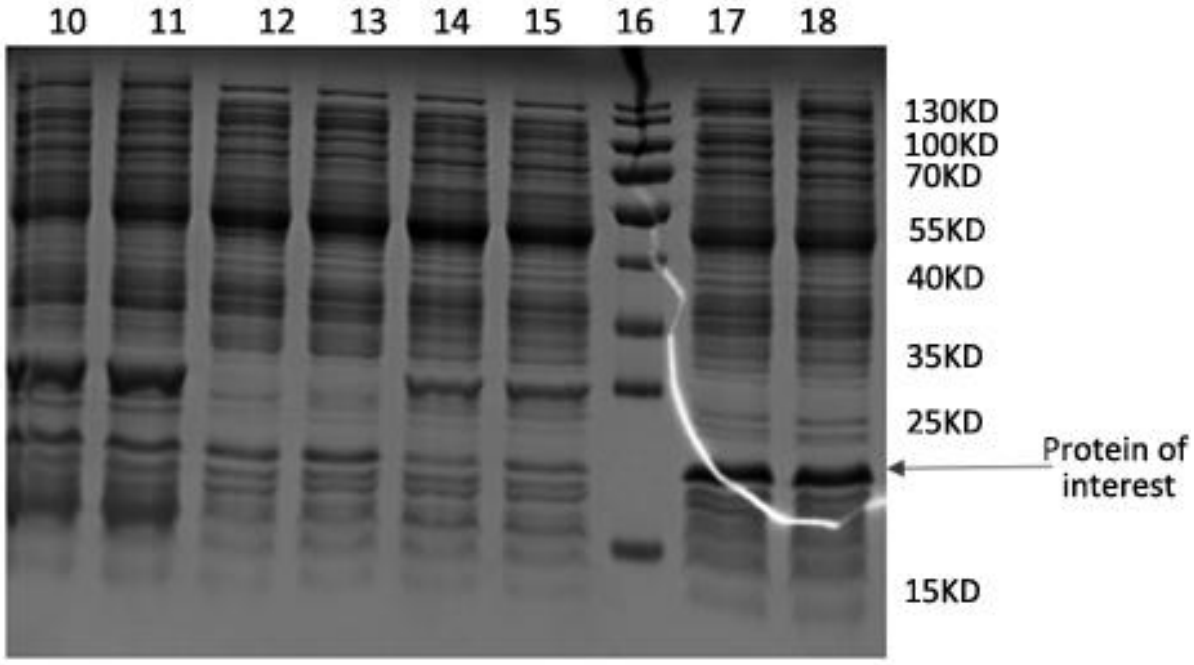

10
11
12
13
14
15
16
17
18
130KD
100KD
70KD
55KD
40KD
35KD
25KD
Protein of interest
15KD

FIG. 10

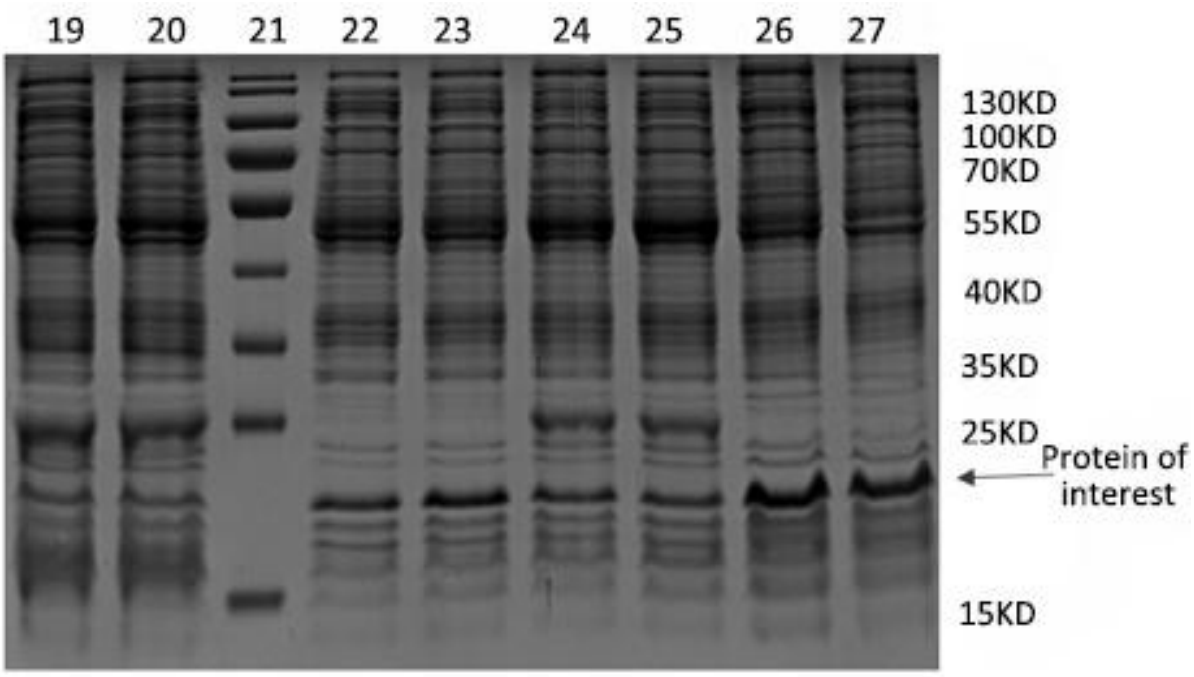

19
20
21
22
23
24
25
26
27
130KD
100KD
70KD
55KD
40KD
35KD
25KD
Protein of interest
15KD

FIG. 11

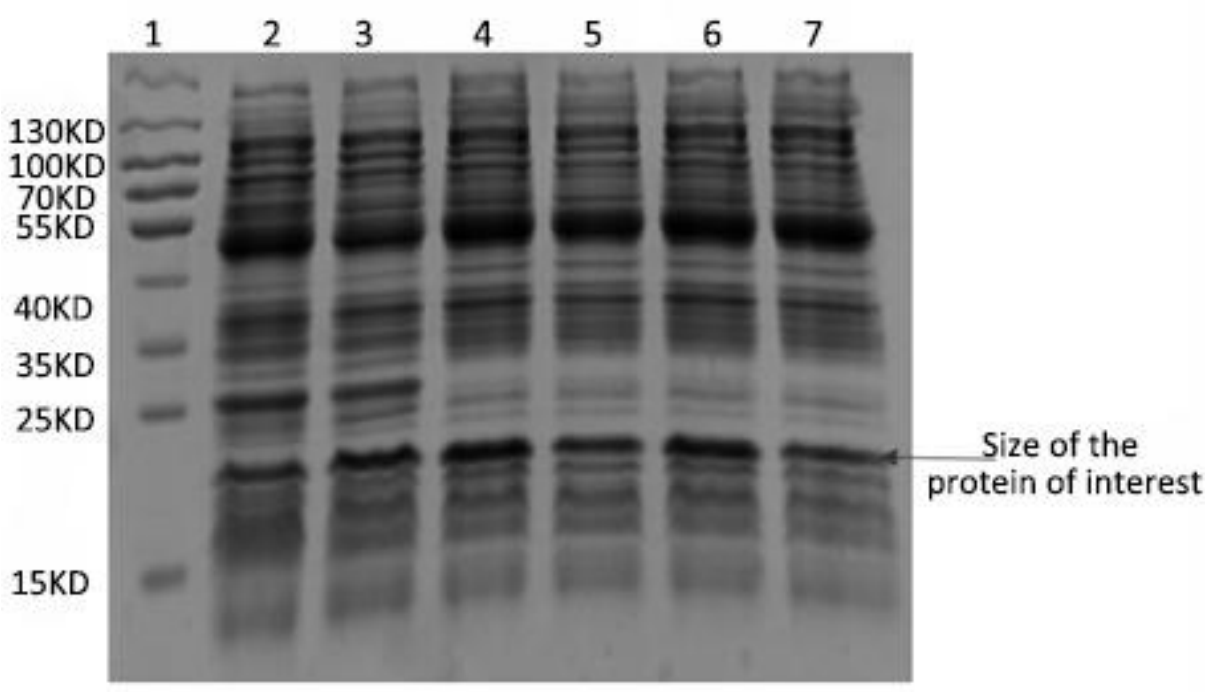

1
2
3
4
5
6
7
130KD
100KD
70KD
55KD
40KD
35KD
25KD
15KD
Size of the protein of interest

FIG. 12

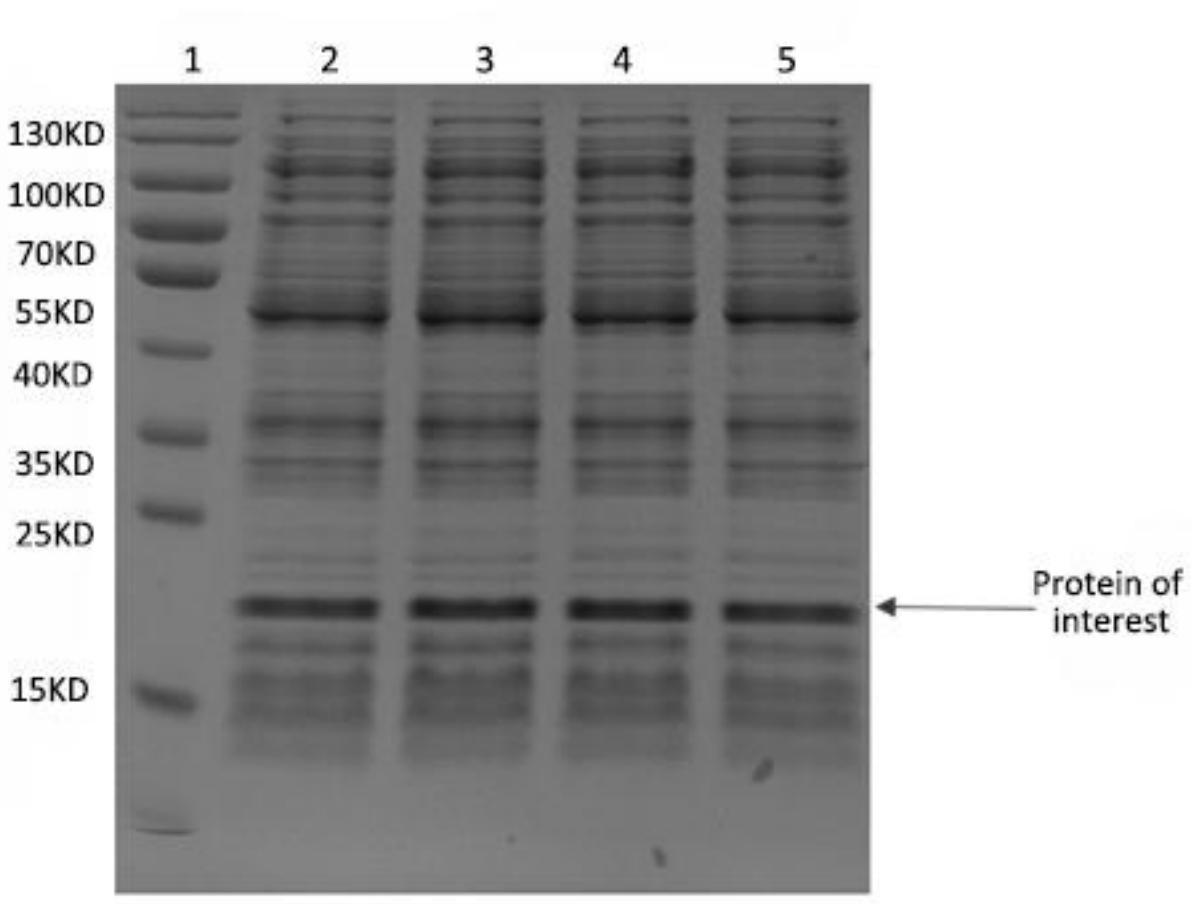

1
2
3
4
5
130KD
100KD
70KD
55KD
40KD
35KD
25KD
15KD
Protein of interest

FIG. 13

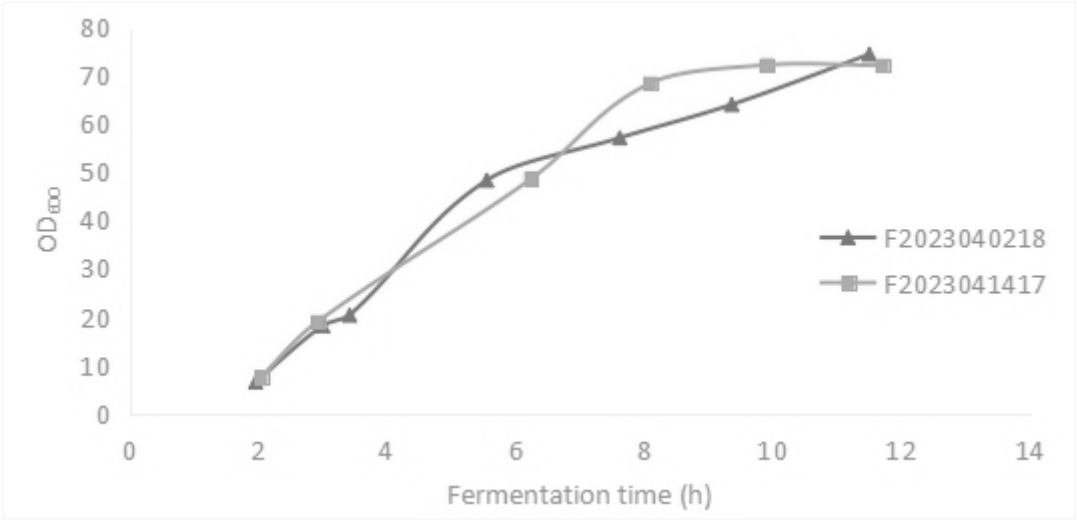

80
70
60
50
40
30
20
10
0
OD600
0
2
4
6
8
10
12
14
Fermentation time (h)
F2023040218
F2023041417

FIG. 14

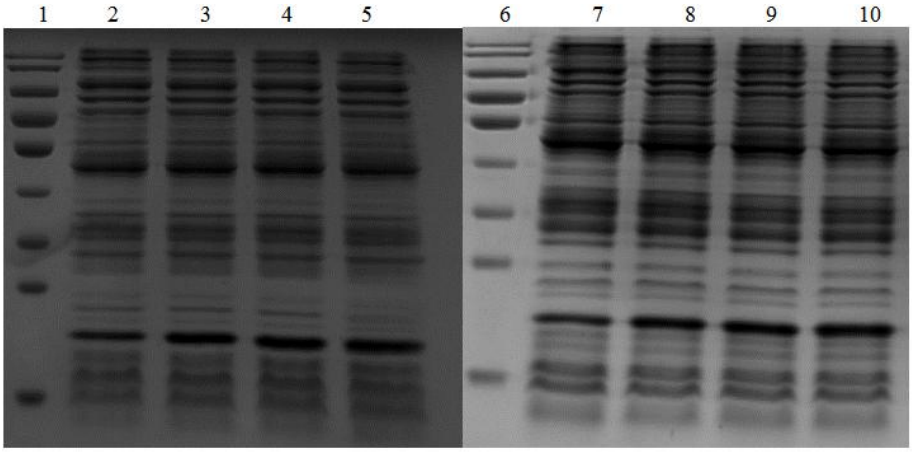


FIG. 15

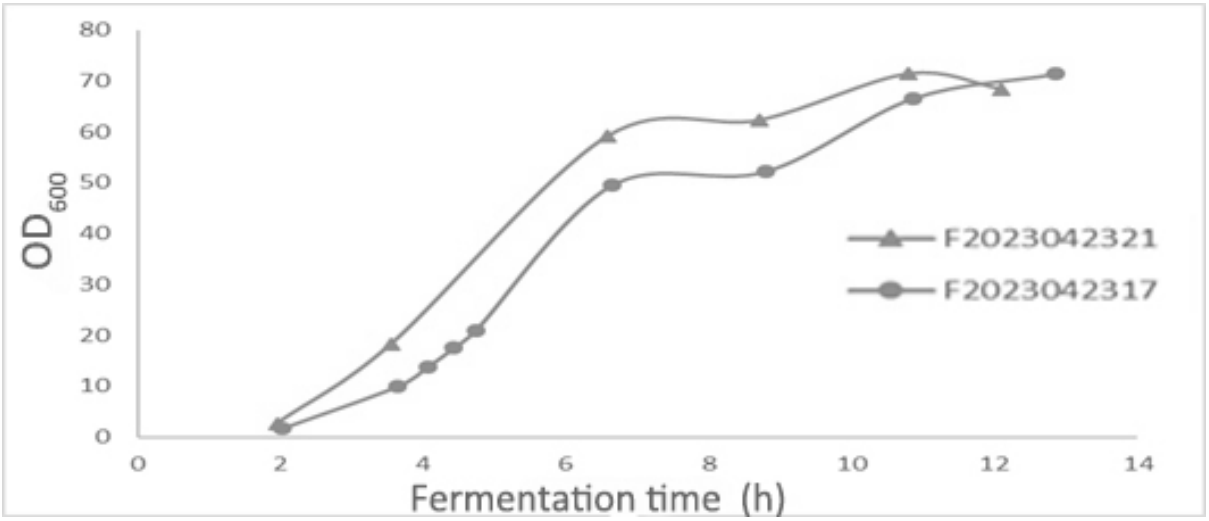


FIG. 16

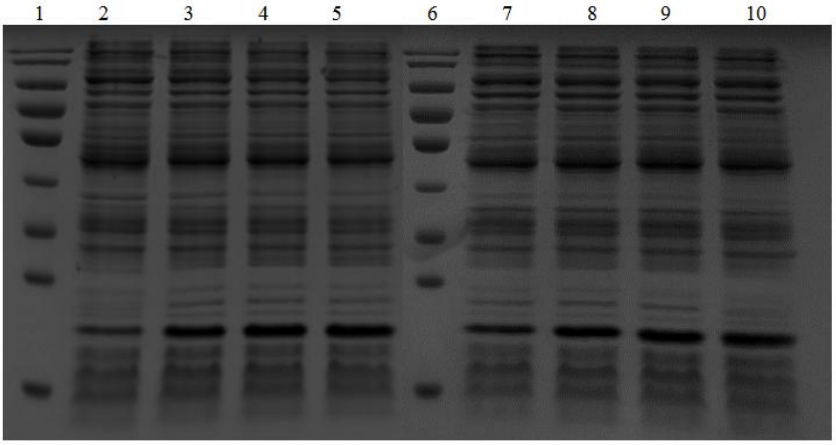


FIG. 17

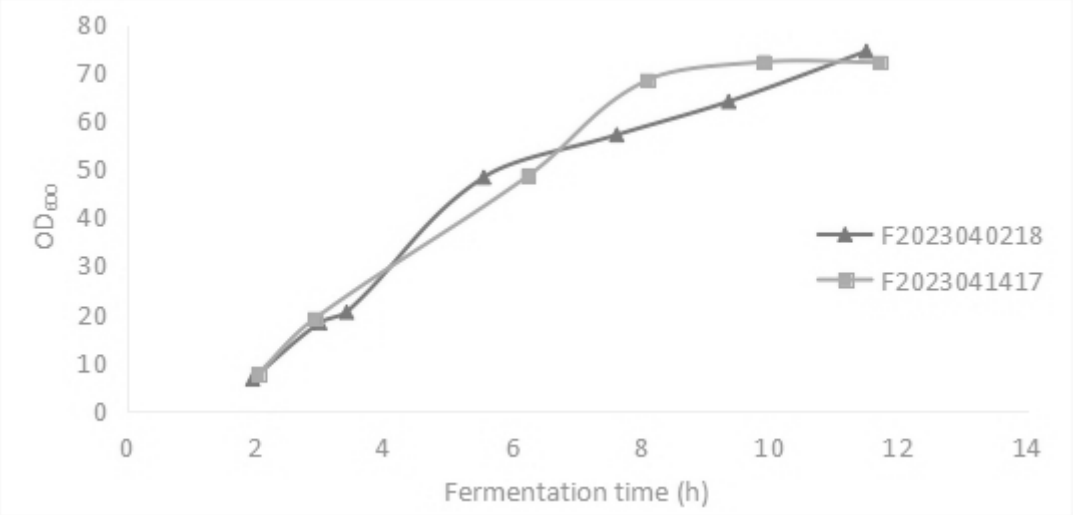


FIG. 18

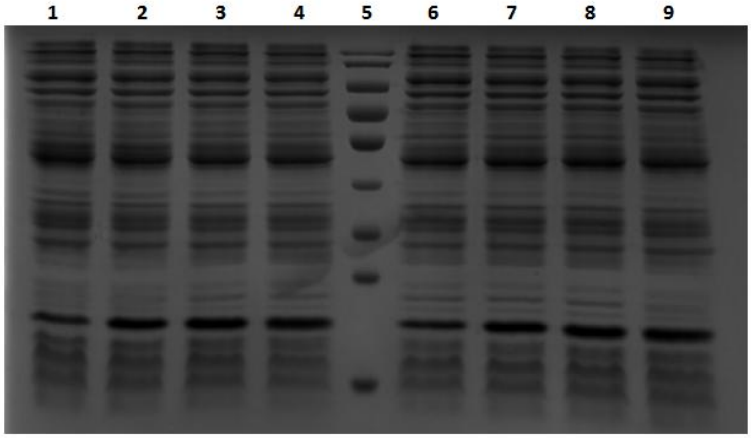


FIG. 19

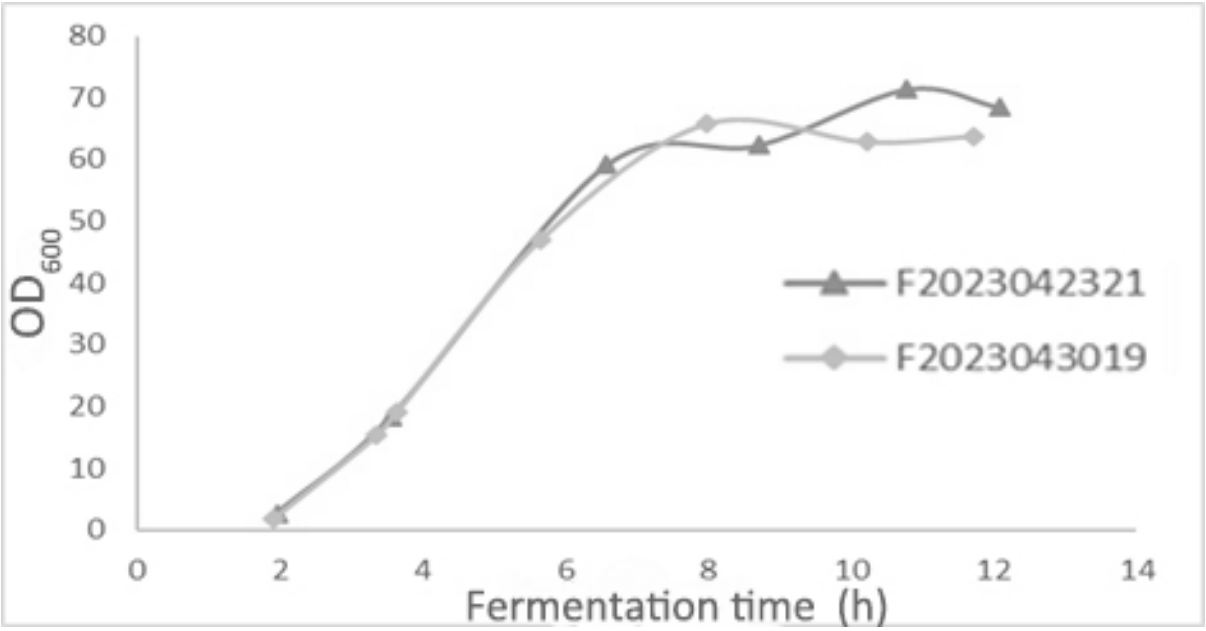


FIG. 20

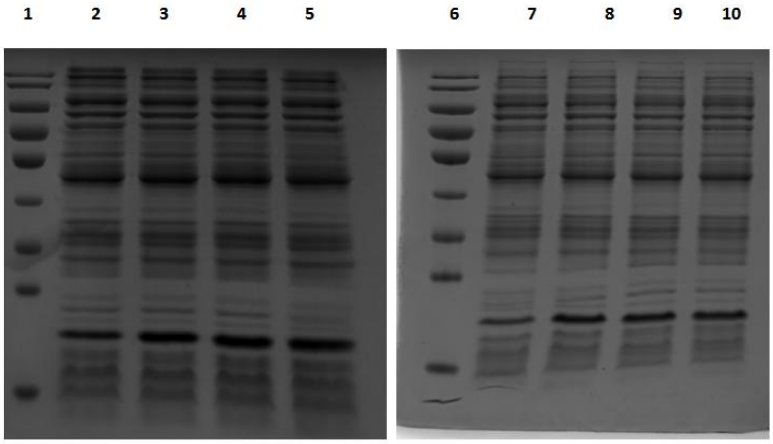


FIG. 21

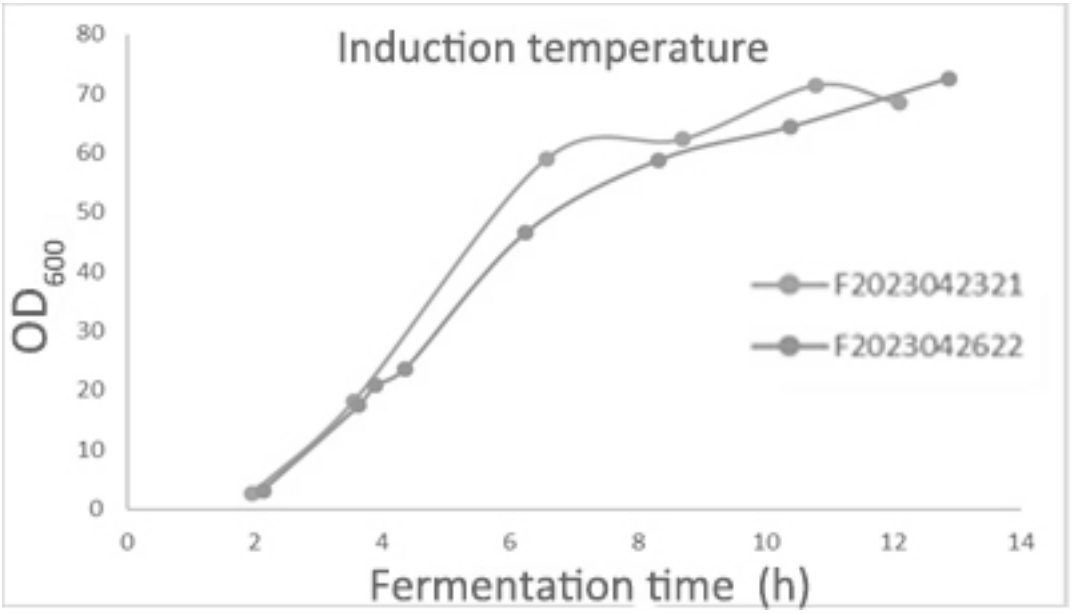


FIG. 22

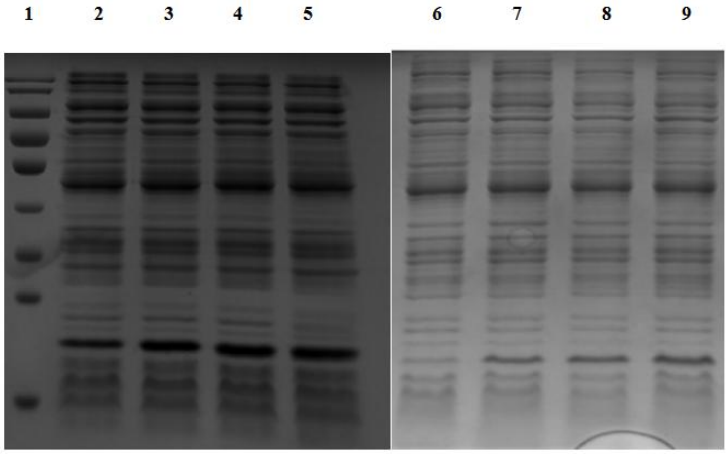


FIG. 23

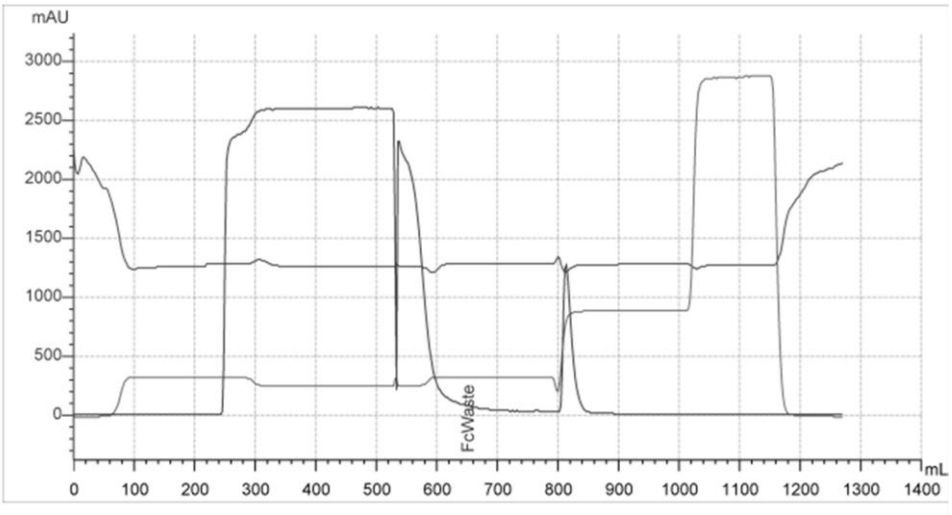

mAU
3000
2500
2000
1500
1000
500
0
FcWaste
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
mL

FIG. 24

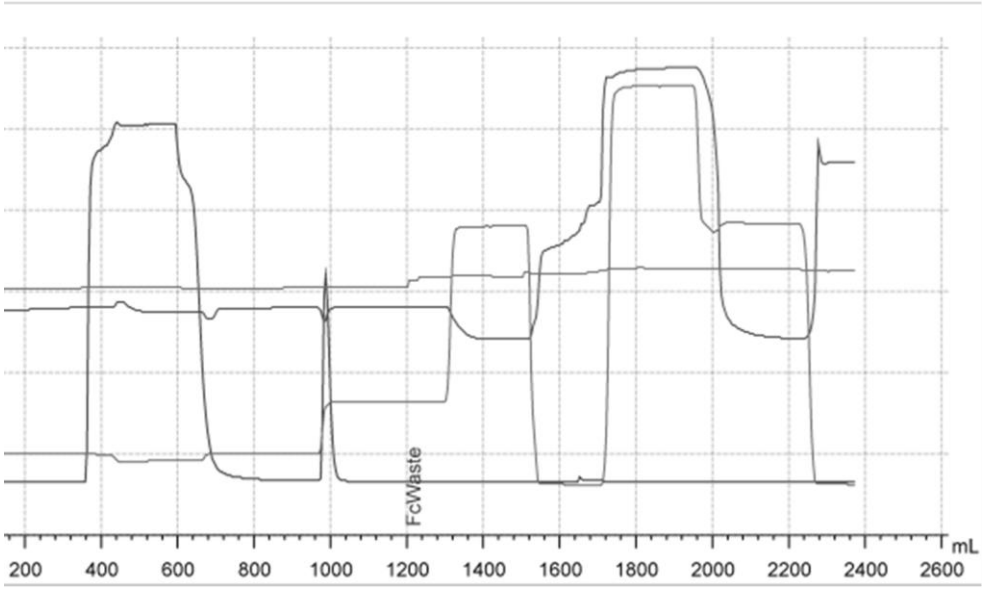

FcWaste
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
mL

FIG. 25

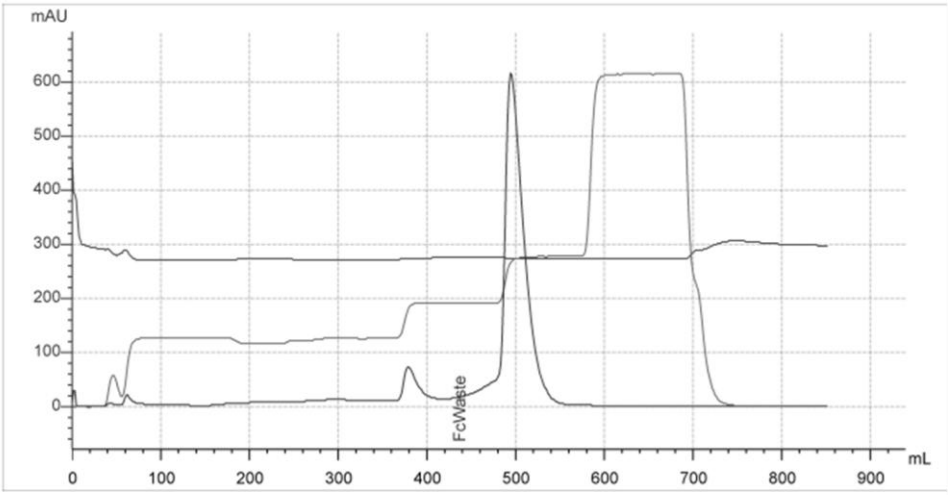


FIG. 26

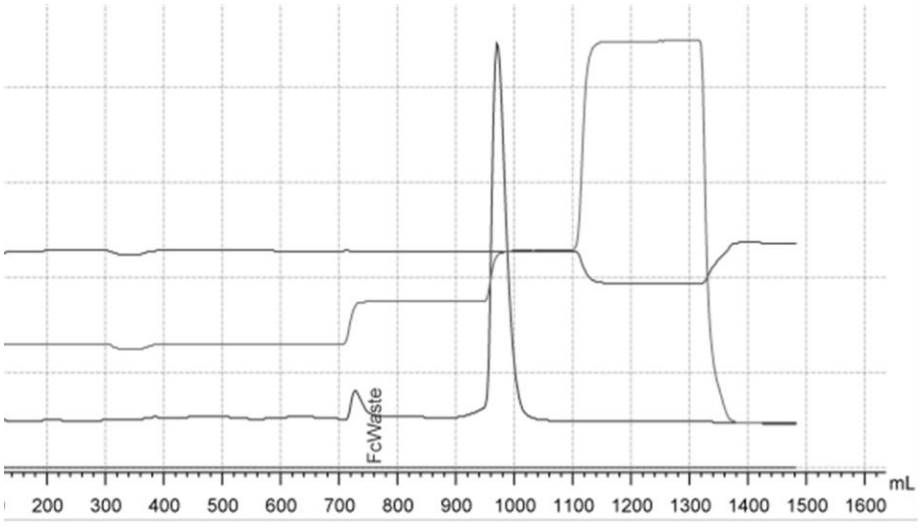


FIG. 27

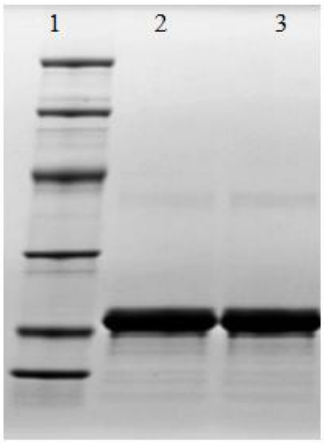


FIG. 28

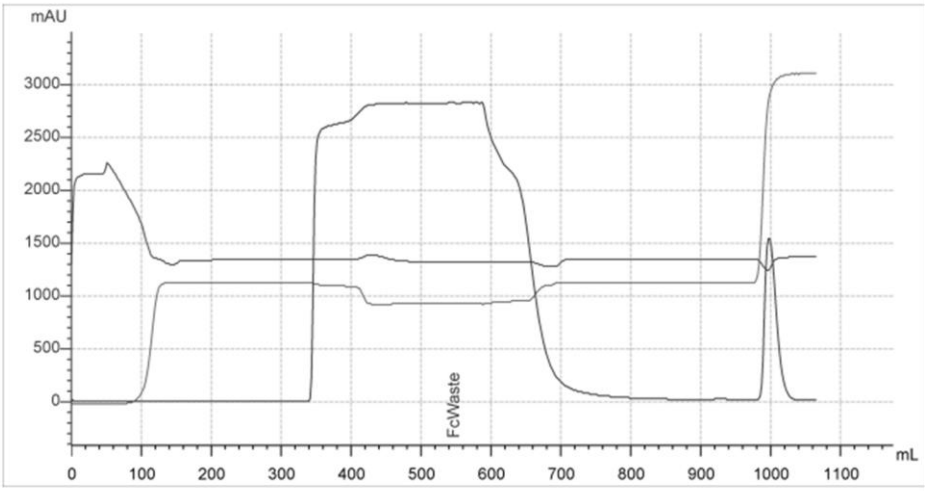

mAU
3000
2500
2000
1500
1000
500
0
FcWaste
0
100
200
300
400
500
600
700
800
900
1000
1100
mL

FIG. 29

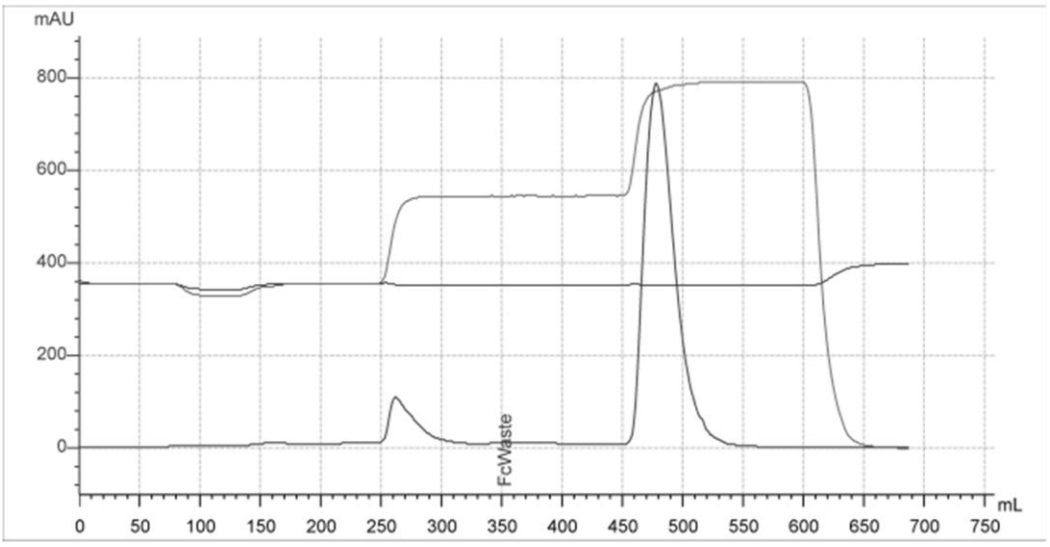

mAU
800
600
400
200
0
FcWaste
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
mL

FIG. 30

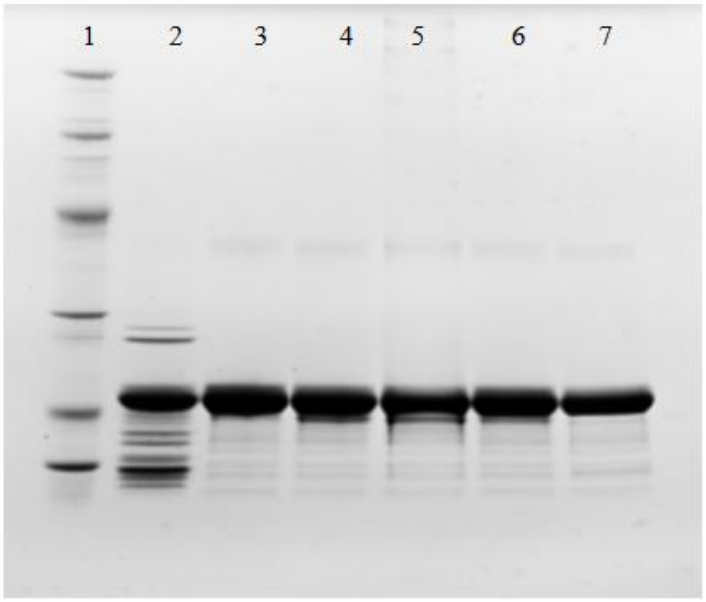

1
2
3
4
5
6
7

FIG. 31

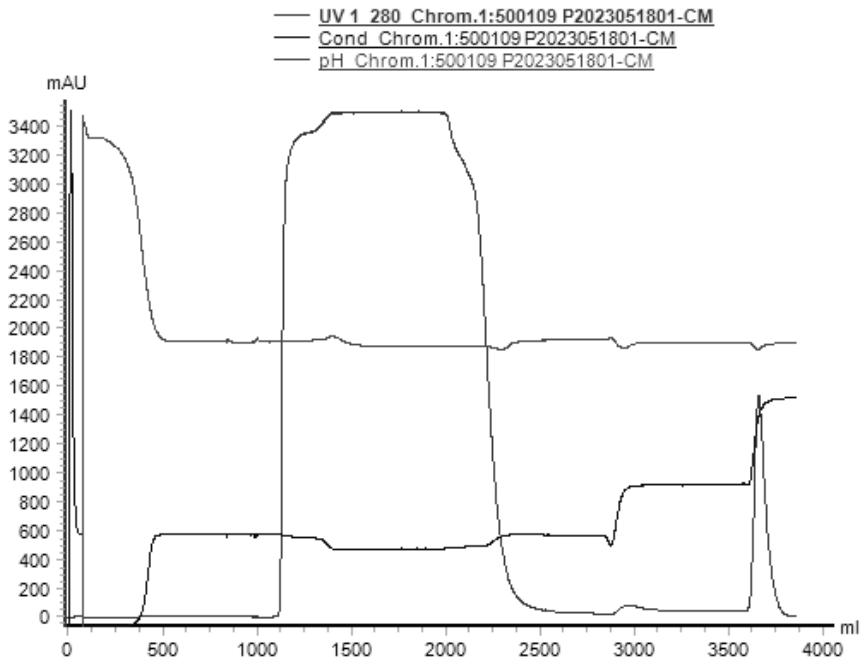

UV 1_280_Chrom.1:500109 P2023051801-CM
Cond_Chrom.1:500109 P2023051801-CM
pH_Chrom.1:500109 P2023051801-CM
mAU
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
500
1000
1500
2000
2500
3000
3500
4000
ml

FIG. 32

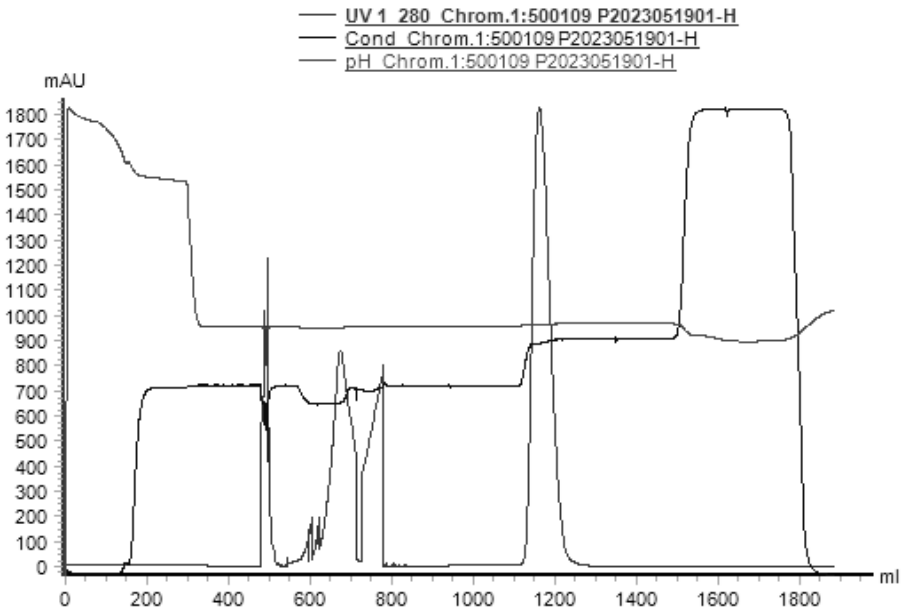

UV 1_280_Chrom.1:500109 P2023051901-H
Cond_Chrom.1:500109 P2023051901-H
pH_Chrom.1:500109 P2023051901-H
mAU
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
200
400
600
800
1000
1200
1400
1600
1800
ml

FIG. 33

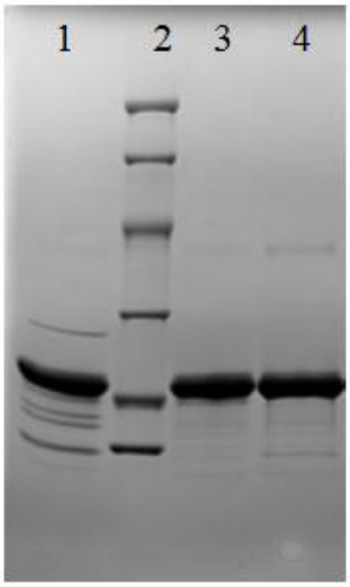

1
2
3
4

FIG. 34

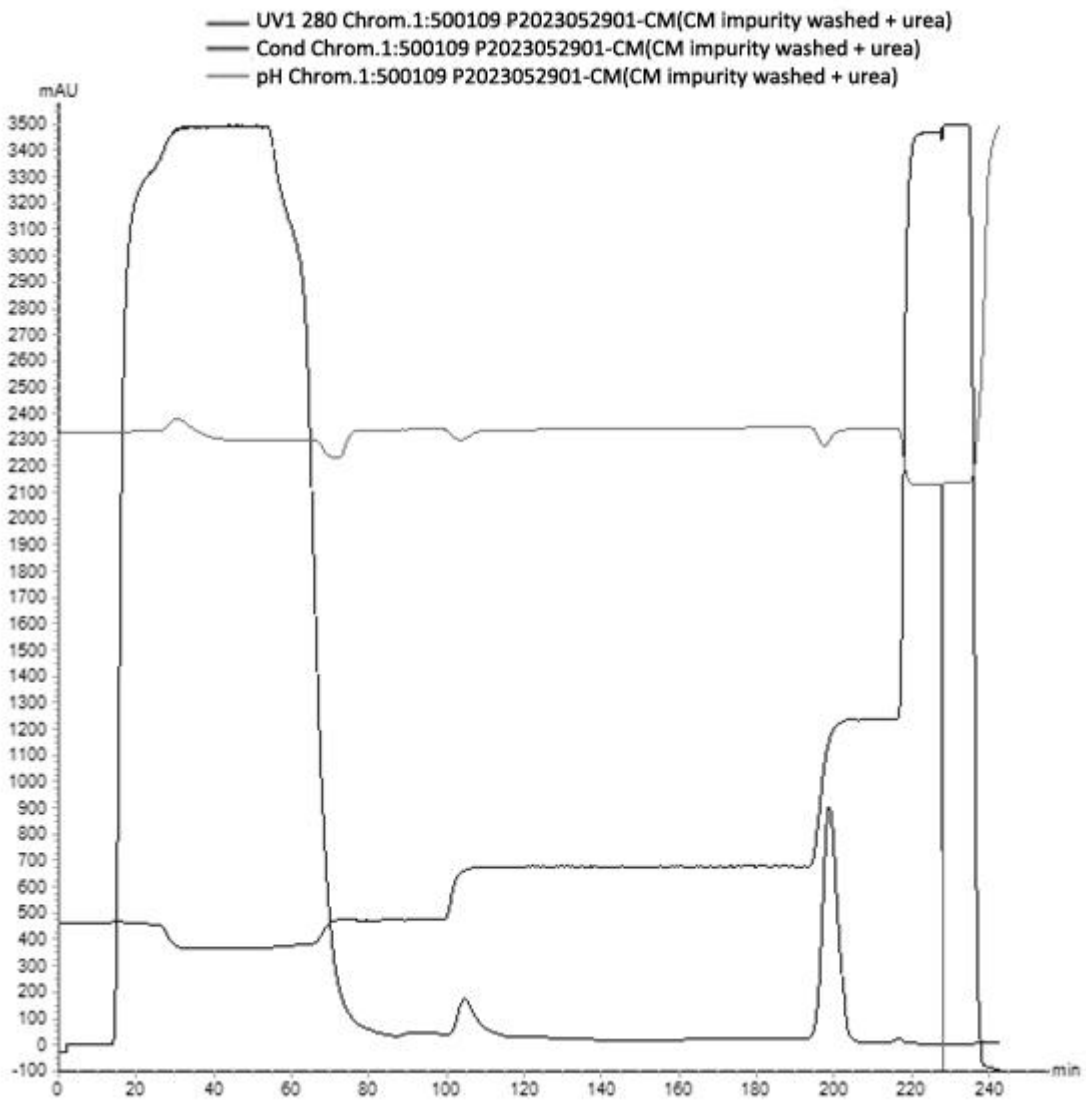

UV1 280 Chrom.1:500109 P2023052901-CM(CM impurity washed + urea)
Cond Chrom.1:500109 P2023052901-CM(CM impurity washed + urea)
pH Chrom.1:500109 P2023052901-CM(CM impurity washed + urea)
mAU
3500
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
0
20
40
60
80
100
120
140
160
180
200
220
240
min

FIG. 35

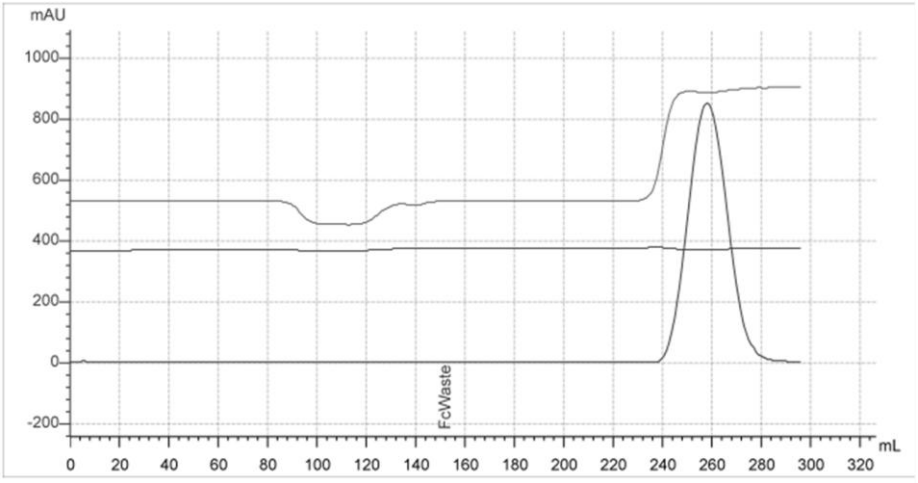

mAU
1000
800
600
400
200
0
-200
FcWaste
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
mL

FIG. 36

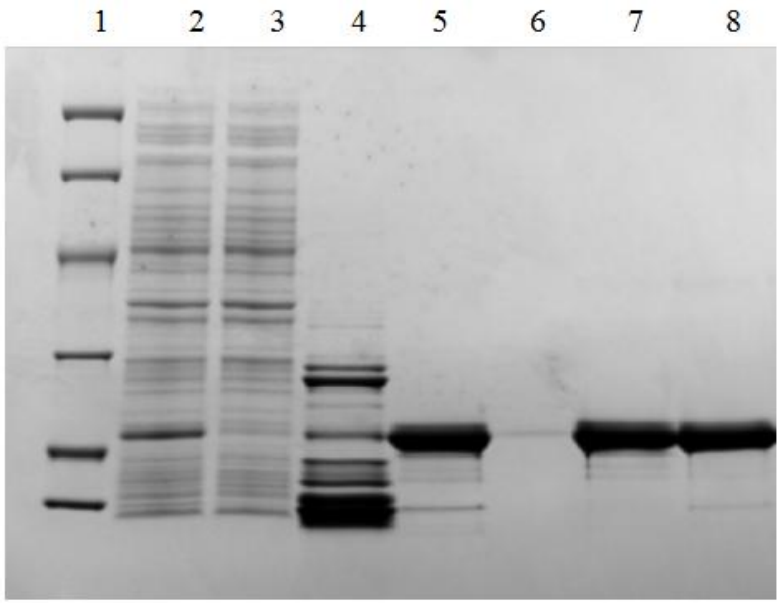

1
2
3
4
5
6
7
8

FIG. 37

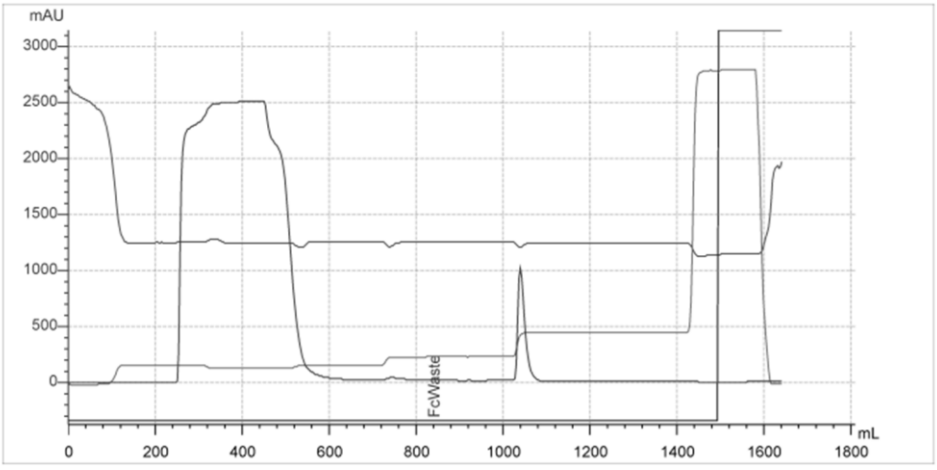

mAU
3000
2500
2000
1500
1000
500
0
FcWaste
0
200
400
600
800
1000
1200
1400
1600
1800
mL

FIG. 38

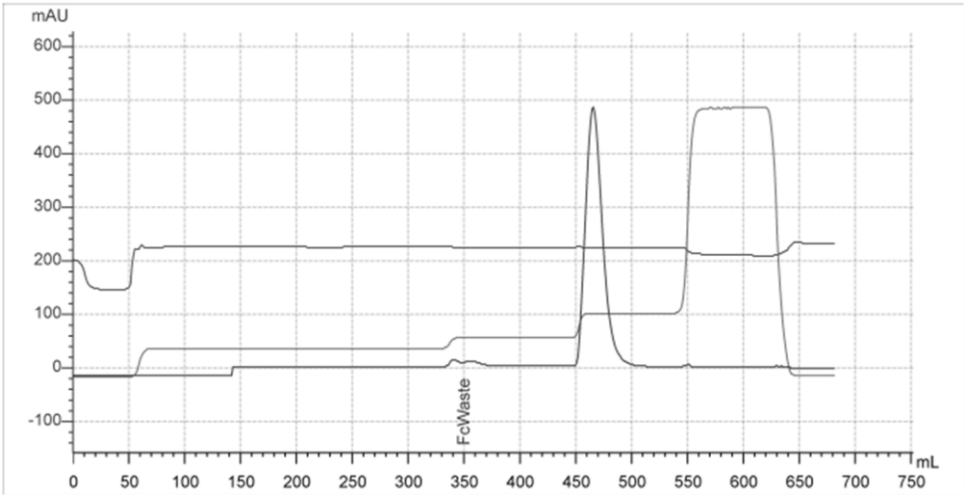

mAU
600
500
400
300
200
100
0
-100
FcWaste
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
mL

FIG. 39

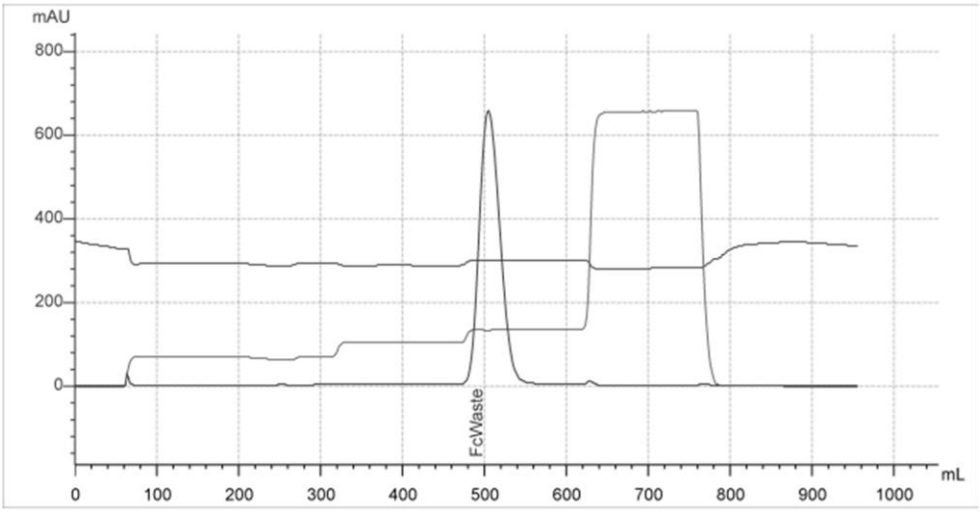

mAU
800
600
400
200
0
FcWaste
0
100
200
300
400
500
600
700
800
900
1000
mL

FIG. 40

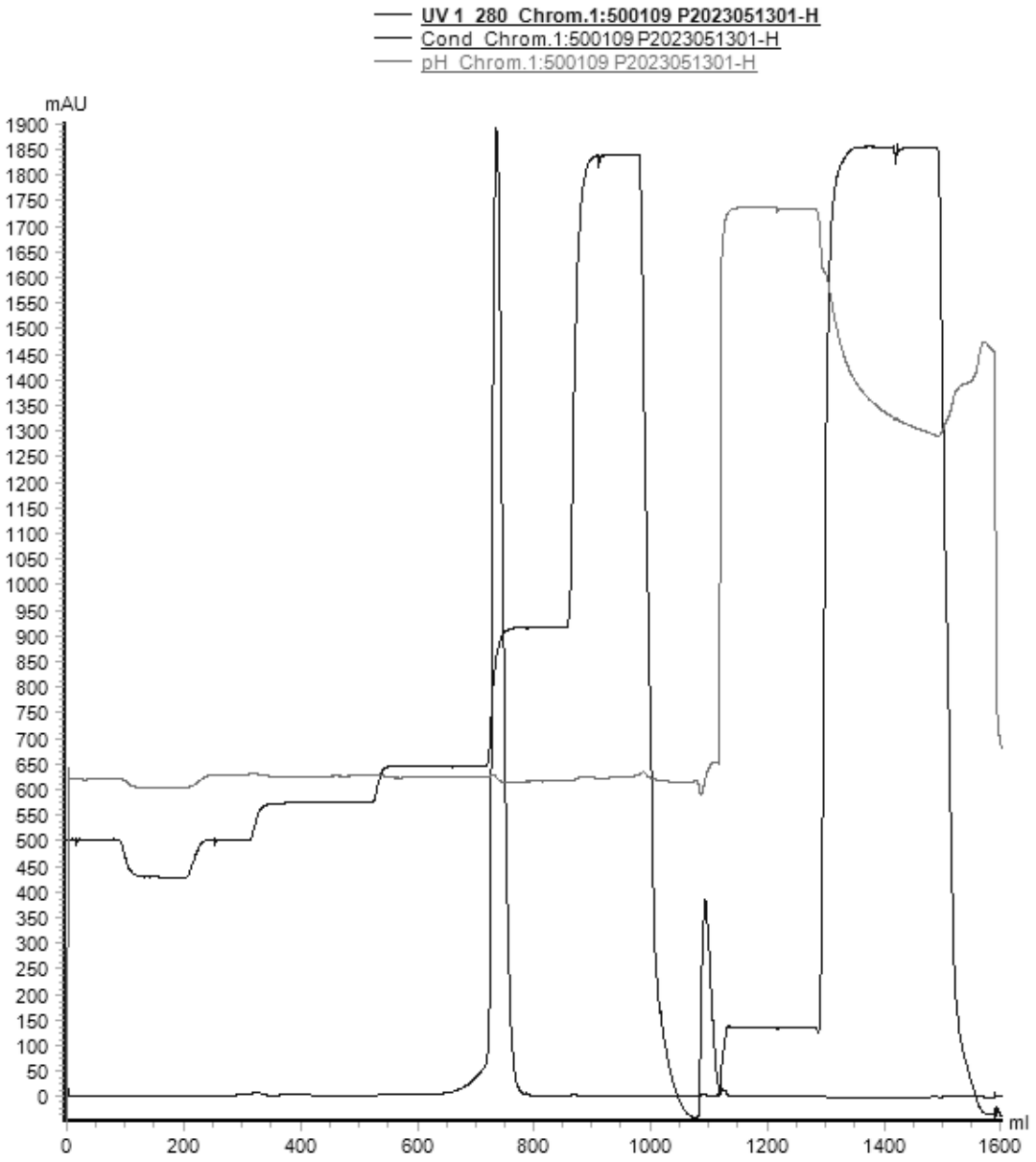

UV 1_280_Chrom.1:500109 P2023051301-H
Cond_Chrom.1:500109 P2023051301-H
pH_Chrom.1:500109 P2023051301-H
mAU
1900
1850
1800
1750
1700
1650
1600
1550
1500
1450
1400
1350
1300
1250
1200
1150
1100
1050
1000
950
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
200
400
600
800
1000
1200
1400
1600
ml

FIG. 41

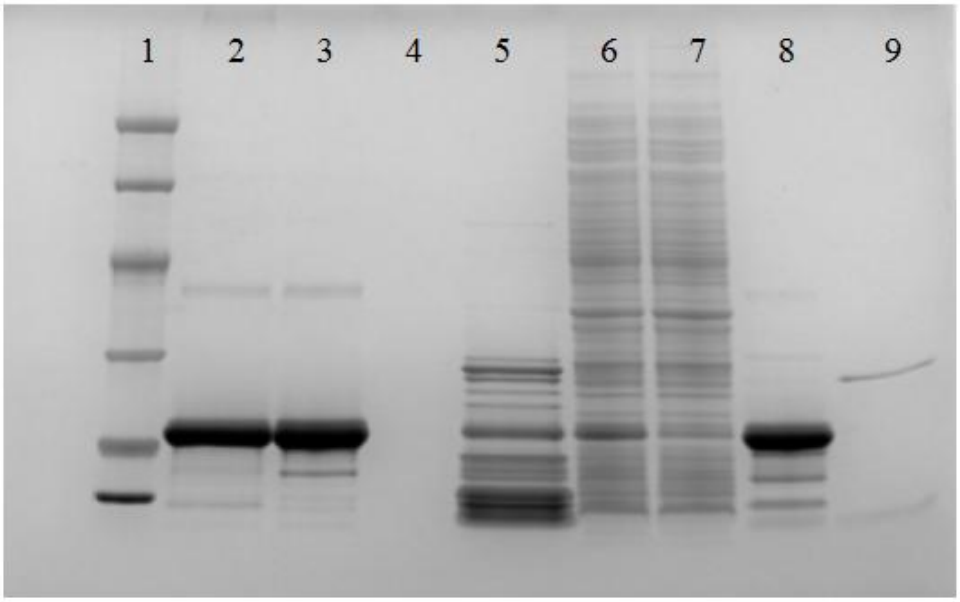


FIG. 42

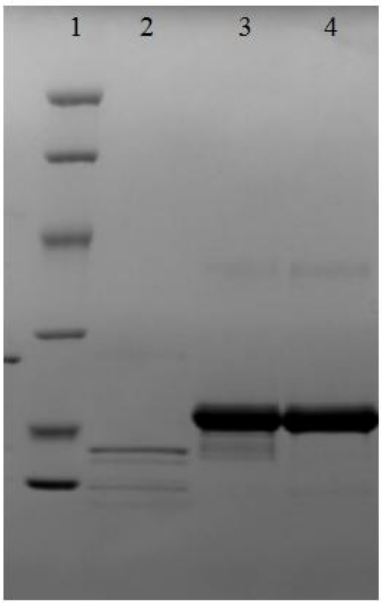


FIG. 43

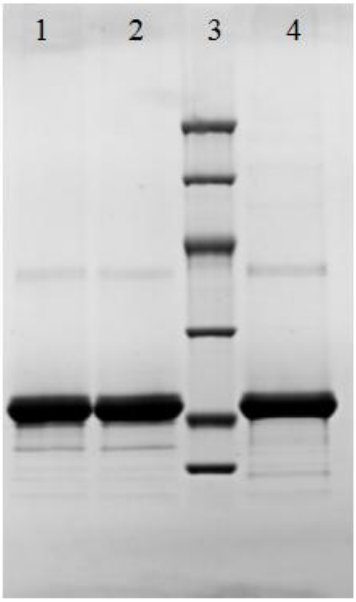

1
2
3
4

FIG. 44

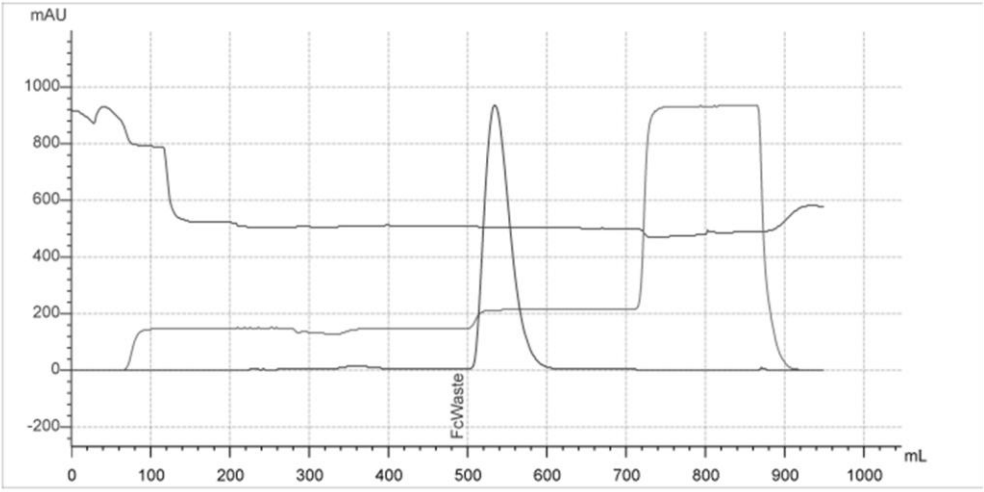

mAU
1000
800
600
400
200
0
-200
FcWaste
0
100
200
300
400
500
600
700
800
900
1000
mL

FIG. 45

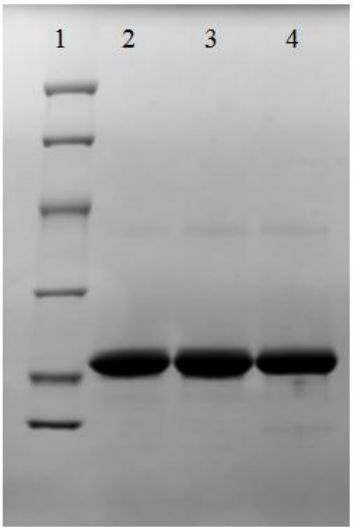


FIG. 46

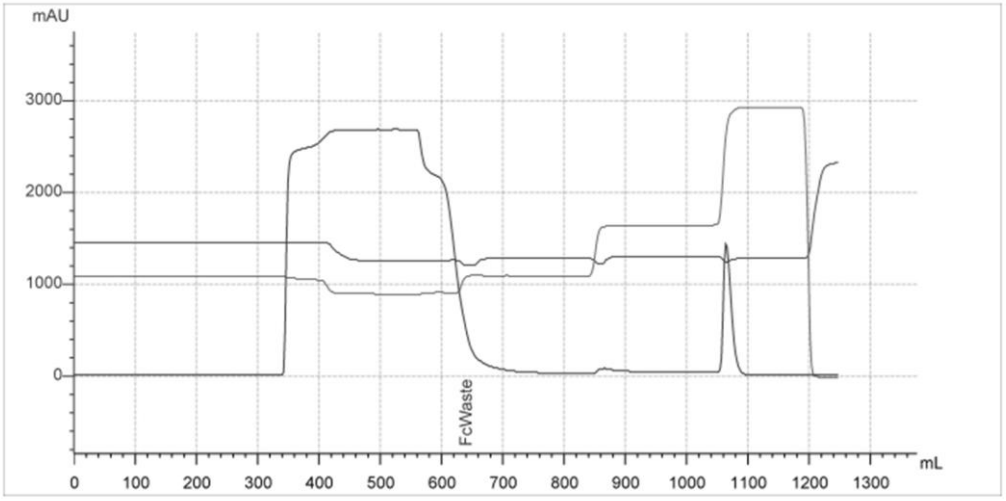


FIG. 47

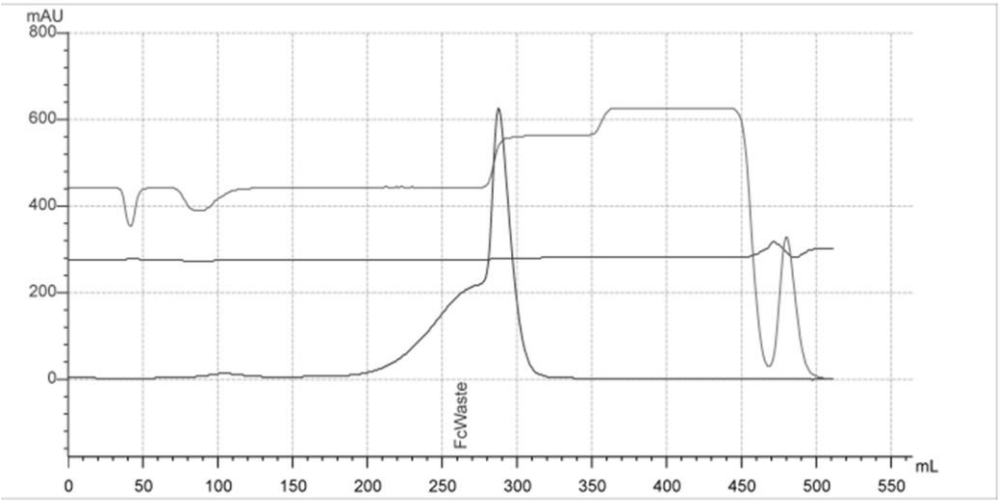


FIG. 48

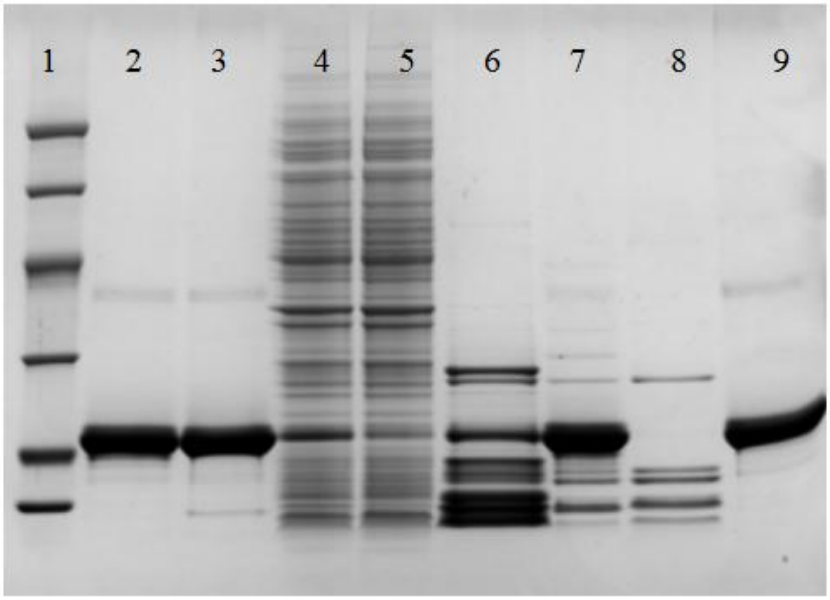

1
2
3
4
5
6
7
8
9

FIG. 49

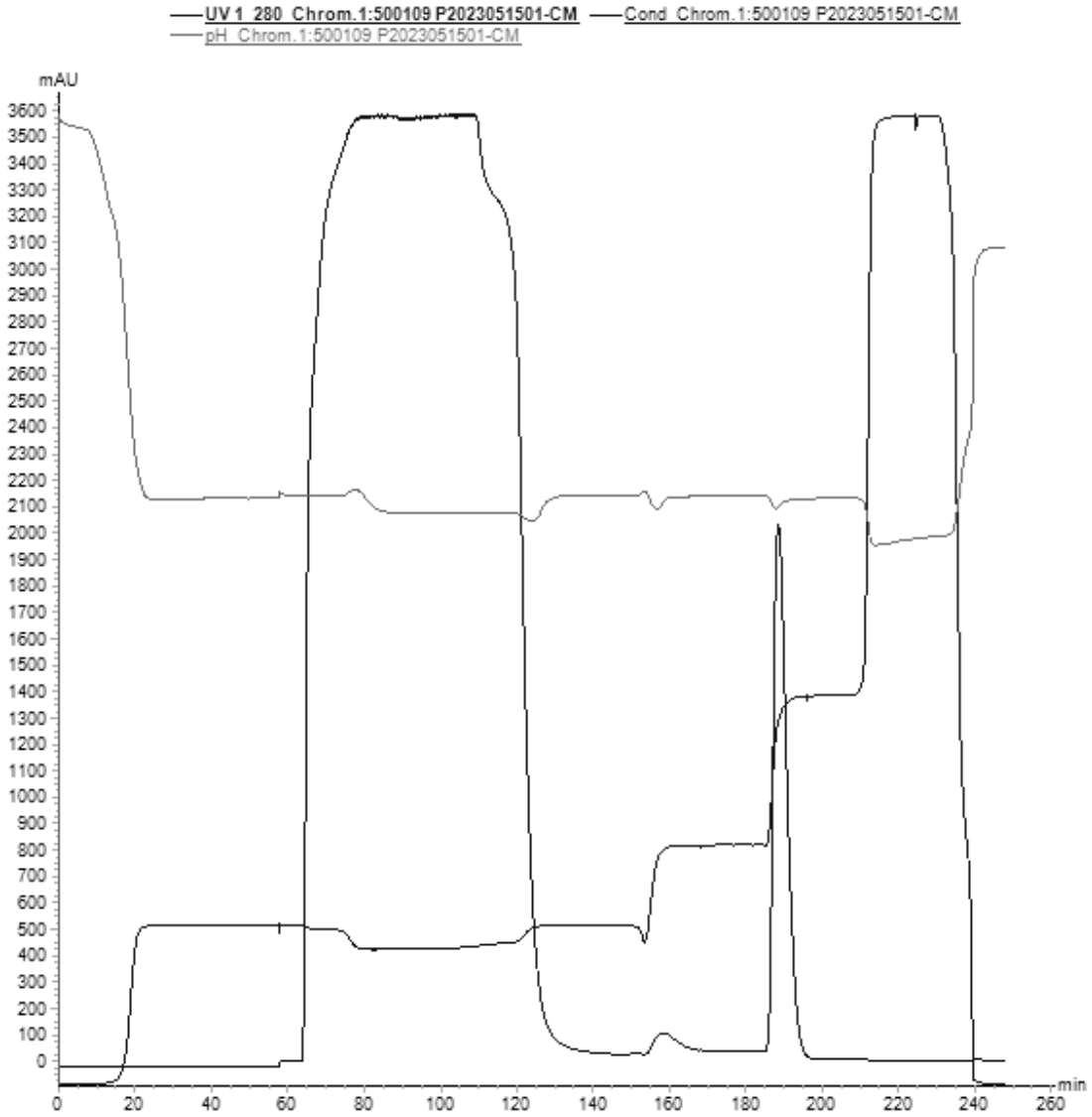

UV 1_280_Chrom.1:500109 P2023051501-CM
Cond_Chrom.1:500109 P2023051501-CM
pH_Chrom.1:500109 P2023051501-CM
mAU
3600
3500
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100
120
140
160
180
200
220
240
260
min

FIG. 50

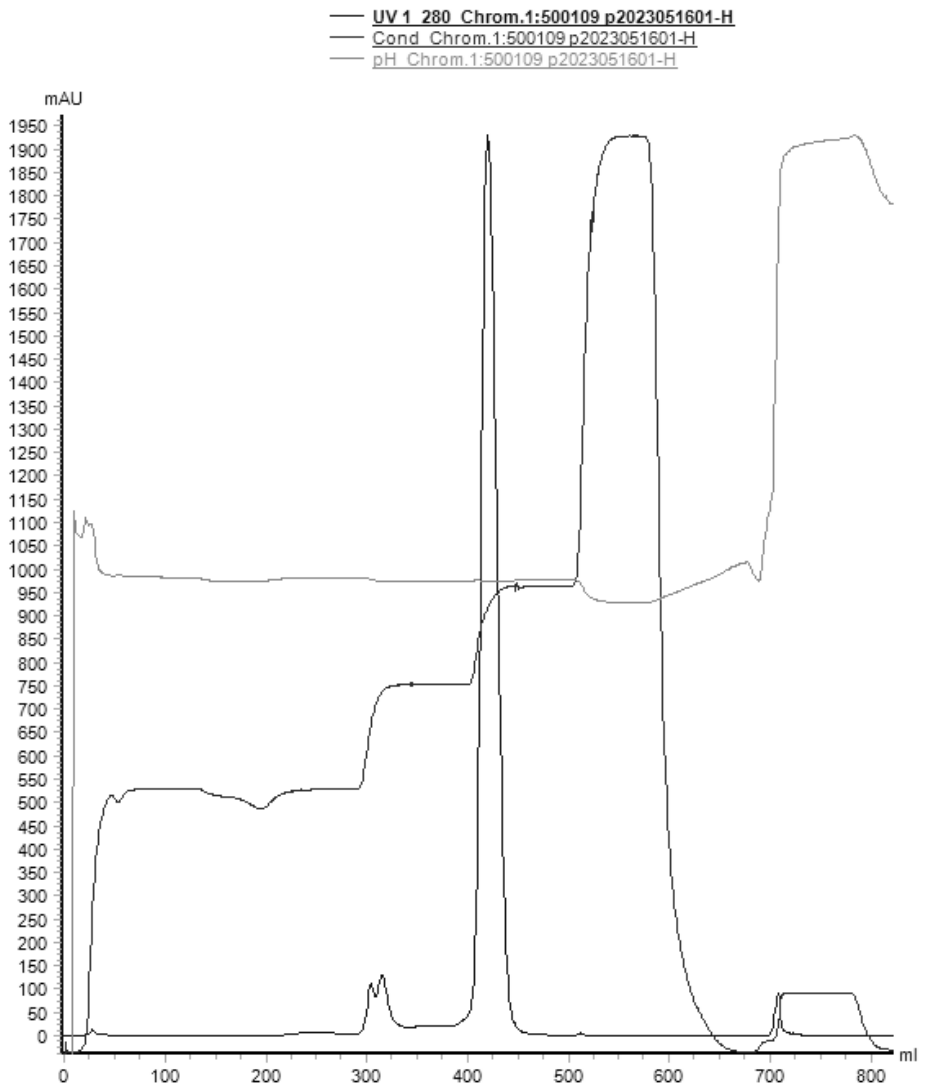

UV 1_280_Chrom.1:500109 p2023051601-H
Cond_Chrom.1:500109 p2023051601-H
pH_Chrom.1:500109 p2023051601-H
mAU
1950
1900
1850
1800
1750
1700
1650
1600
1550
1500
1450
1400
1350
1300
1250
1200
1150
1100
1050
1000
950
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
ml

FIG. 51

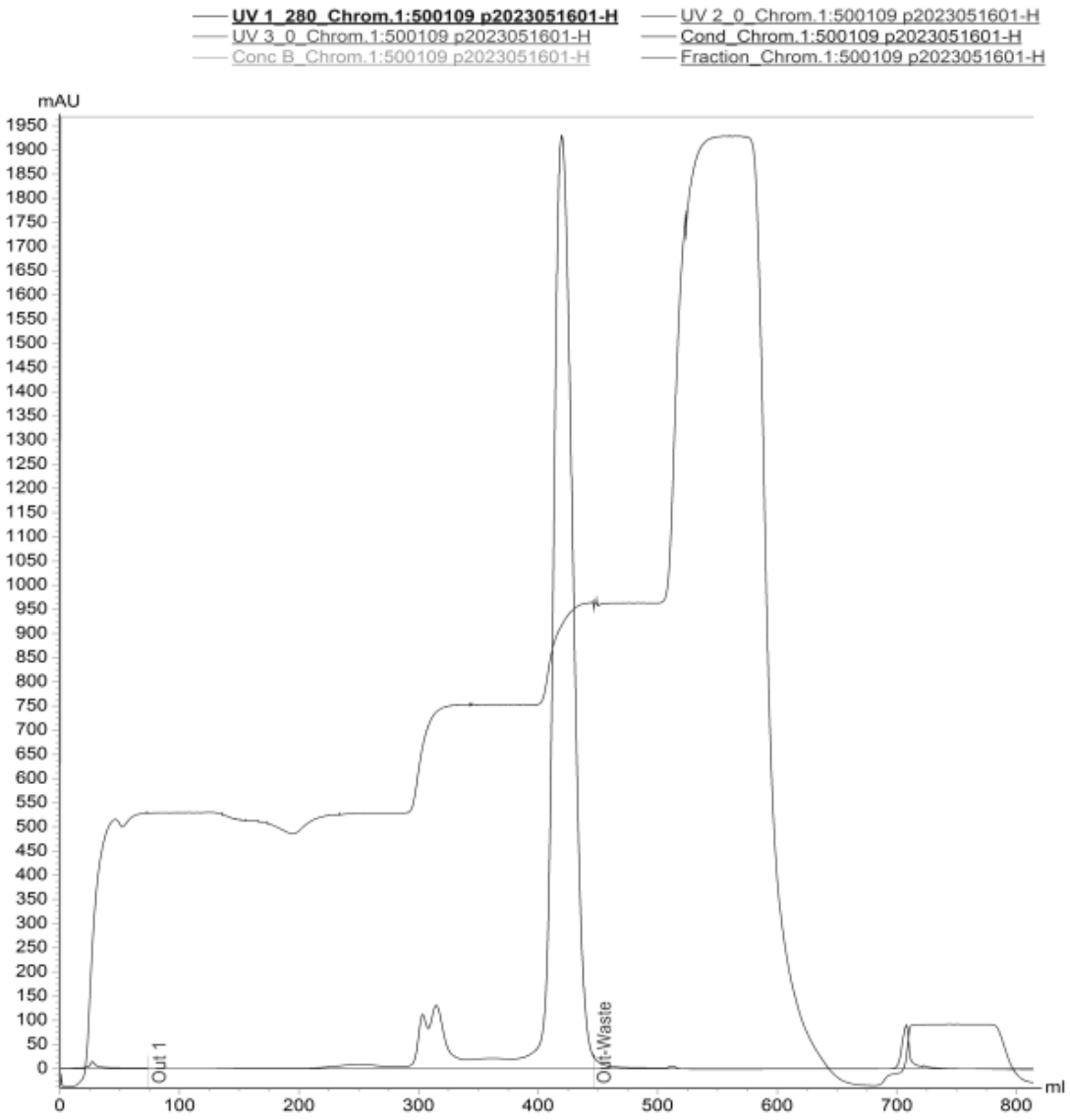

UV 1_280_Chrom.1:500109 p2023051601-H
UV 2_0_Chrom.1:500109 p2023051601-H
UV 3_0_Chrom.1:500109 p2023051601-H
Cond_Chrom.1:500109 p2023051601-H
Conc B_Chrom.1:500109 p2023051601-H
Fraction_Chrom.1:500109 p2023051601-H
mAU
1950
1900
1850
1800
1750
1700
1650
1600
1550
1500
1450
1400
1350
1300
1250
1200
1150
1100
1050
1000
950
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
Out 1
Out-Waste
ml
0
100
200
300
400
500
600
700
800

FIG. 52

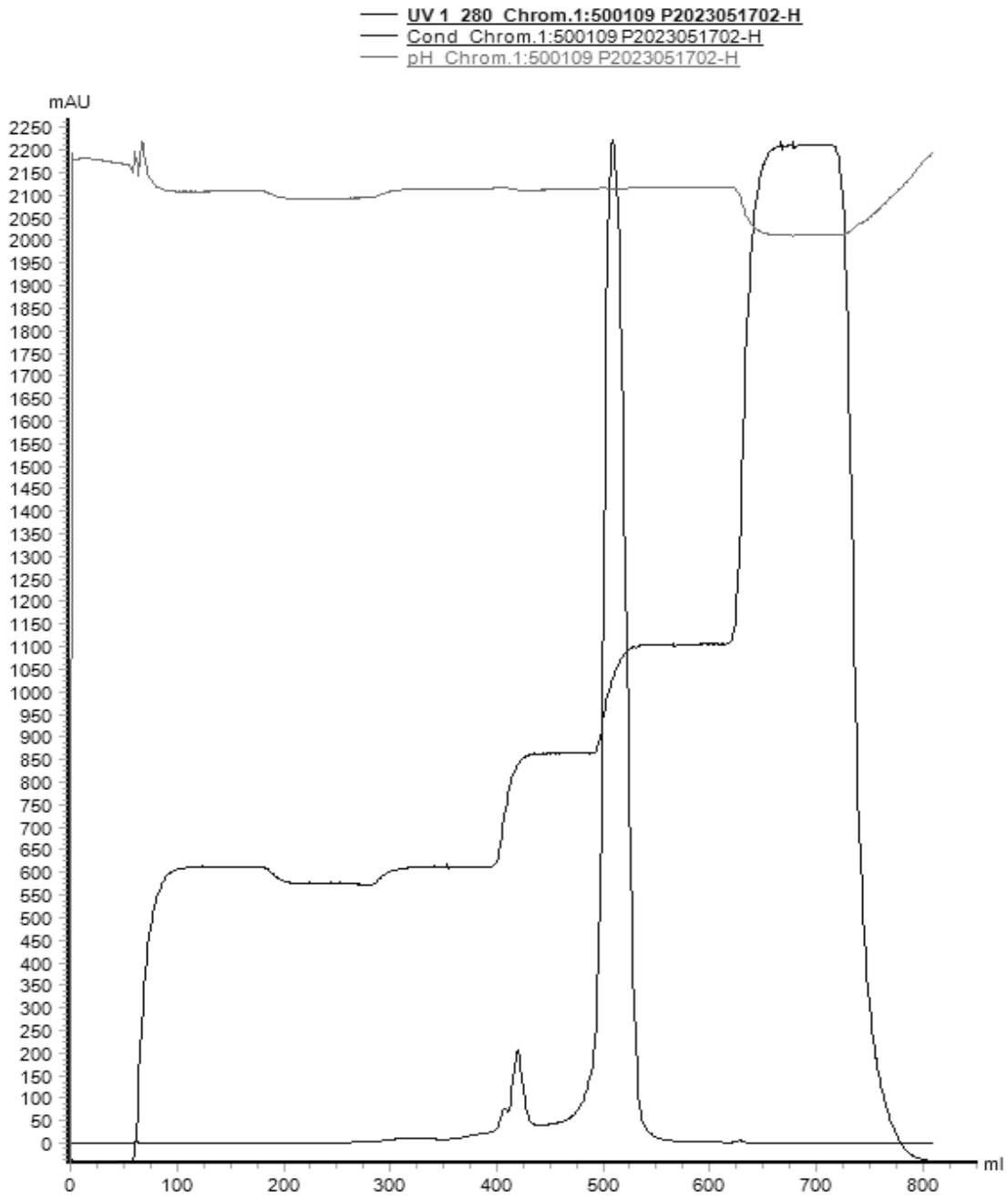

UV 1_280_Chrom.1:500109 P2023051702-H
Cond_Chrom.1:500109 P2023051702-H
pH_Chrom.1:500109 P2023051702-H
mAU
2250
2200
2150
2100
2050
2000
1950
1900
1850
1800
1750
1700
1650
1600
1550
1500
1450
1400
1350
1300
1250
1200
1150
1100
1050
1000
950
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
ml

FIG. 53

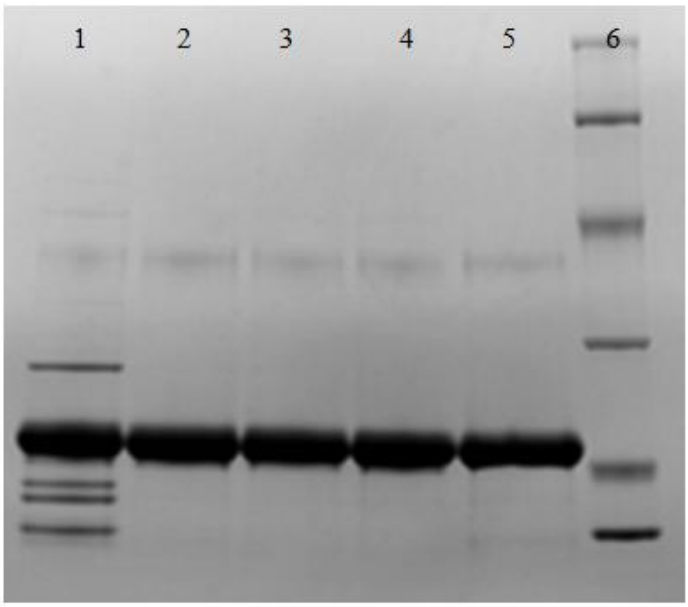


FIG. 54

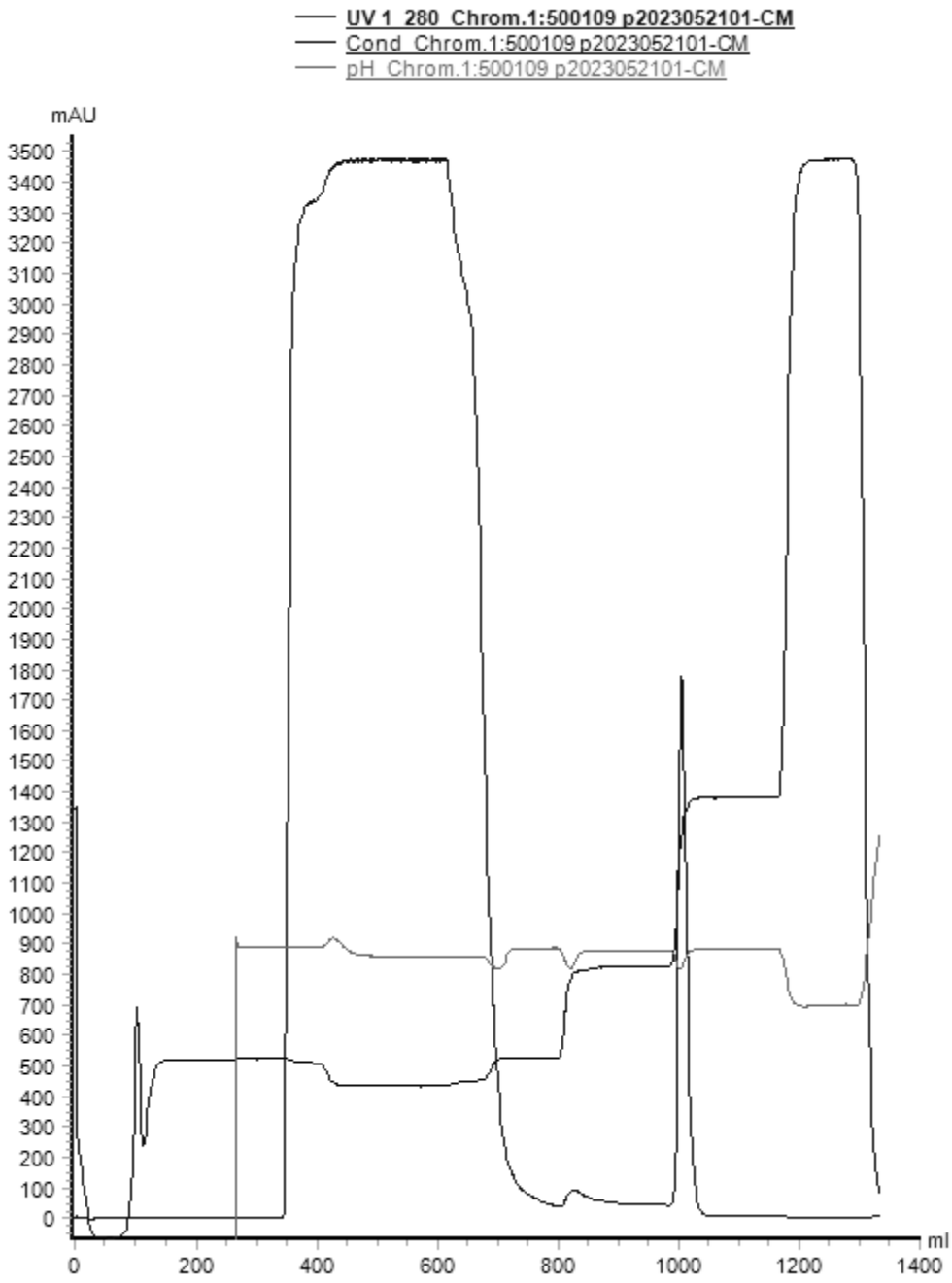

UV 1_280_Chrom.1:500109 p2023052101-CM
Cond_Chrom.1:500109 p2023052101-CM
pH_Chrom.1:500109 p2023052101-CM
mAU
3500
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
ml
0
200
400
600
800
1000
1200
1400

FIG. 55

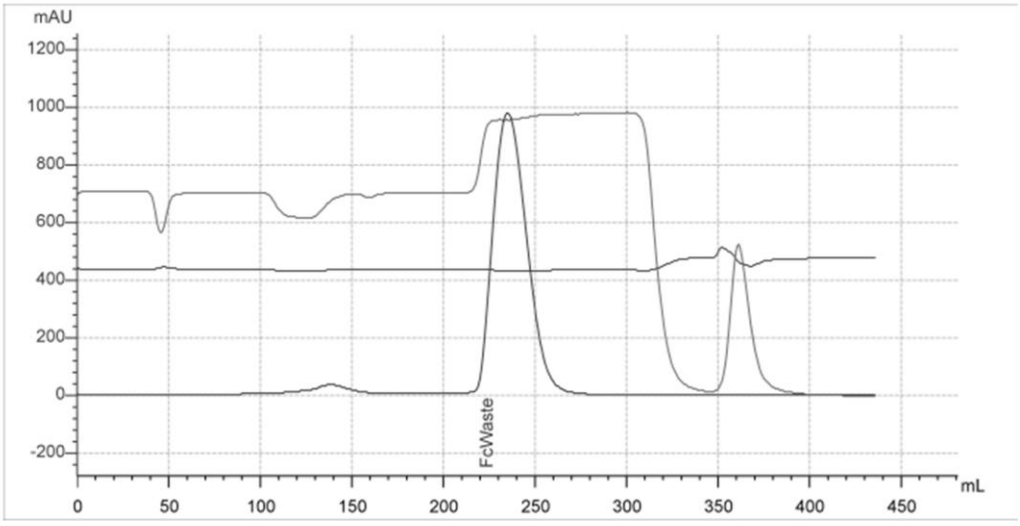

mAU
1200
1000
800
600
400
200
0
-200
FcWaste
0
50
100
150
200
250
300
350
400
450
mL

FIG. 56

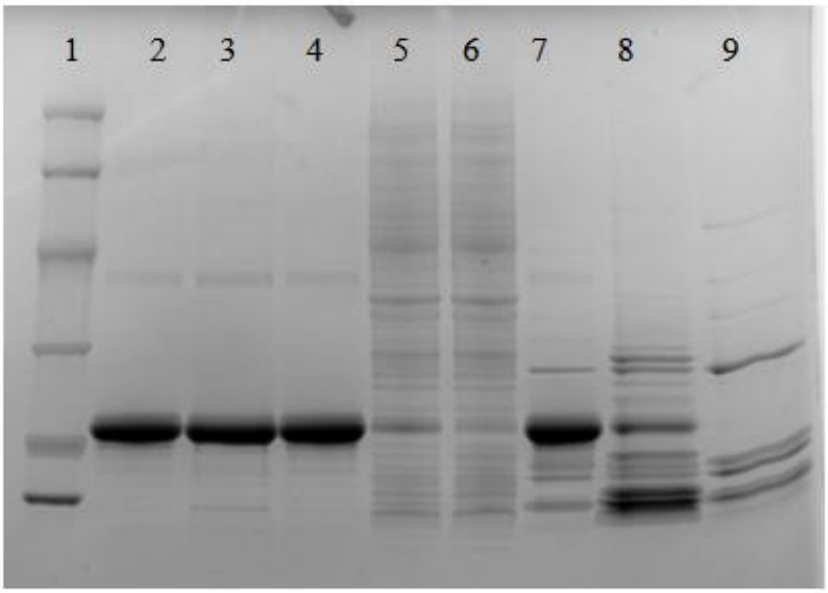

1
2
3
4
5
6
7
8
9

FIG. 57

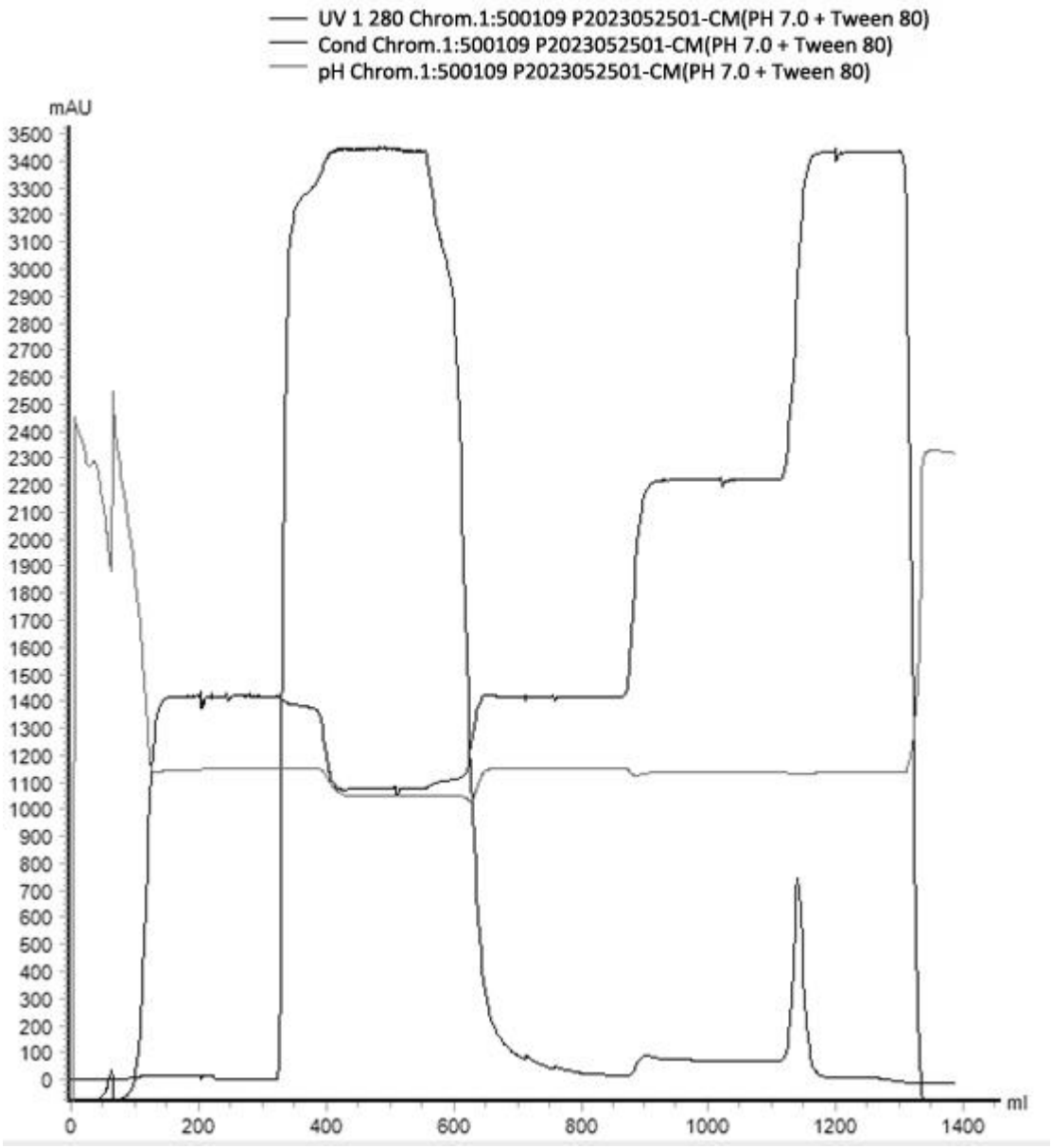

UV 1 280 Chrom.1:500109 P2023052501-CM(PH 7.0 + Tween 80)
Cond Chrom.1:500109 P2023052501-CM(PH 7.0 + Tween 80)
pH Chrom.1:500109 P2023052501-CM(PH 7.0 + Tween 80)
mAU
3500
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
200
400
600
800
1000
1200
1400
ml

FIG. 58

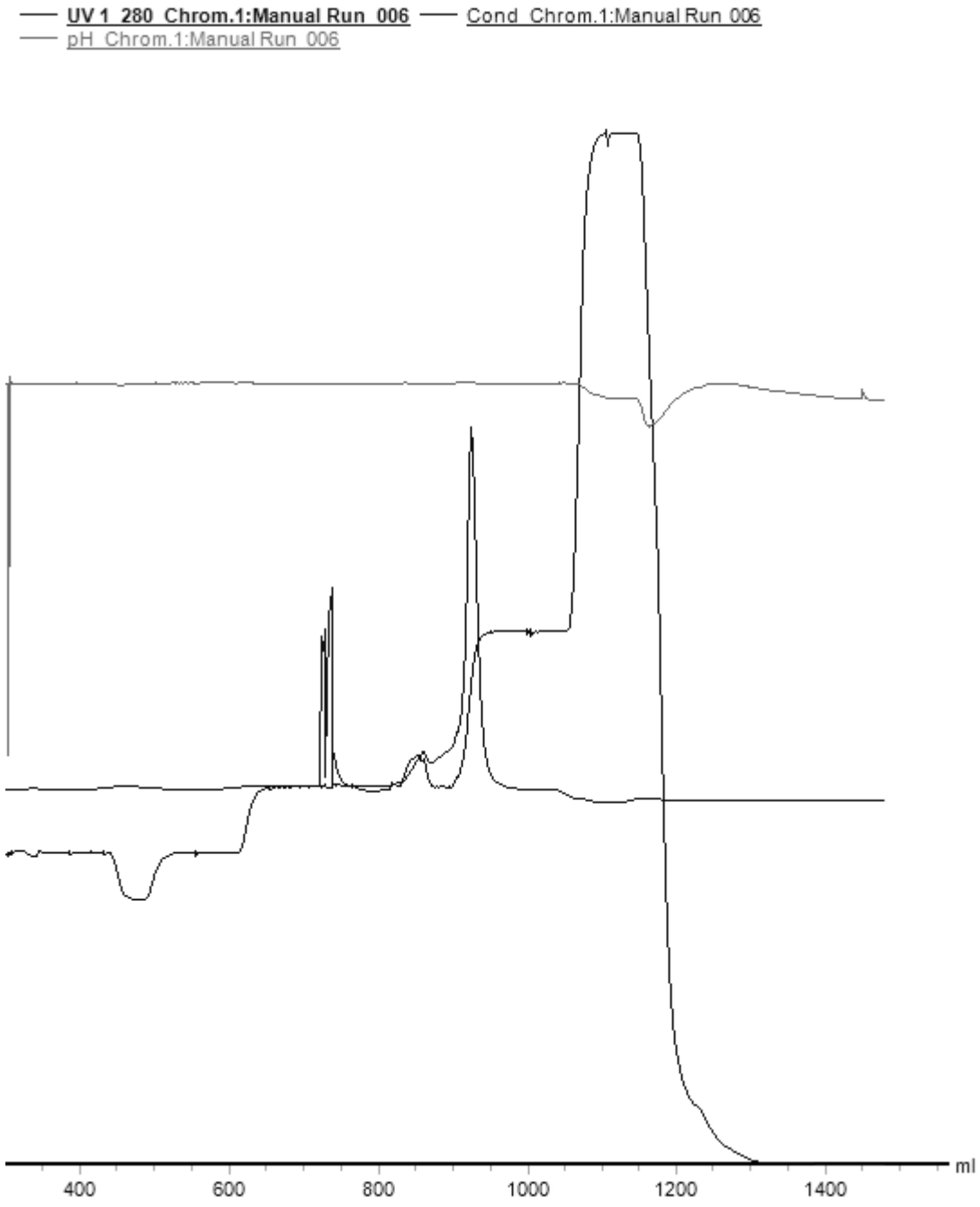

UV 1_280_Chrom.1:Manual Run_006
Cond_Chrom.1:Manual Run_006
pH_Chrom.1:Manual Run_006
ml
400
600
800
1000
1200
1400

FIG. 59

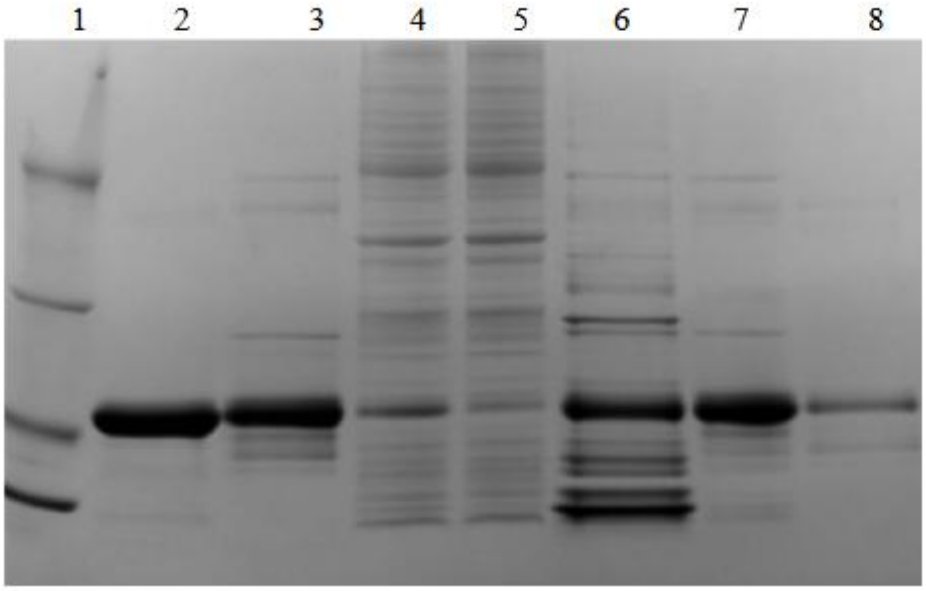


FIG. 60

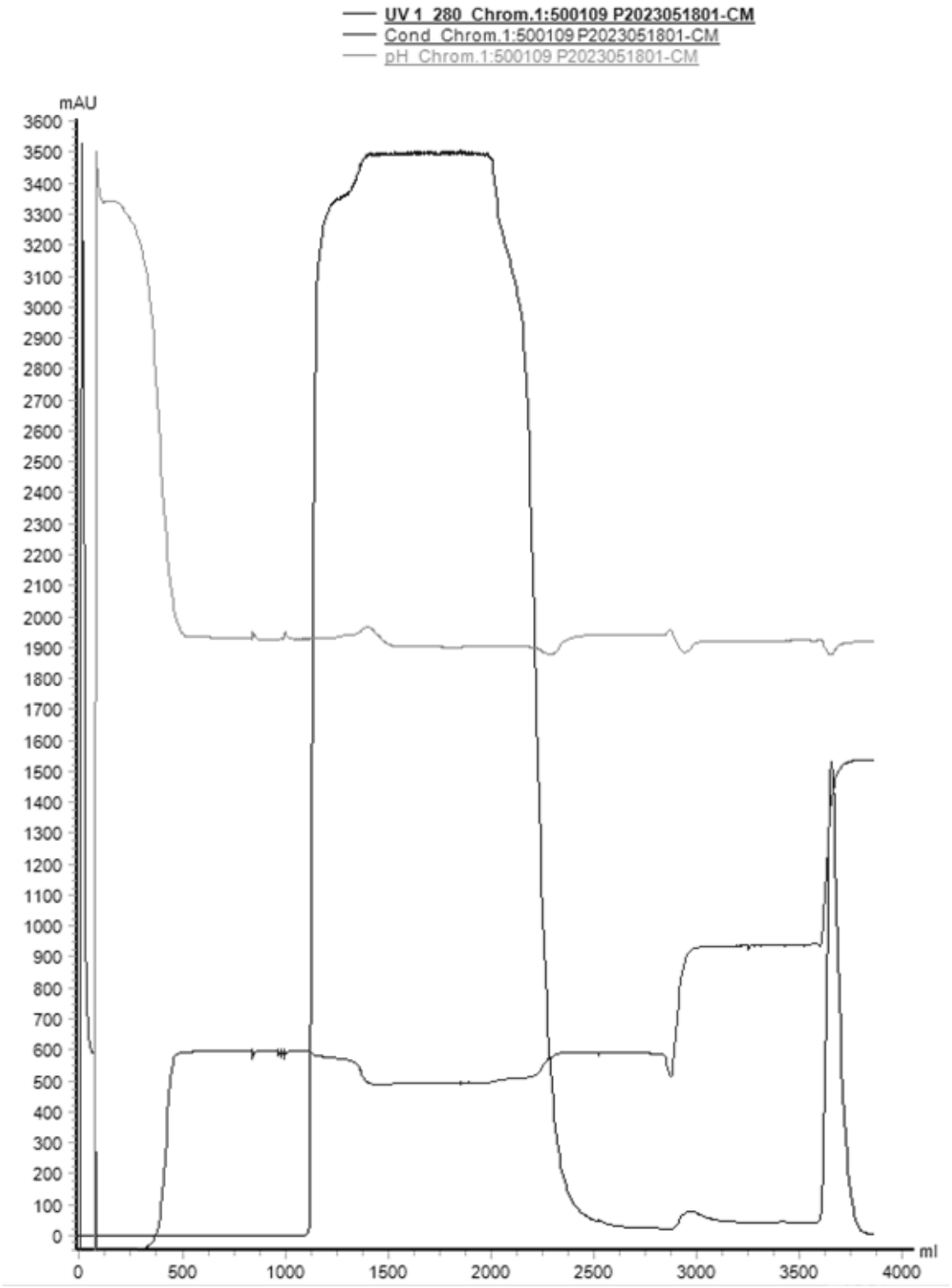

UV 1_280_Chrom.1:500109 P2023051801-CM
Cond_Chrom.1:500109 P2023051801-CM
pH_Chrom.1:500109 P2023051801-CM
mAU
3600
3500
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
500
1000
1500
2000
2500
3000
3500
4000
ml

FIG. 61

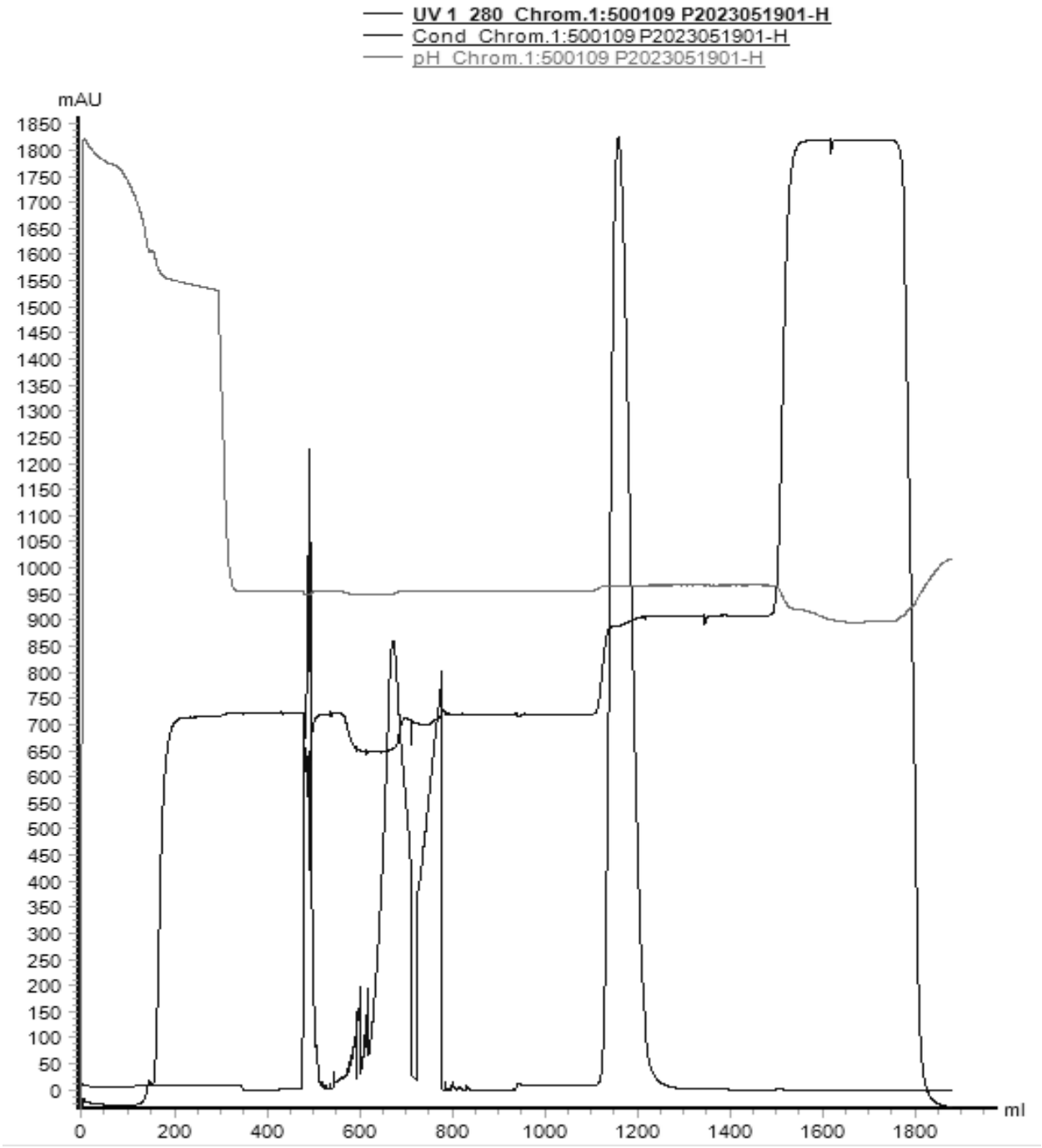

UV 1_280_Chrom.1:500109 P2023051901-H
Cond_Chrom.1:500109 P2023051901-H
pH_Chrom.1:500109 P2023051901-H
mAU
1850
1800
1750
1700
1650
1600
1550
1500
1450
1400
1350
1300
1250
1200
1150
1100
1050
1000
950
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
200
400
600
800
1000
1200
1400
1600
1800
ml

FIG. 62

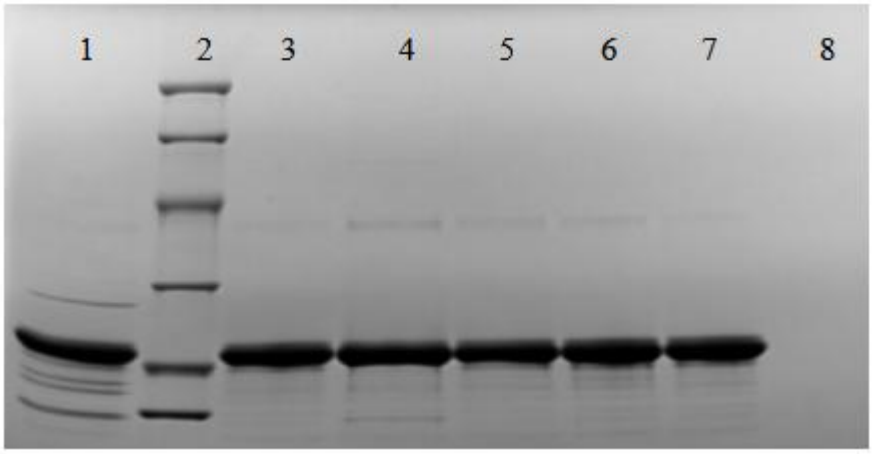


FIG. 63

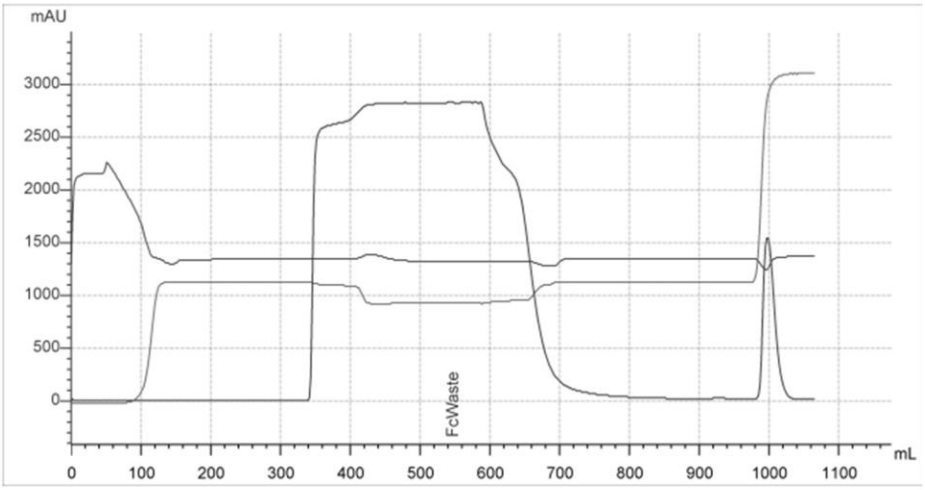


FIG. 64

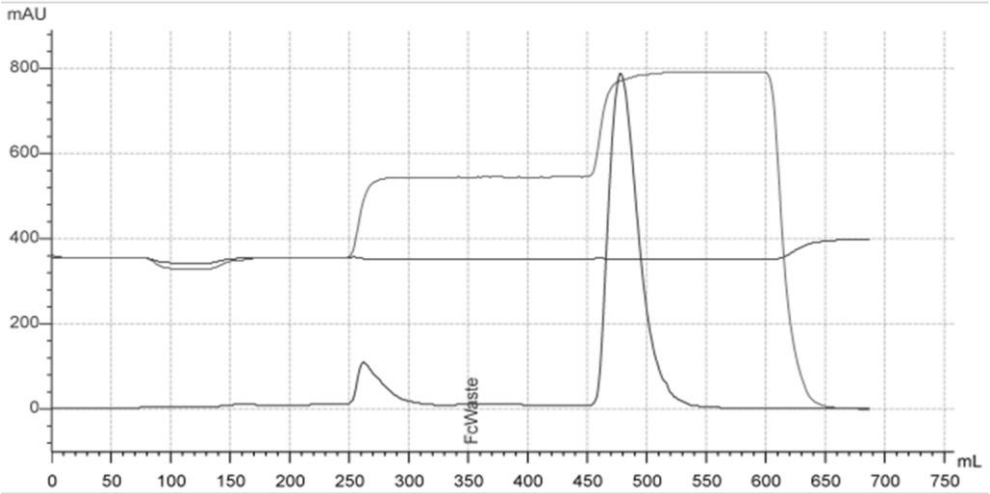


FIG. 65

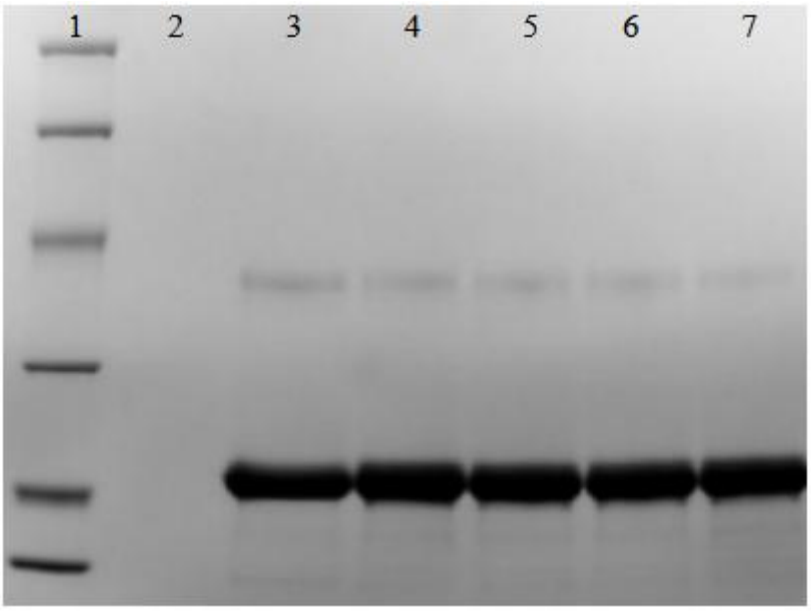


FIG. 66

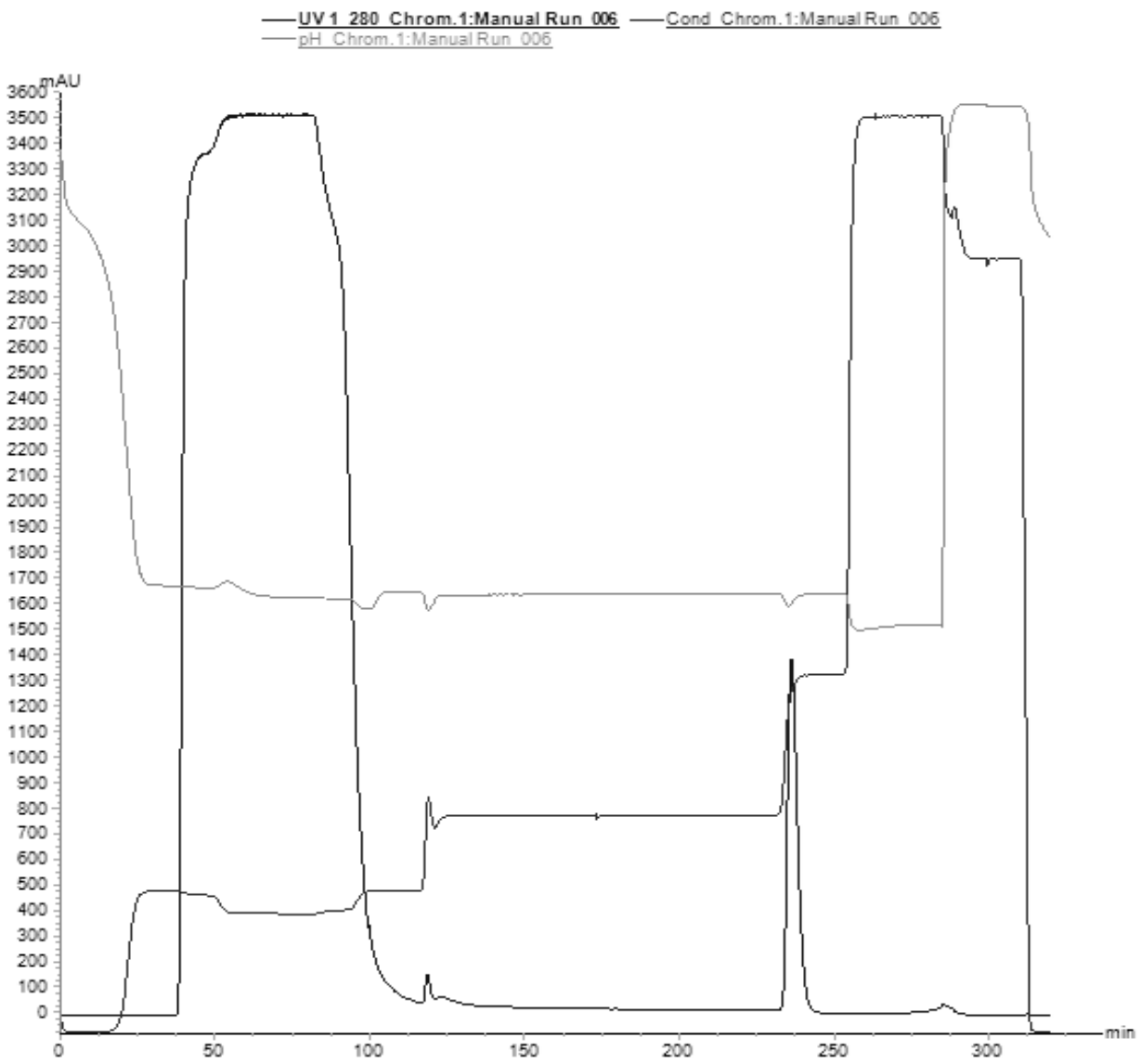

UV 1_280_Chrom.1:Manual Run_006
Cond_Chrom.1:Manual Run_006
pH_Chrom.1:Manual Run_006
mAU
3600
3500
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
300
min

FIG. 67

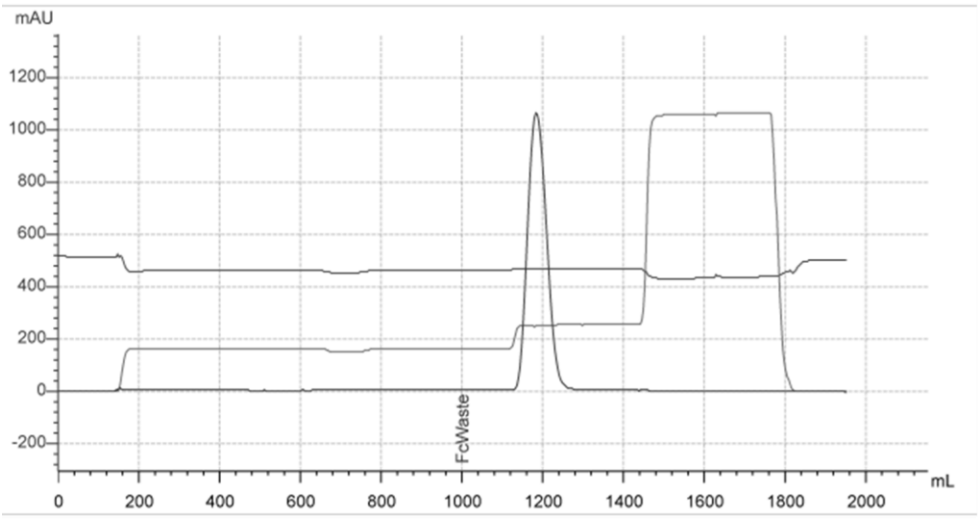

mAU
1200
1000
800
600
400
200
0
-200
FcWaste
0
200
400
600
800
1000
1200
1400
1600
1800
2000
mL

FIG. 68

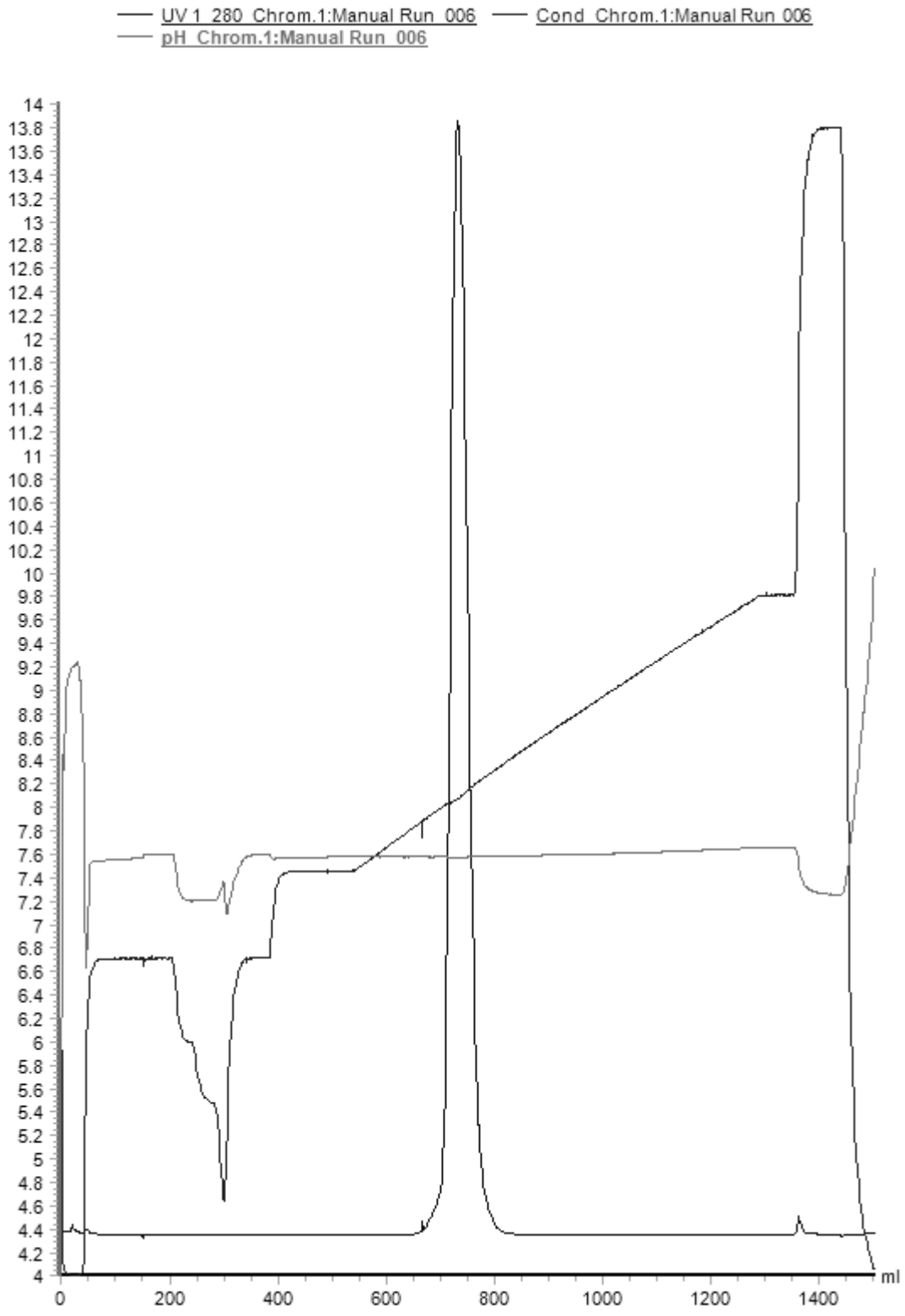

UV 1_280_Chrom.1:Manual Run_006
Cond_Chrom.1:Manual Run_006
pH_Chrom.1:Manual Run_006
14
13.8
13.6
13.4
13.2
13
12.8
12.6
12.4
12.2
12
11.8
11.6
11.4
11.2
11
10.8
10.6
10.4
10.2
10
9.8
9.6
9.4
9.2
9
8.8
8.6
8.4
8.2
8
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
5.8
5.6
5.4
5.2
5
4.8
4.6
4.4
4.2
4
0
200
400
600
800
1000
1200
1400
ml

FIG. 69

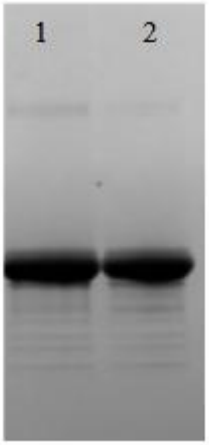


FIG. 70

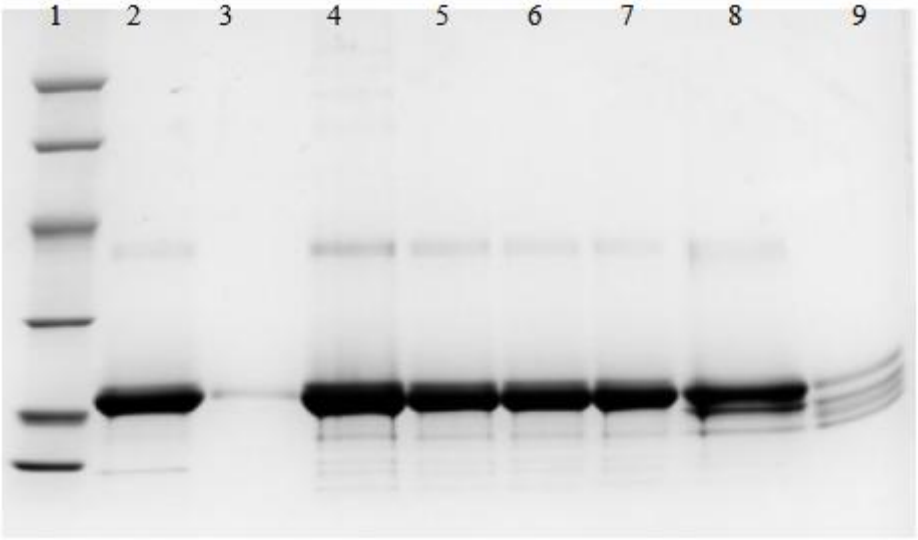


FIG. 71

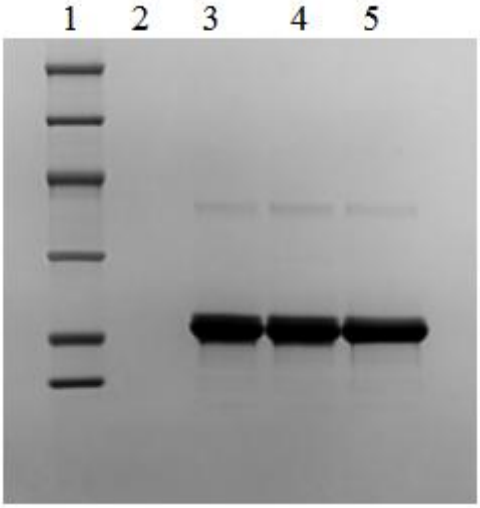


FIG. 72



FIG. 73