Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

		A	B	C	D	E	F
		20mM Sodium Acetate pH 4.5	20mM Sodium Citrate pH 6.0	50mM Histidine pH 6.5	50mM Sodium Phosphate pH 7.0	50mM Sodium Bicarbonate pH 7.3	50mM HEPES pH 7.5
1	No additions	4.56	5.92	6.59	7.01	9.02	7.49
2	15mM Trehalose	4.51	5.88	6.54	7.00	9.02	7.47
3	15mM Sucrose	4.47	5.87	6.58	6.97	8.92	7.49
4	15mM Mannitol	4.46	5.86	6.64	6.99	8.52	7.49
5	75mM $(NH_4)_2SO_4$	4.54	5.86	6.50	7.05	8.14	7.54
6	250mM Glycine	4.47	5.83	6.70	7.02	7.88	7.51
7	250mM Arginine	4.50	5.79	6.51	6.96	7.65	7.47
8	100mM Glutamate	4.54	5.76	6.45	7.03	8.64	7.56

Figure 1

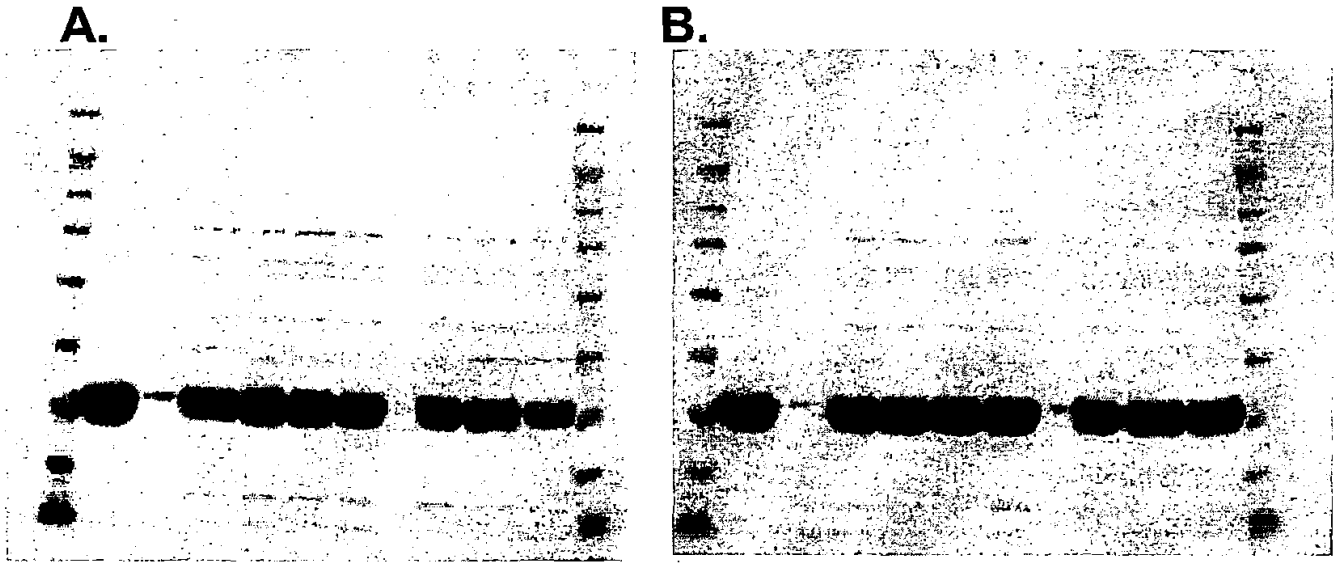
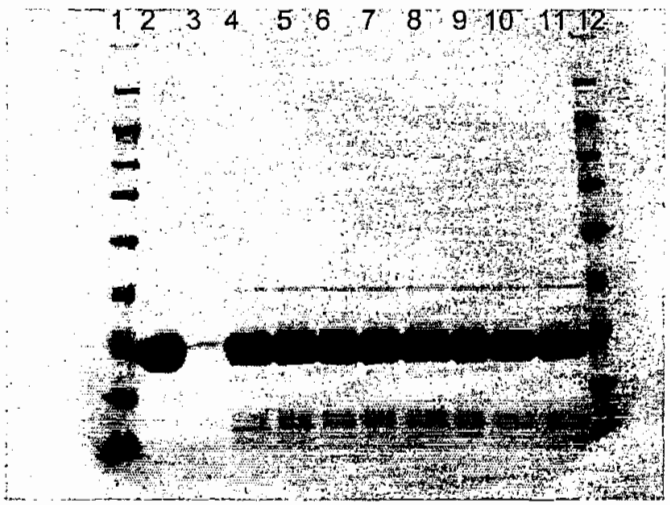


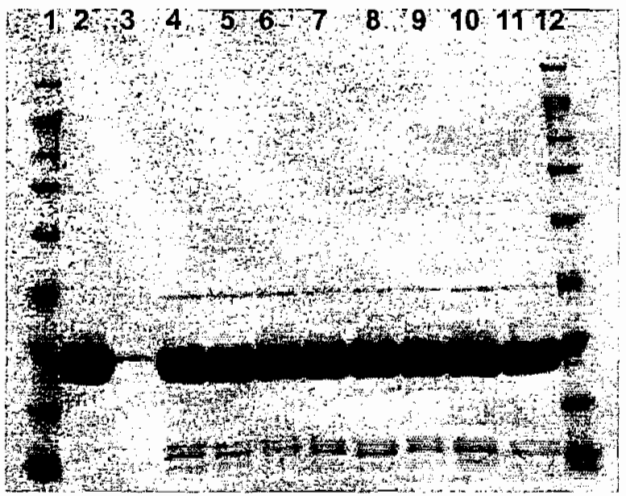
Figure 2

C.



t = 0

D.



t = 4w

Figure 2 cont.

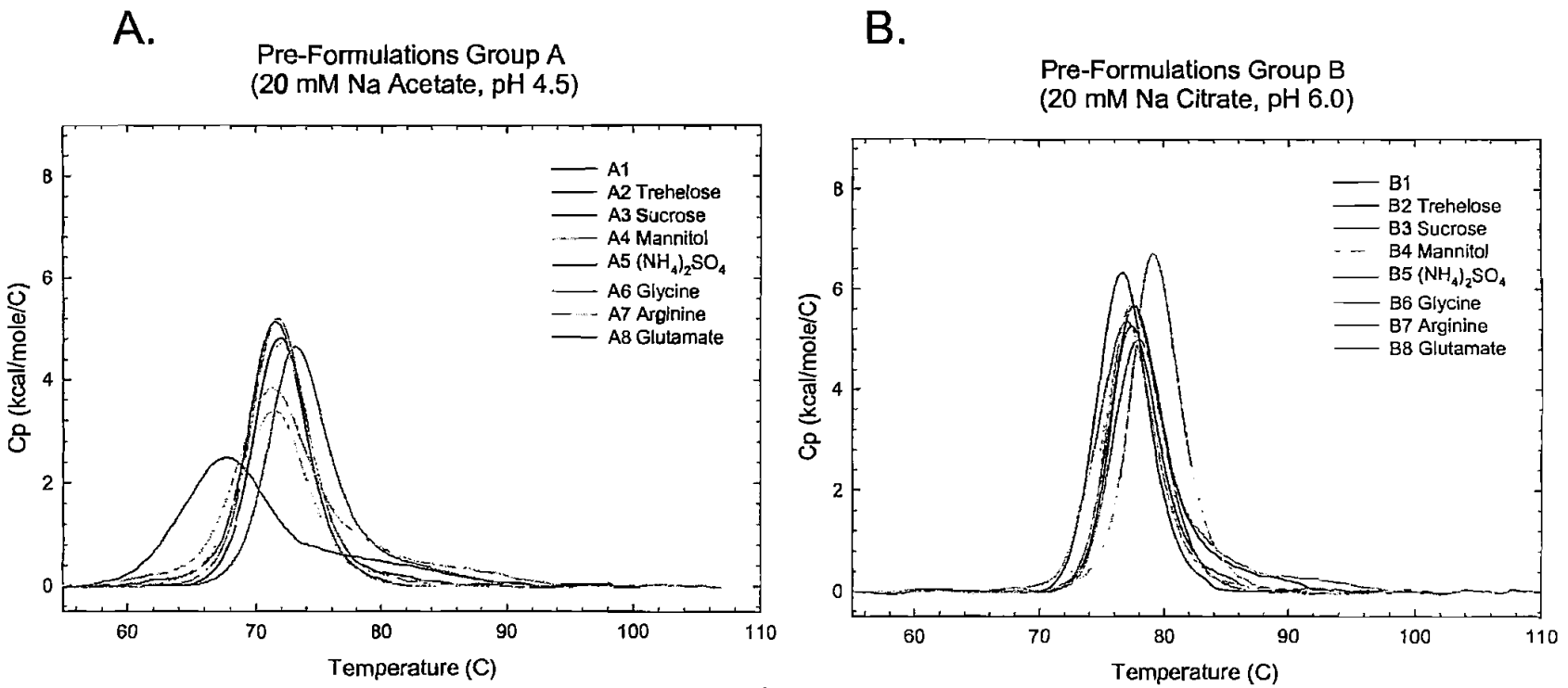

A.
Pre-Formulations Group A
(20 mM Na Acetate, pH 4.5)
A1
A2 Trehelose
A3 Sucrose
A4 Mannitol
A5 $(NH_4)_2SO_4$
A6 Glycine
A7 Arginine
A8 Glutamate
Cp (kcal/mole/C)
Temperature (C)
B.
Pre-Formulations Group B
(20 mM Na Citrate, pH 6.0)
B1
B2 Trehelose
B3 Sucrose
B4 Mannitol
B5 $(NH_4)_2SO_4$
B6 Glycine
B7 Arginine
B8 Glutamate
Cp (kcal/mole/C)
Temperature (C)

Figure 3

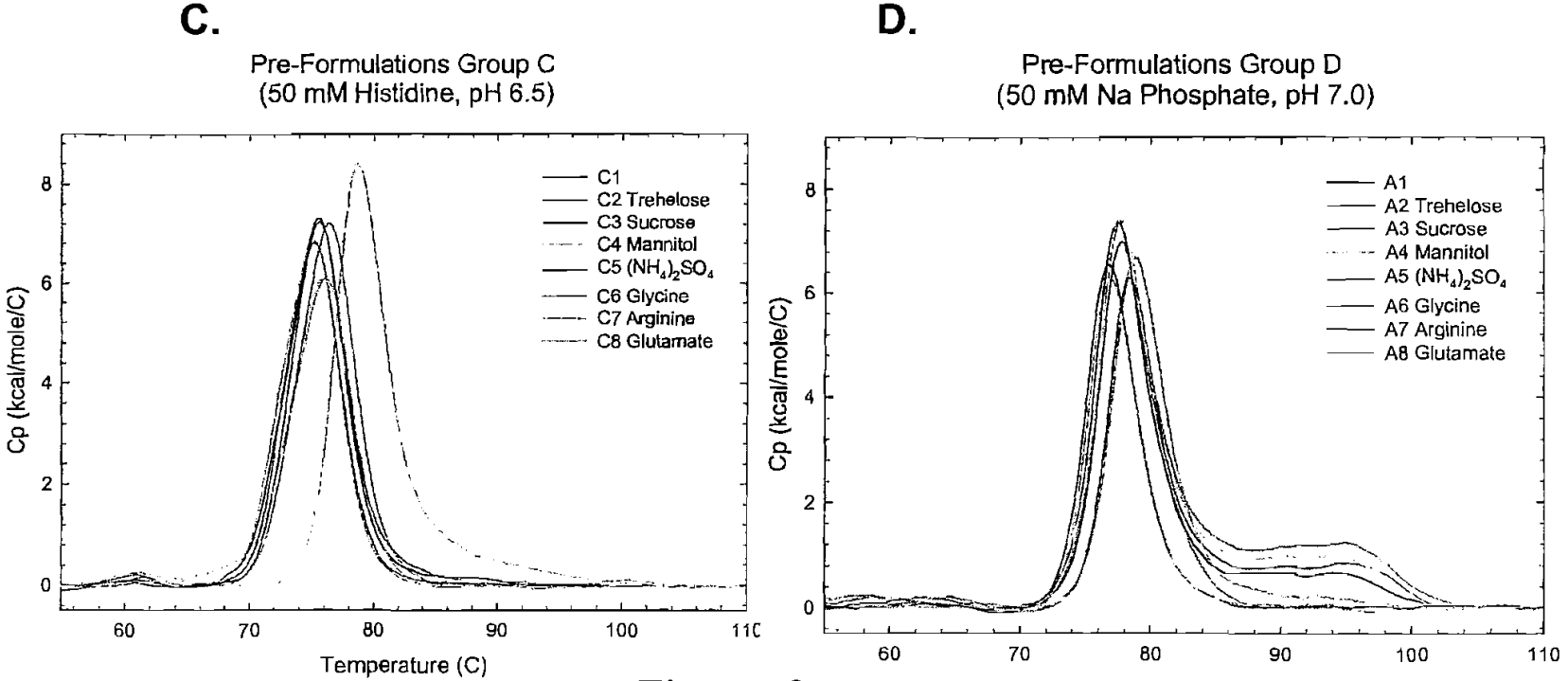
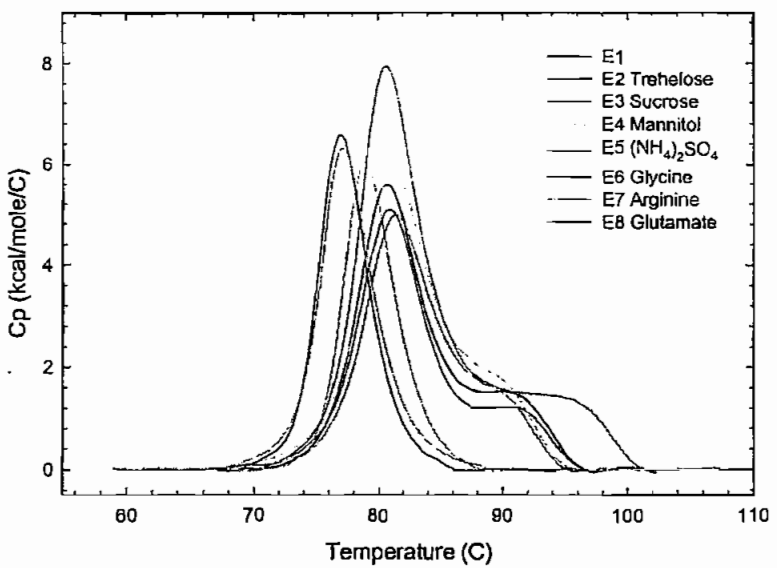


Figure 3 cont.

E.



F.

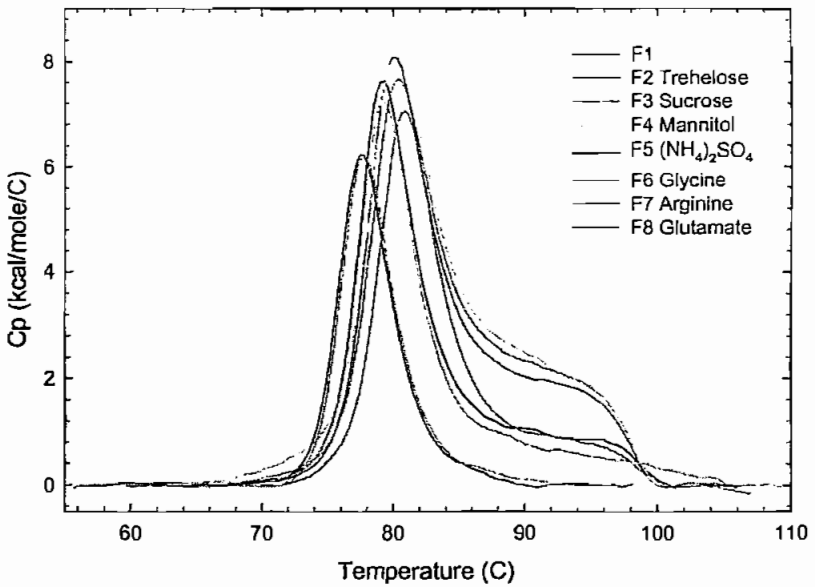


Figure 3 cont.

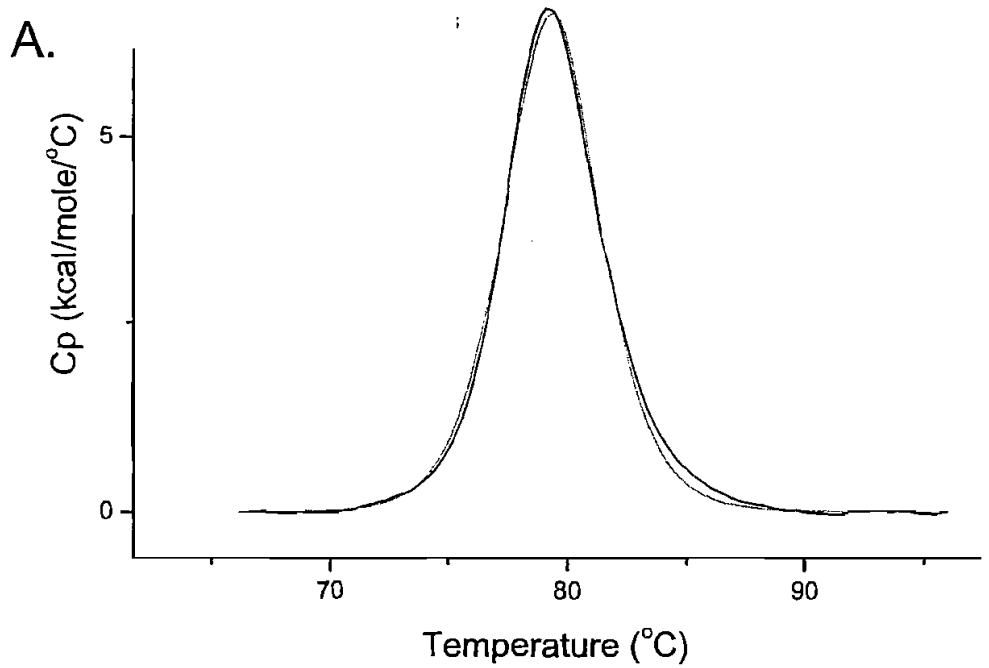


Figure 4

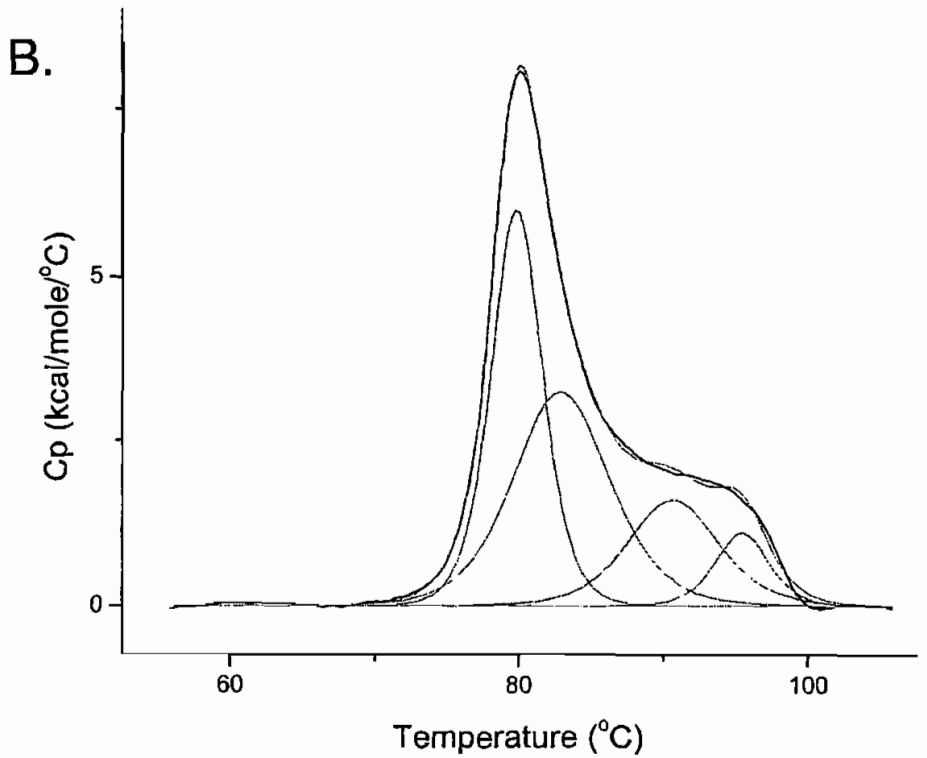


Figure 4

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

		A		B		C		D		E		F	
		20 mM Sodium Acetate pH 4.5		20 mM Sodium Citrate pH 6.0		50 mM Histidine pH 6.5		50 mM Sodium Phosphate pH 7.0		50 mM Sodium Bicarbonate pH 7.3		50 mM HEPES pH 7.5	
		T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m
1	No additions	72.06	0	77.47	0	75.45	0	77.45	0	80.78	0	79.29	0
2	15 mM Trehalose	71.67	- 0.39	77.48	+ 0.01	75.2	- 0.25	77.77	+ 0.32	81.21	+ 0.43	80.02	+ 0.73
3	15 mM Sucrose	71.79	- 0.27	77.31	- 0.16	75.25	- 0.2	77.67	+ 0.22	81.59	+ 0.81	80.32	+ 1.03
4	15 mM Mannitol	72.08	+ 0.02	77.25	- 0.22	75.8	+ 0.35	77.69	+ 0.24	81.46	+ 0.68	80.05	+ 0.76
5	150 mM $(NH_4)SO_4$*	67.61	- 4.45	76.6	- 0.87	75.62	+ 0.17	76.9	- 0.55	77.09	- 3.69	77.53	- 1.76
6	250 mM Glycine	73.18	+ 1.12	78.09	+ 0.62	76.34	+ 0.89	78.62	+ 1.17	80.71	- 0.07	80.9	+ 1.61
7	250 mM Arginine	71.47	- 0.59	79.32	+ 1.85	78.73	+ 3.28	79.04	+ 1.59	79.15	- 1.63	79.22	- 0.07
8	250 mM Glutamate	71.36	- 0.7	76.95	- 0.52	76.07	+ 0.62	76.97	- 0.48	77.14	- 3.64	77.71	- 1.58

Figure 5

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

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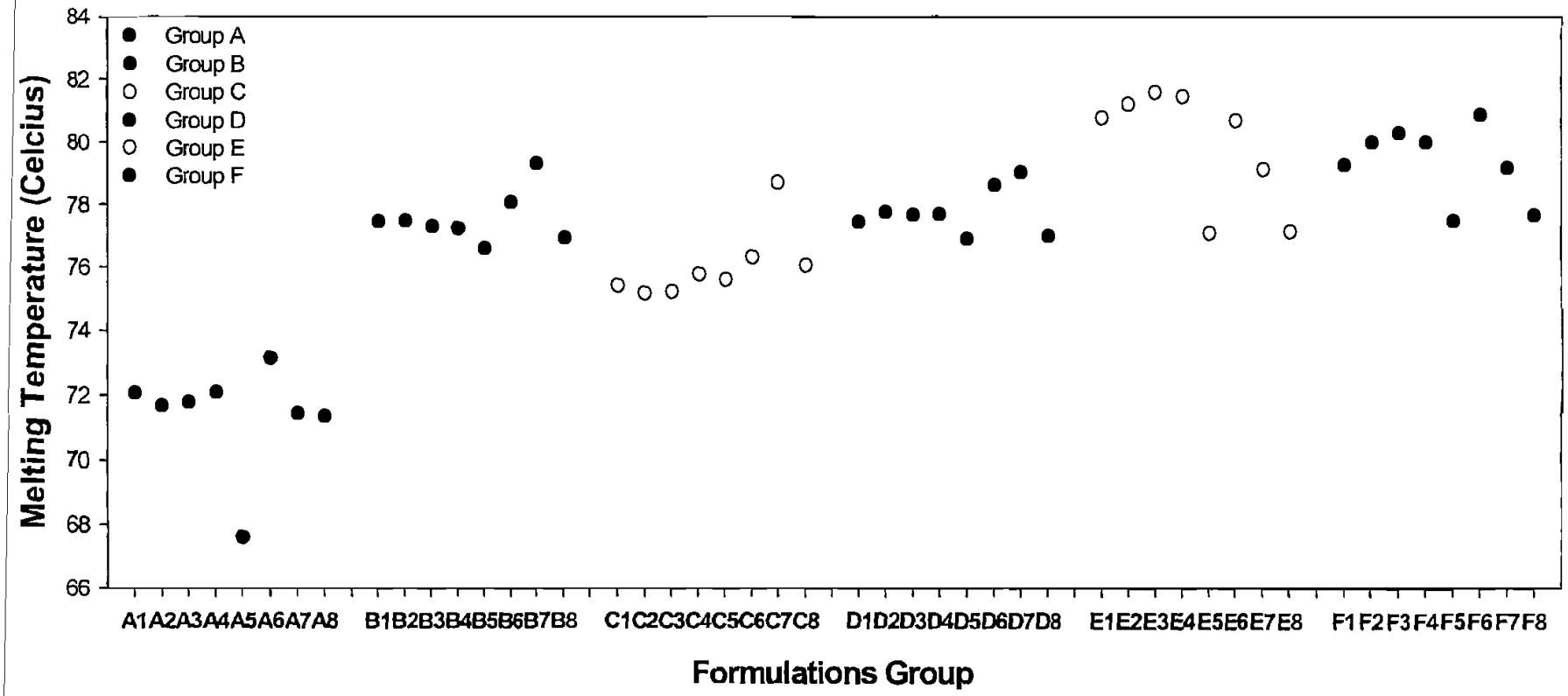


Figure 6

10/88

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

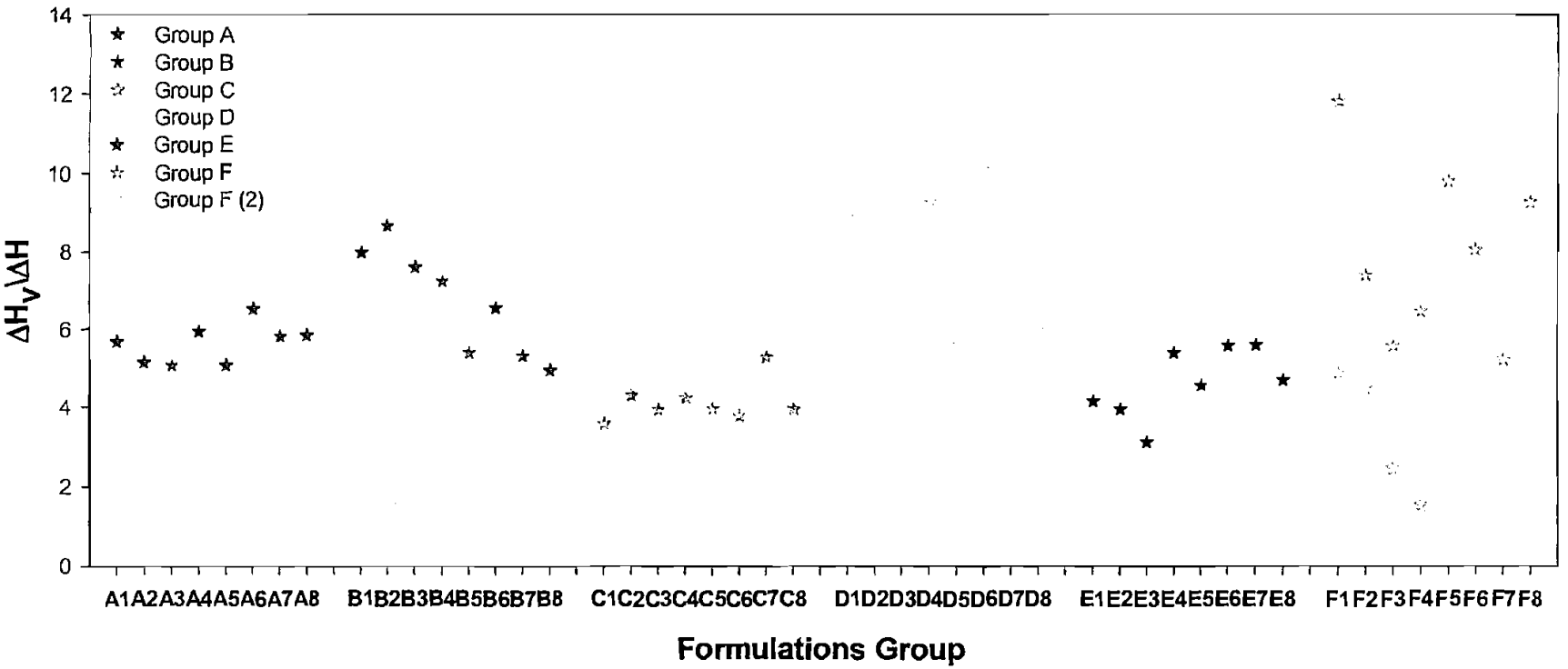


Figure 7

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

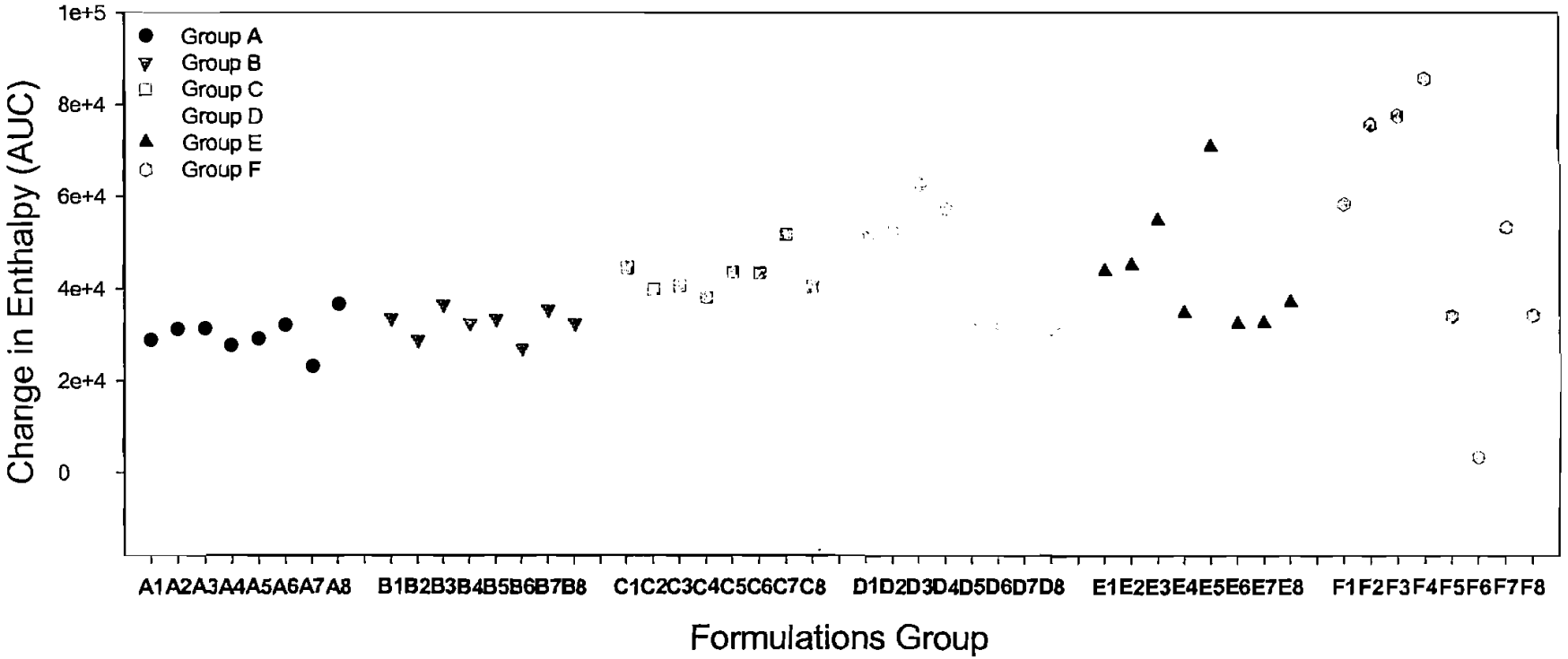


Figure 8

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

Fig. 9A.

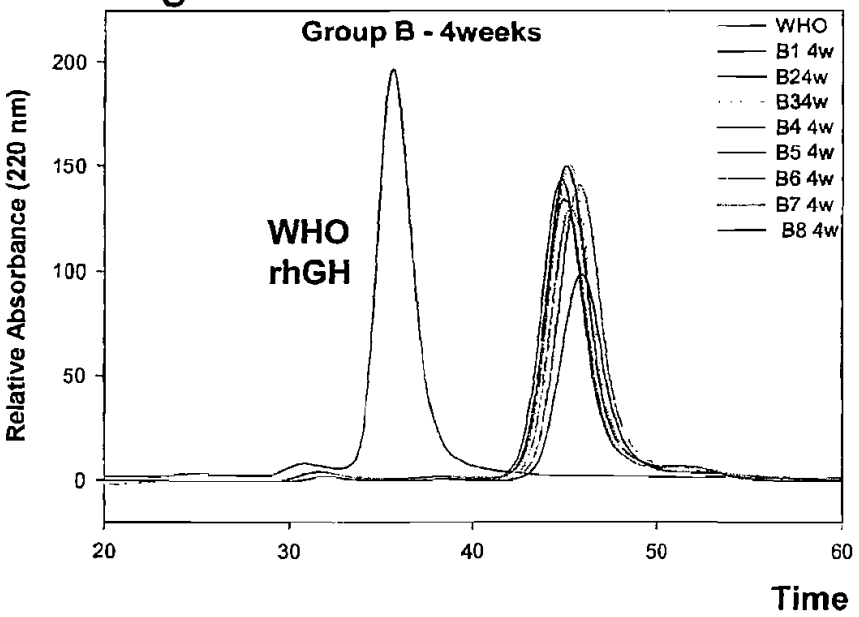
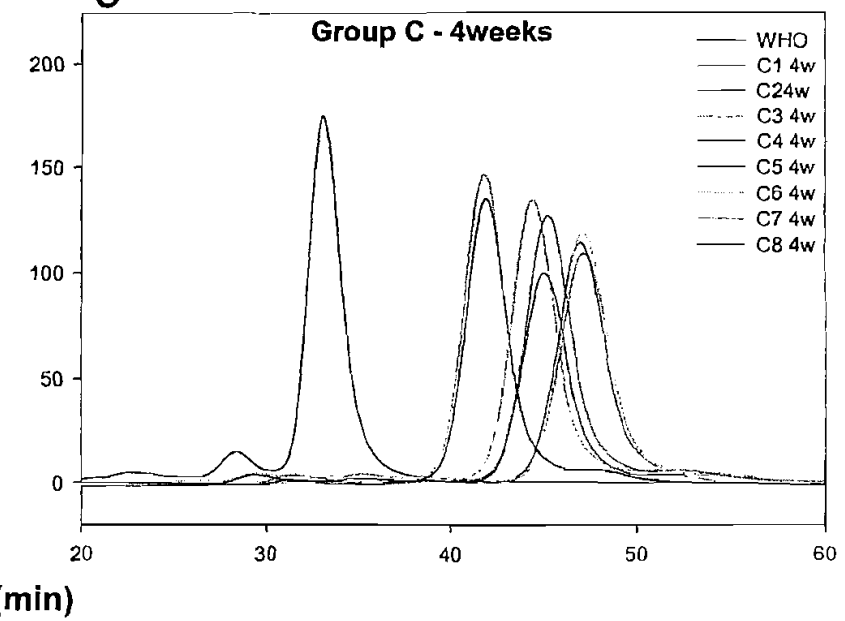


Fig. 9B.



Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

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Fig. 9C.

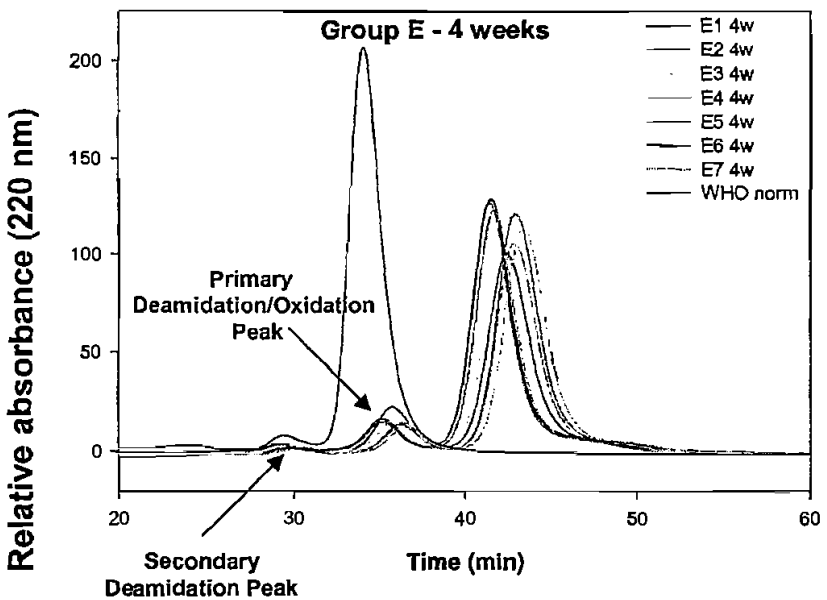
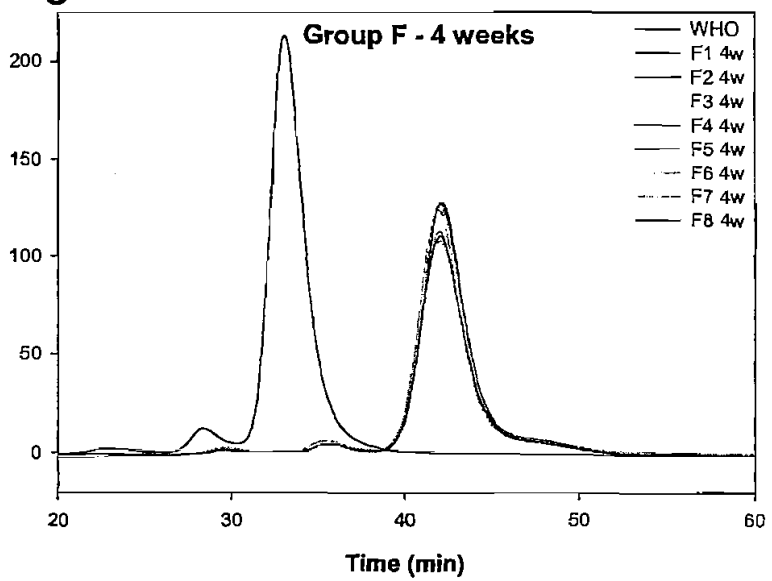
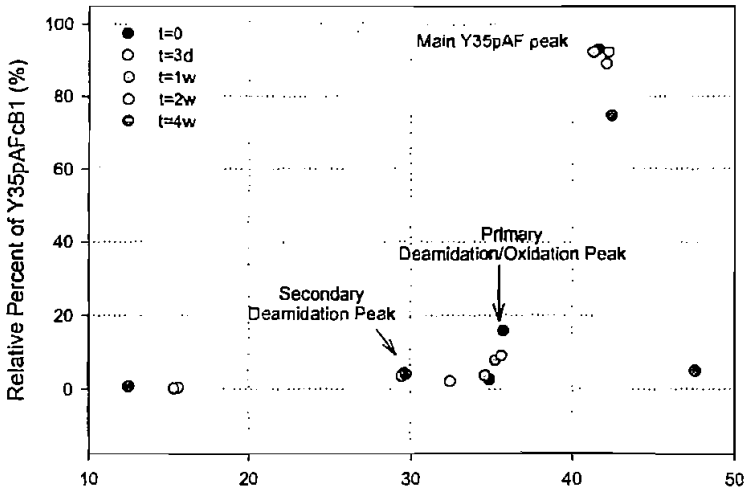


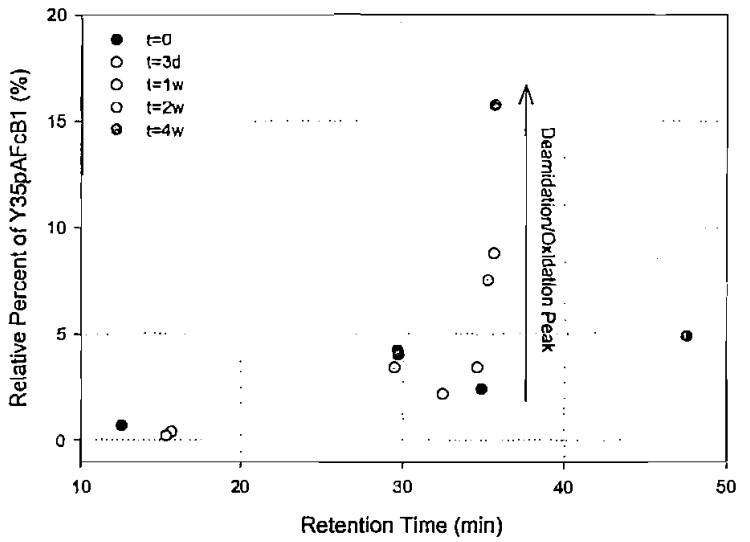
Fig. 9D.



A.



B.



Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al.

Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT

Atty: John W. Wallen, III

Telephone: (858) 875-2403

Figure 10

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

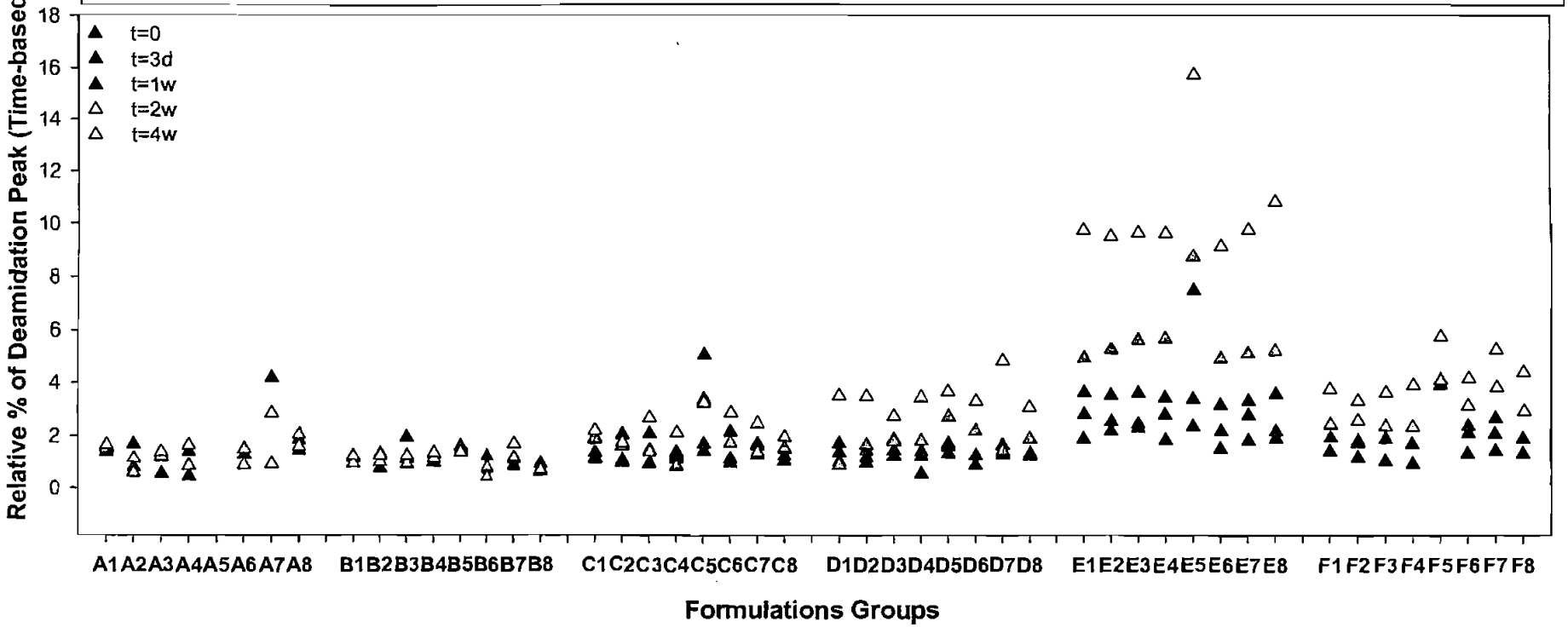


Figure 11

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

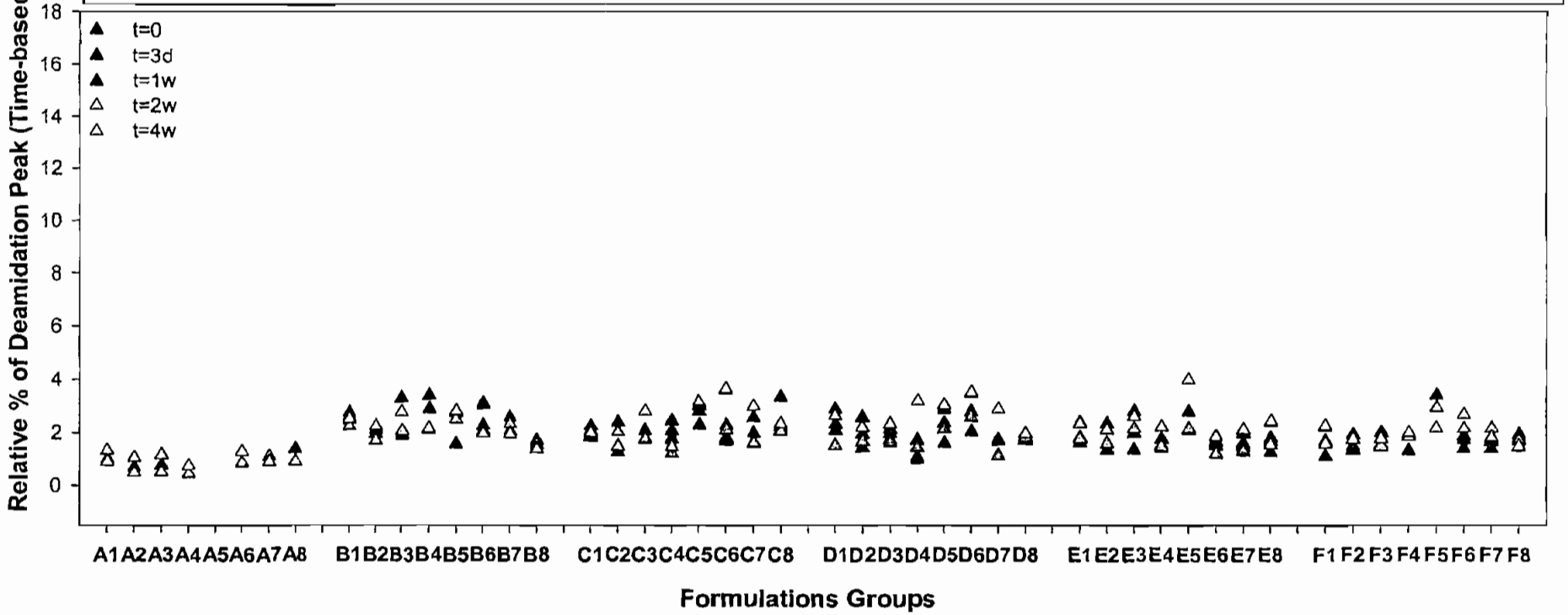


Figure 12

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

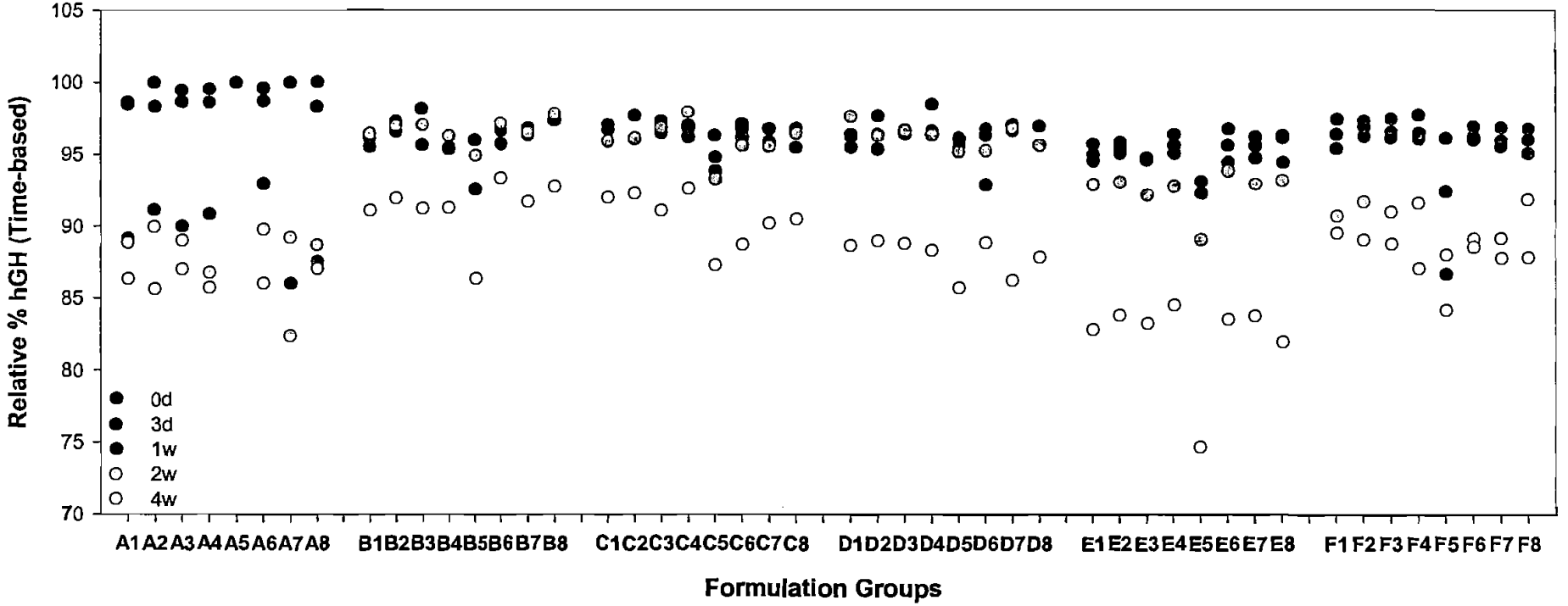


Figure 13

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

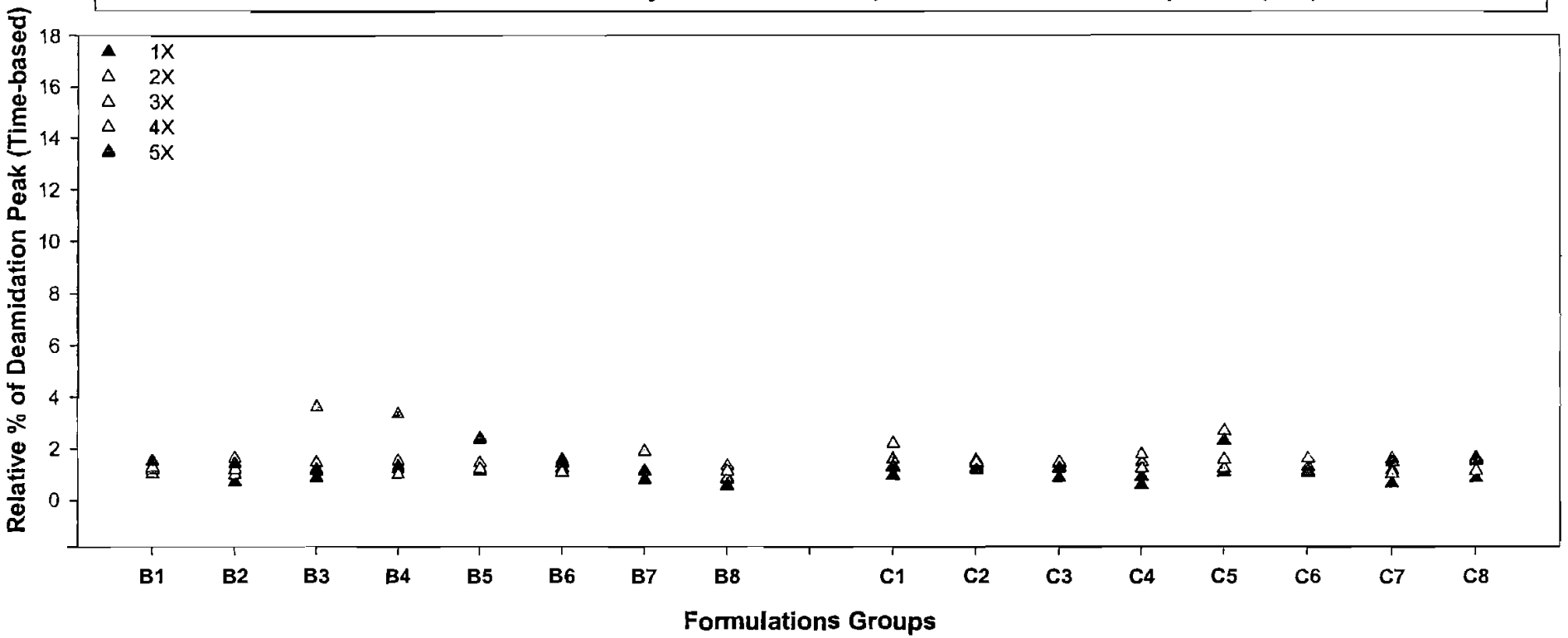


Figure 14

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid

Inventors: Anna-Maria Hays et al. Appl. No.: Not Assigned

Docket Number: AMBX-0077.00PCT Atty: John W. Wallen, III Telephone: (858) 875-2403

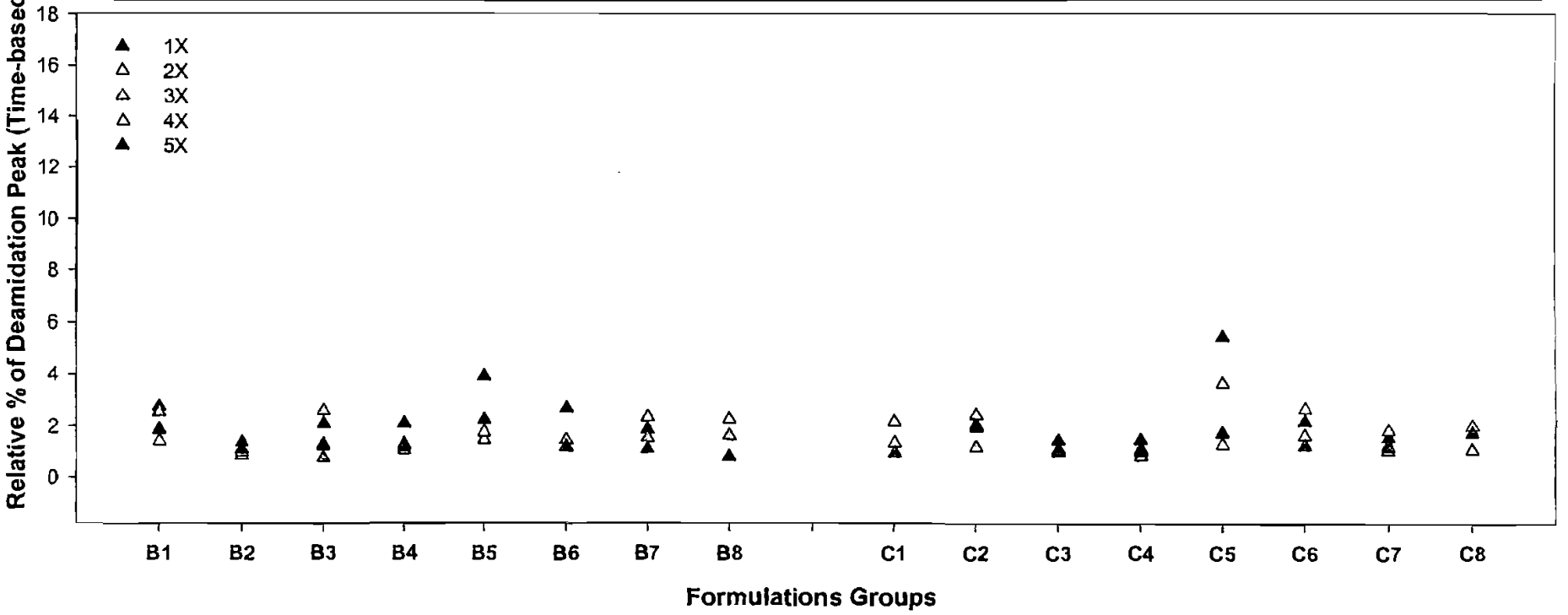


Figure 15

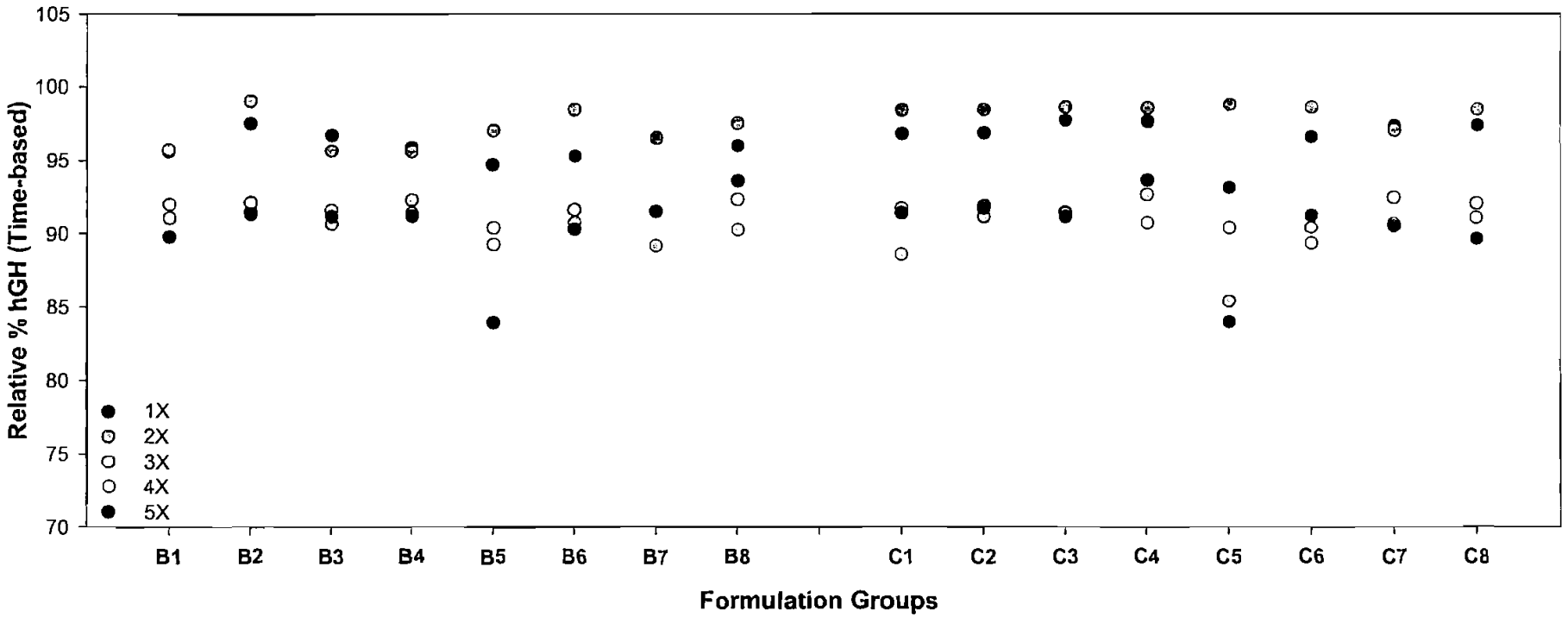

Relative % hGH (Time-based)
105
100
95
90
85
80
75
70
1X
2X
3X
4X
5X
B1
B2
B3
B4
B5
B6
B7
B8
C1
C2
C3
C4
C5
C6
C7
C8
Formulation Groups

Figure 16

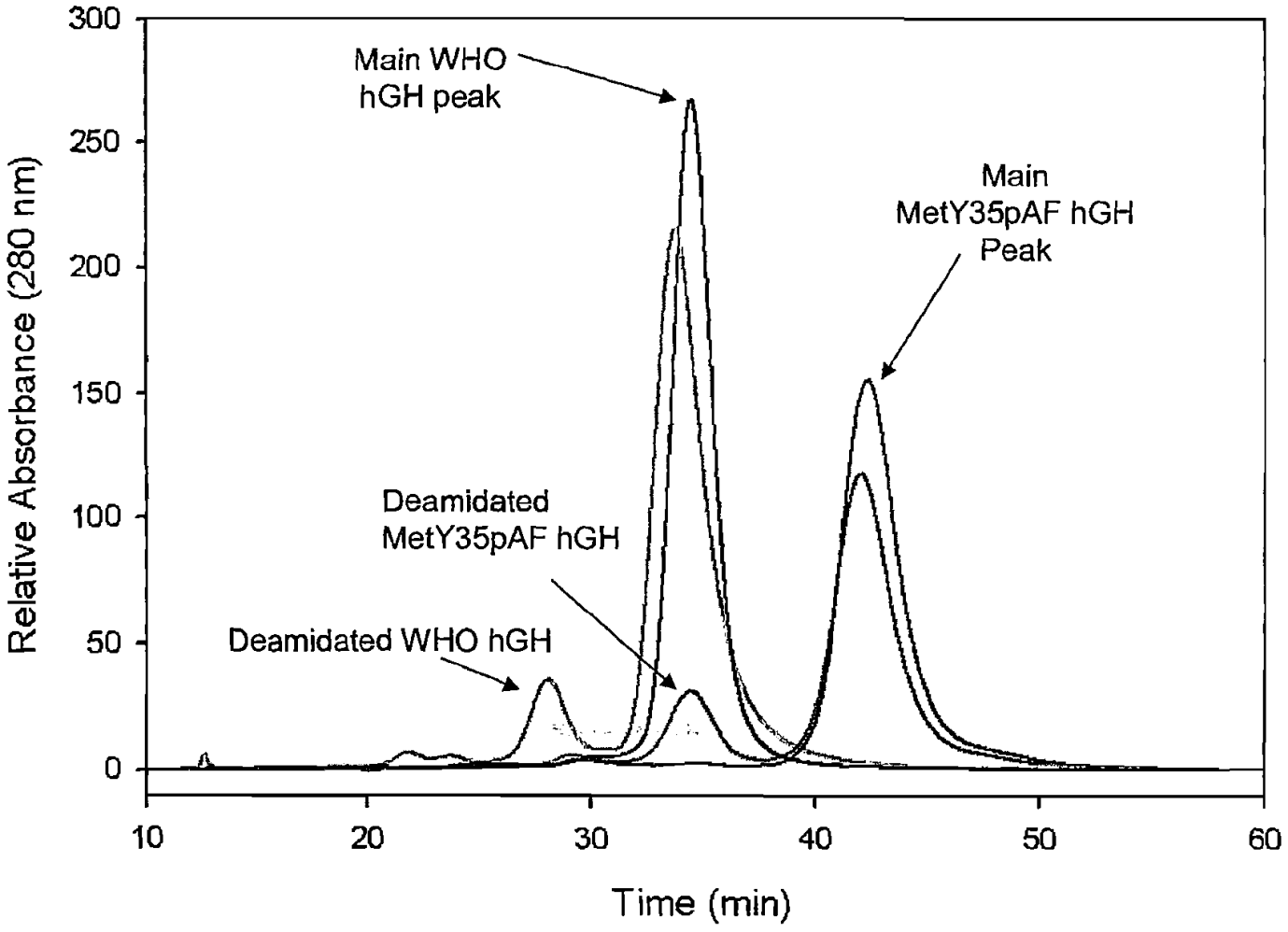

Main WHO hGH peak
Main MetY35pAF hGH Peak
Deamidated MetY35pAF hGH
Deamidated WHO hGH
Relative Absorbance (280 nm)
300
250
200
150
100
50
0
10
20
30
40
50
60
Time (min)

Figure 17

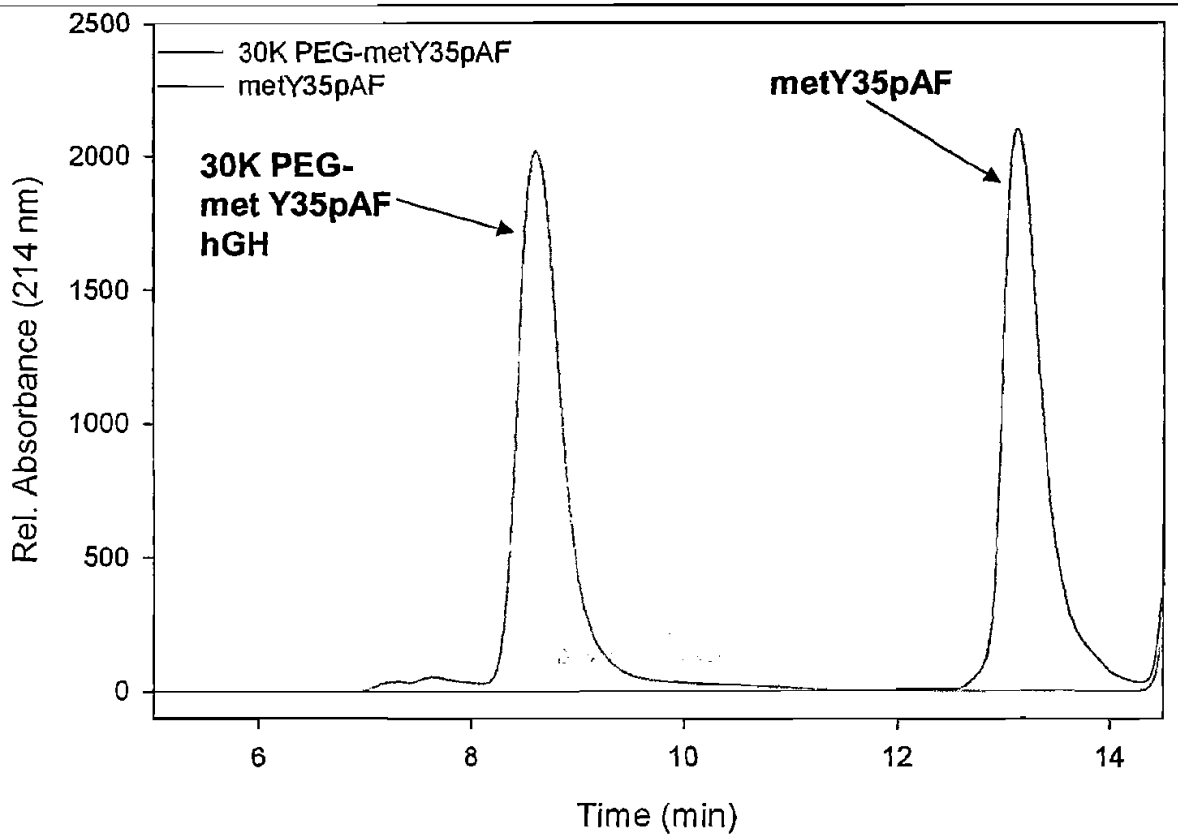

30K PEG-metY35pAF
metY35pAF
metY35pAF
30K PEG-
met Y35pAF
hGH
Rel. Absorbance (214 nm)
2500
2000
1500
1000
500
0
6
8
10
12
14
Time (min)

Figure 18

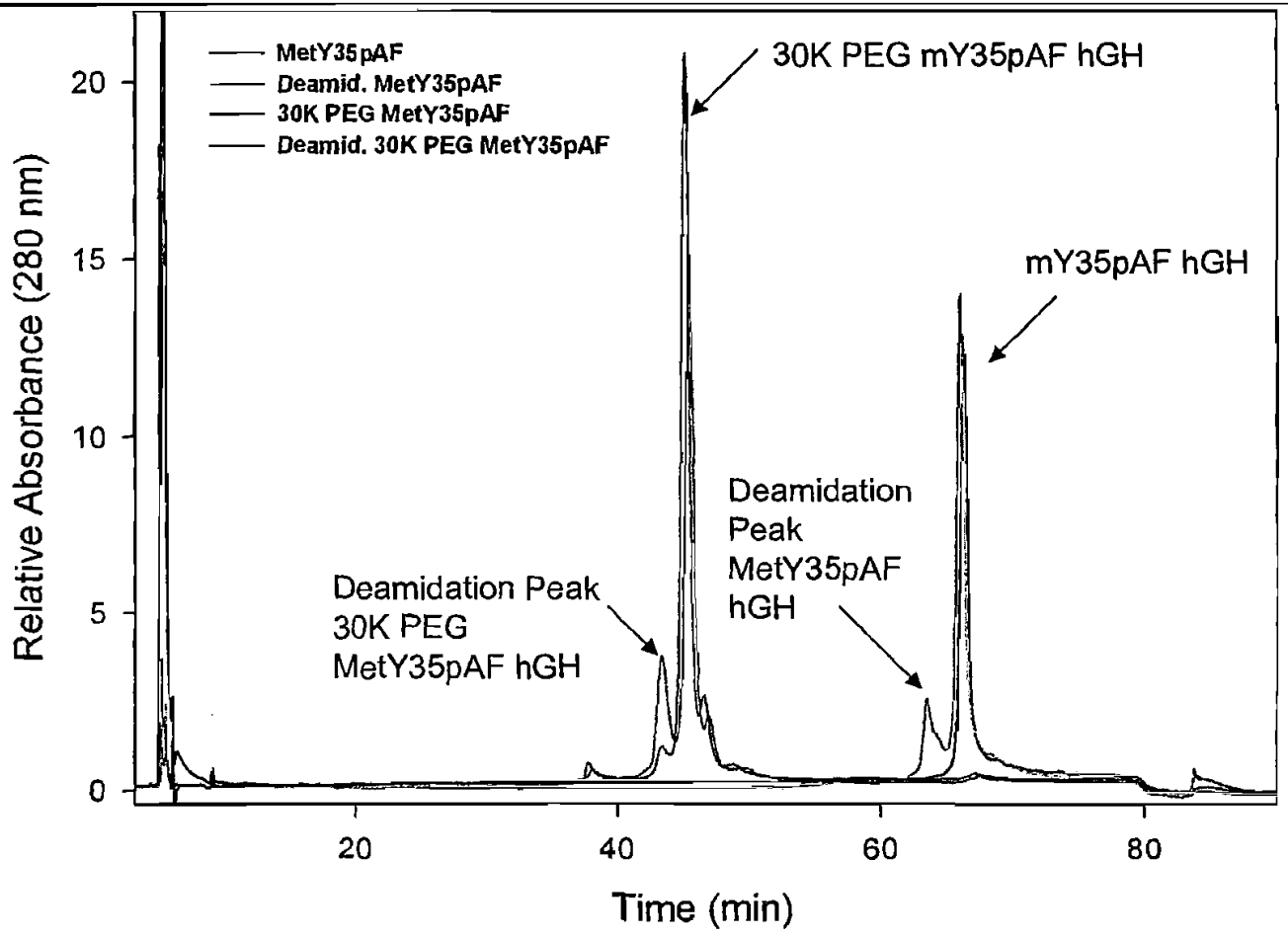

MetY35pAF
Deamid. MetY35pAF
30K PEG MetY35pAF
Deamid. 30K PEG MetY35pAF
30K PEG mY35pAF hGH
mY35pAF hGH
Deamidation Peak 30K PEG MetY35pAF hGH
Deamidation Peak MetY35pAF hGH
Relative Absorbance (280 nm)
0
5
10
15
20
20
40
60
80
Time (min)

Figure 19

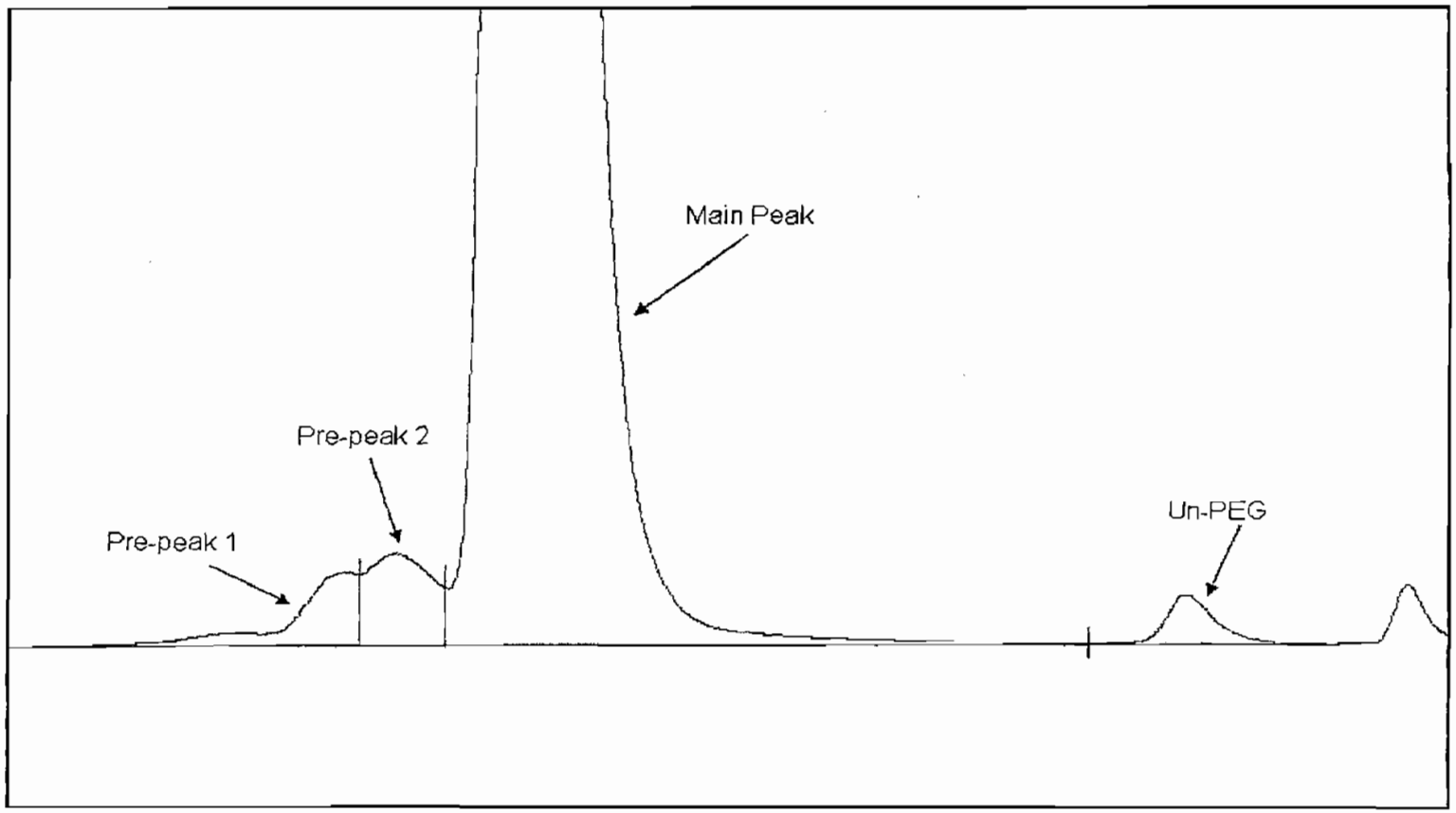


Figure 20

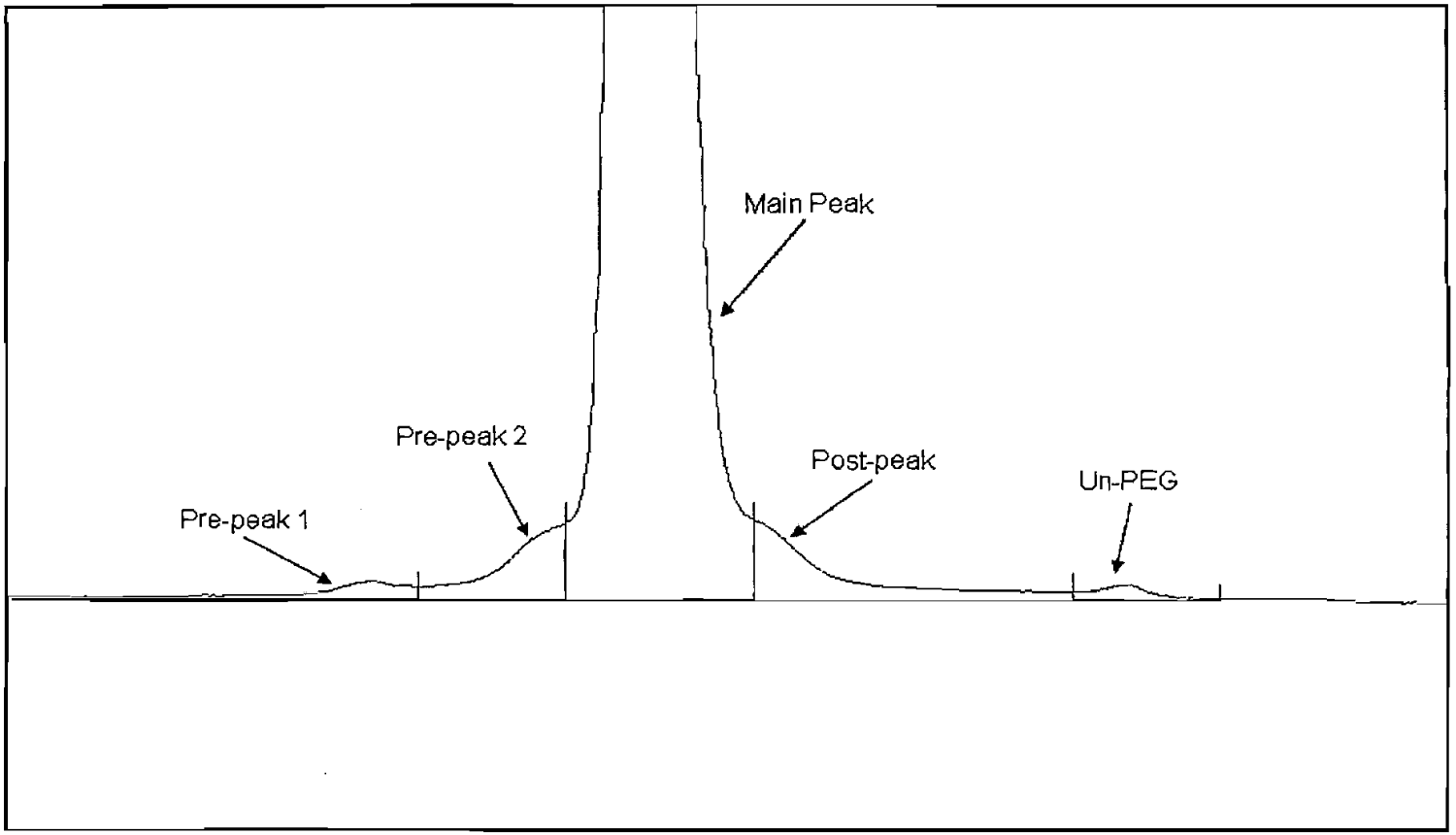


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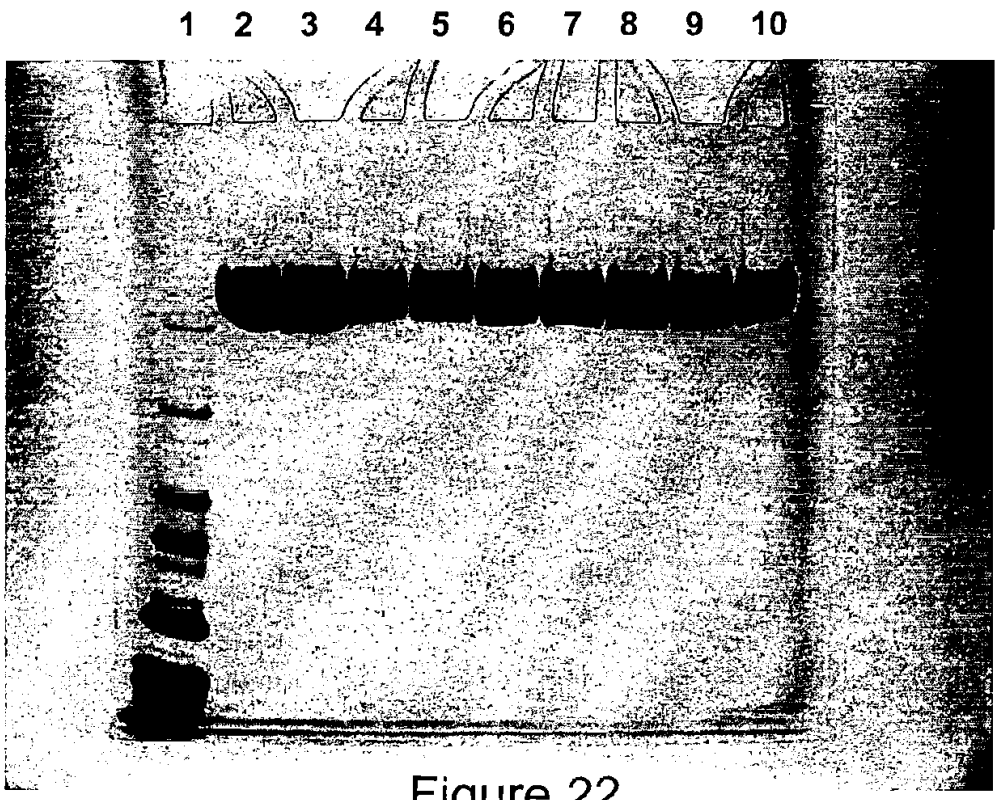


Figure 22

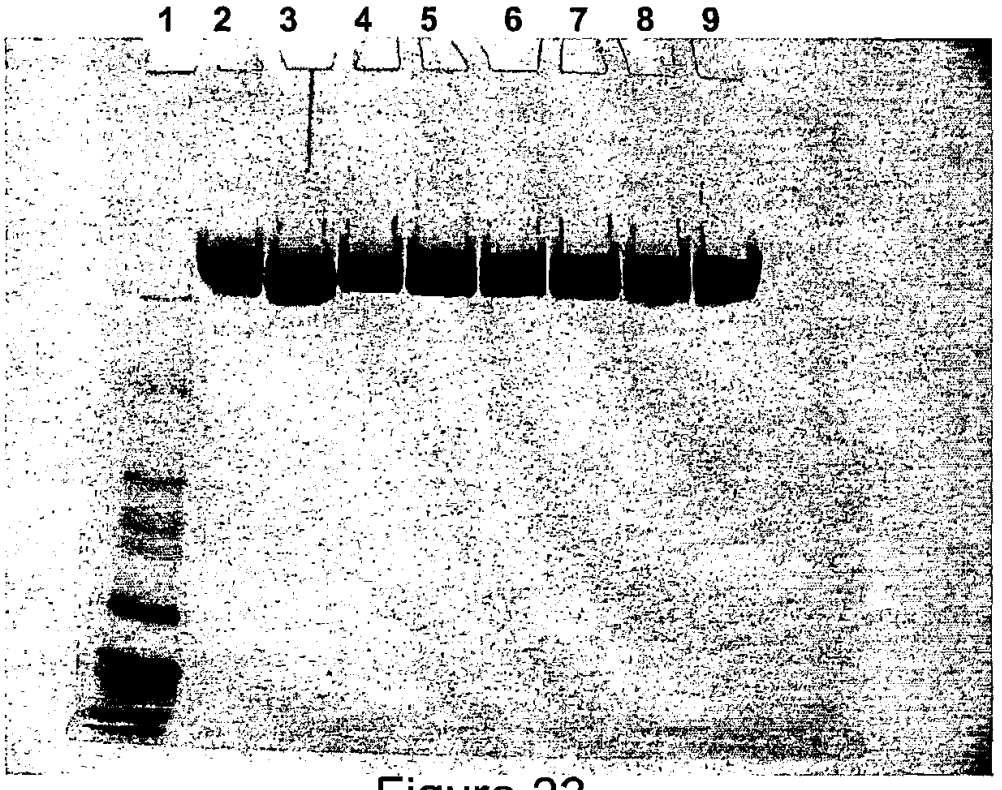


Figure 23

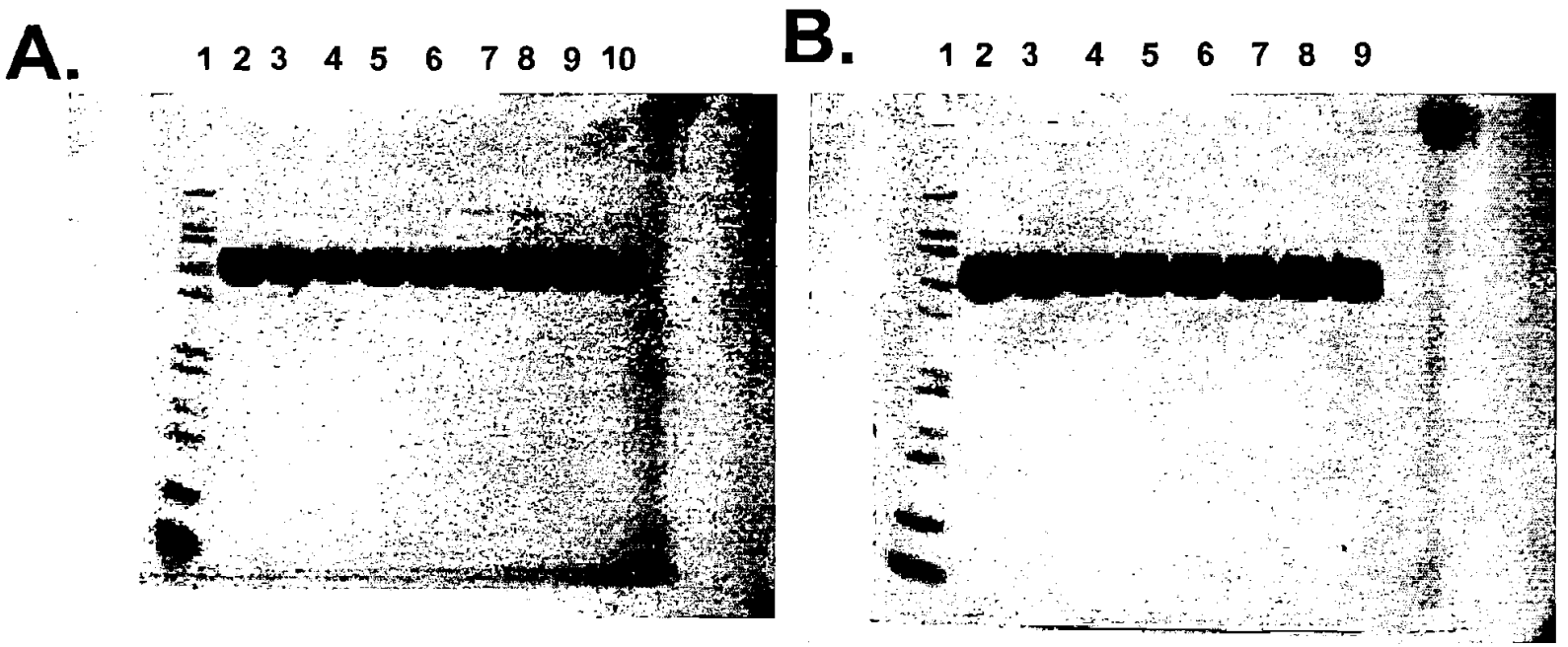


Figure 24

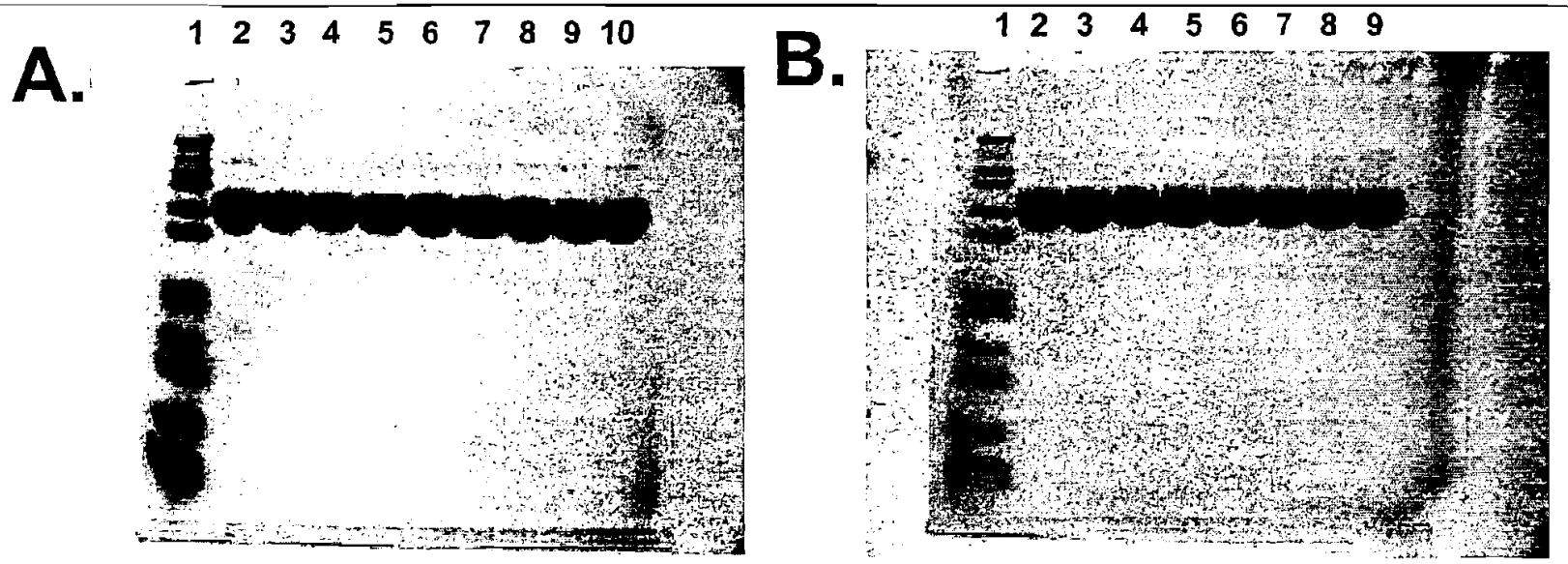


Figure 25

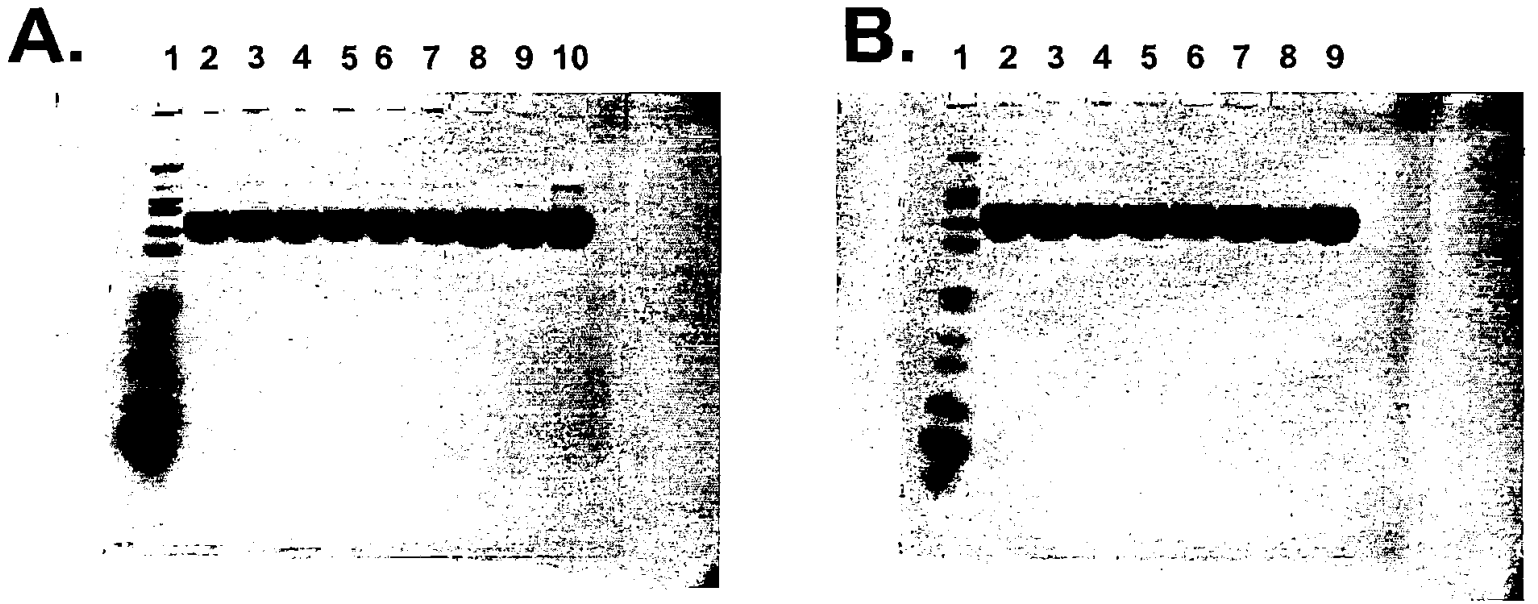


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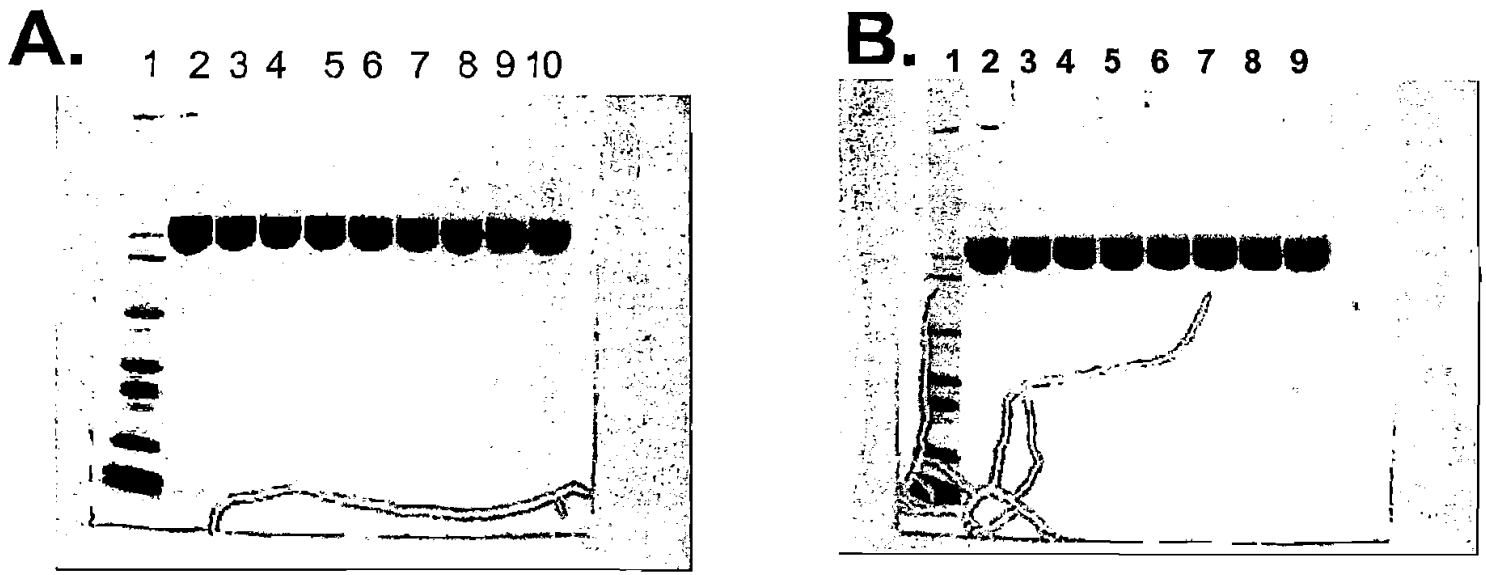


Figure 27

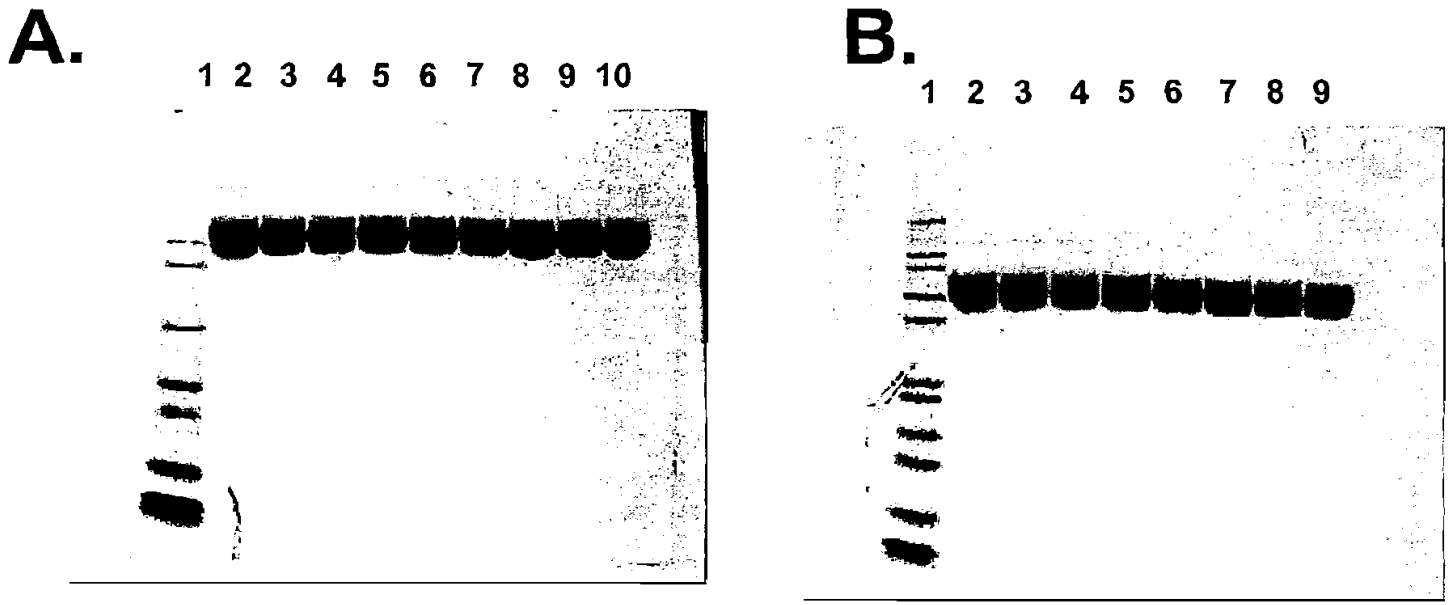


Figure 28

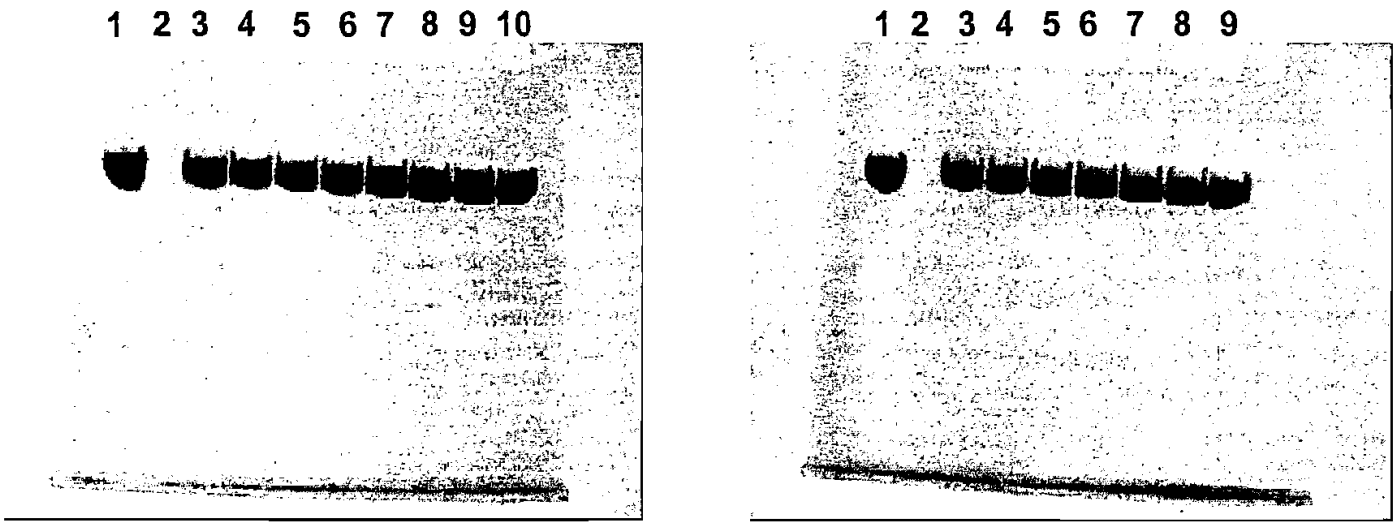


Figure 29

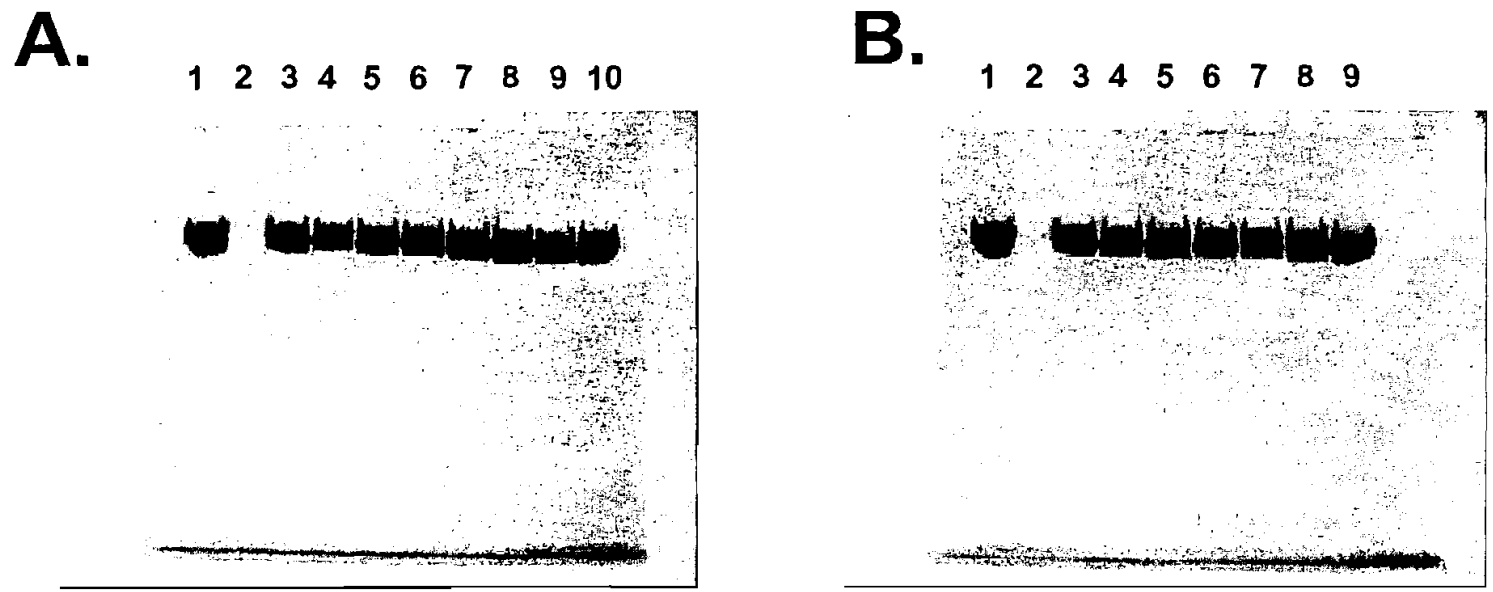


Figure 30

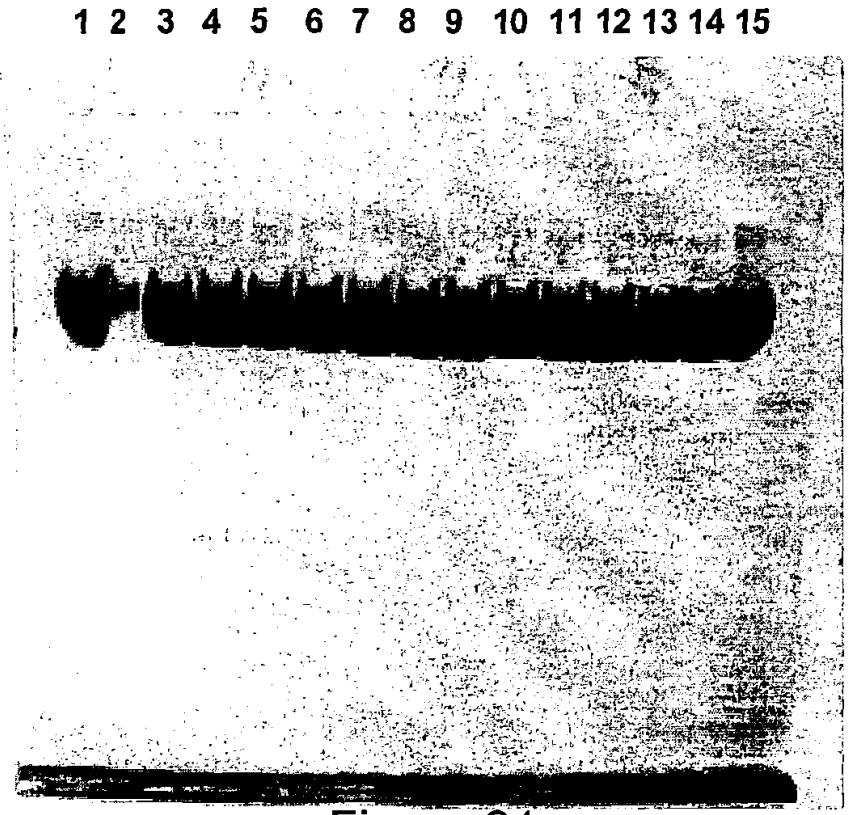


Figure 31

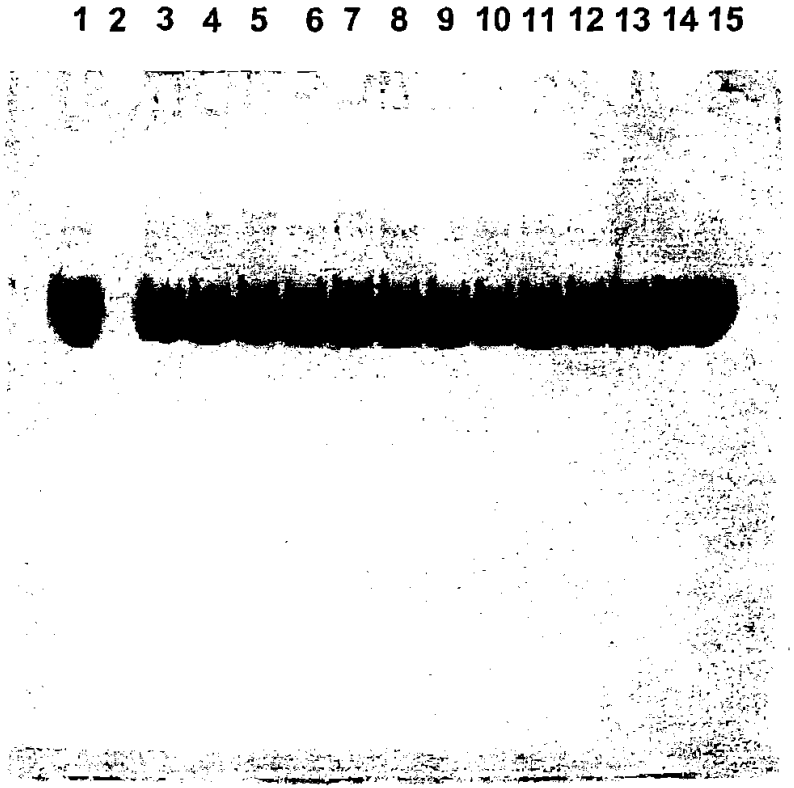


Figure 32

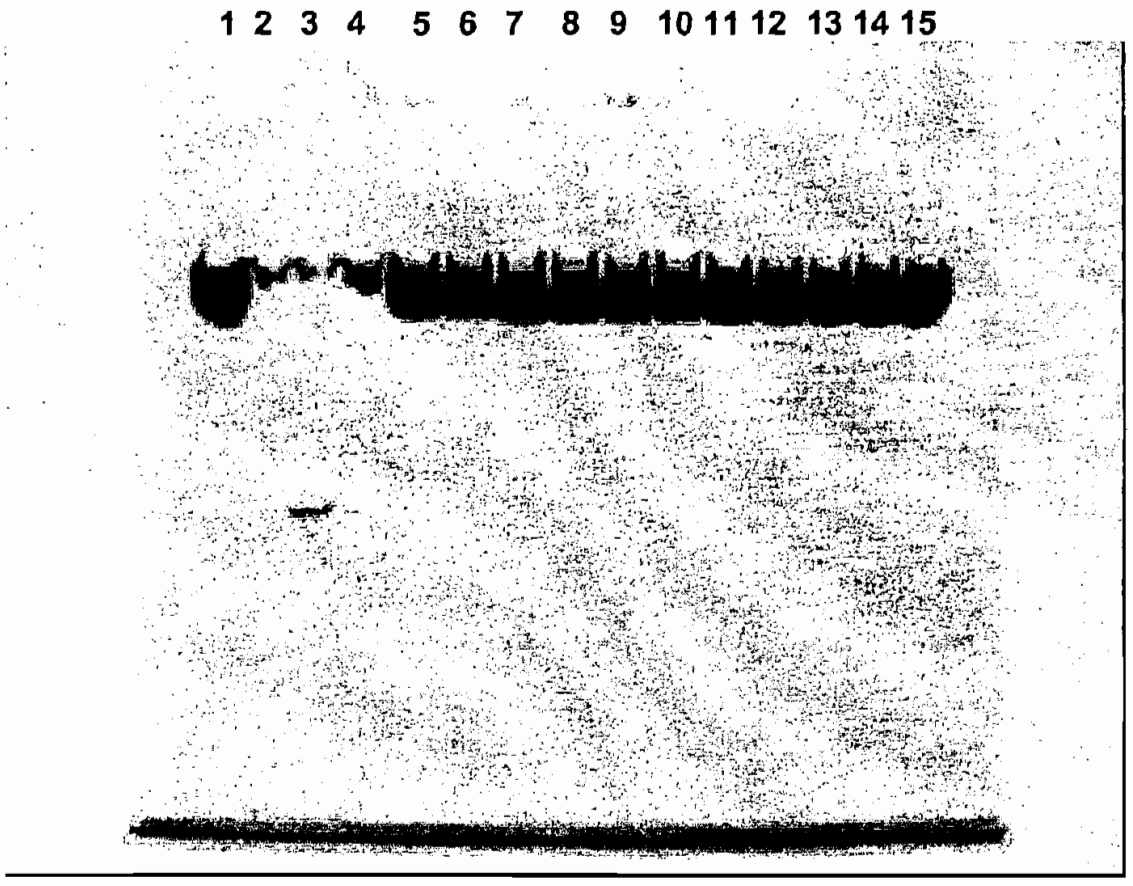


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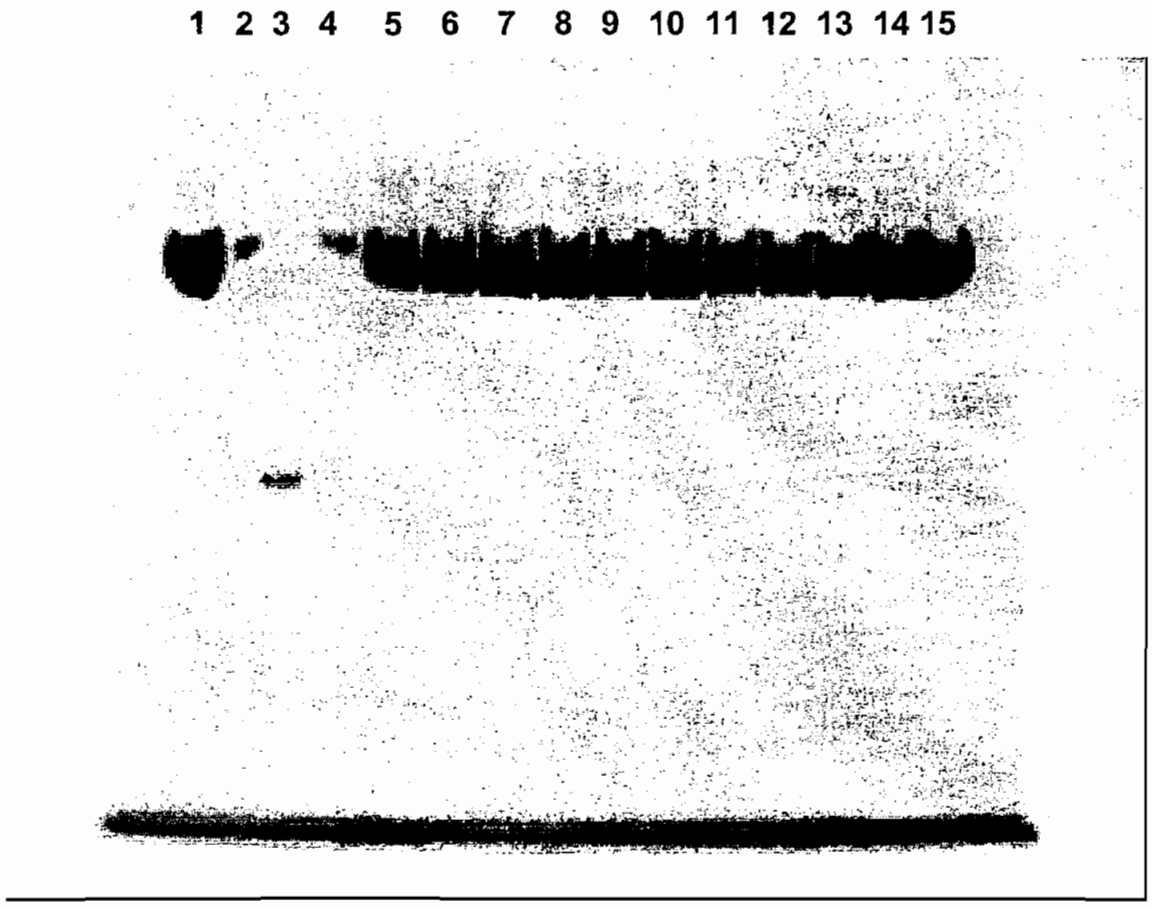


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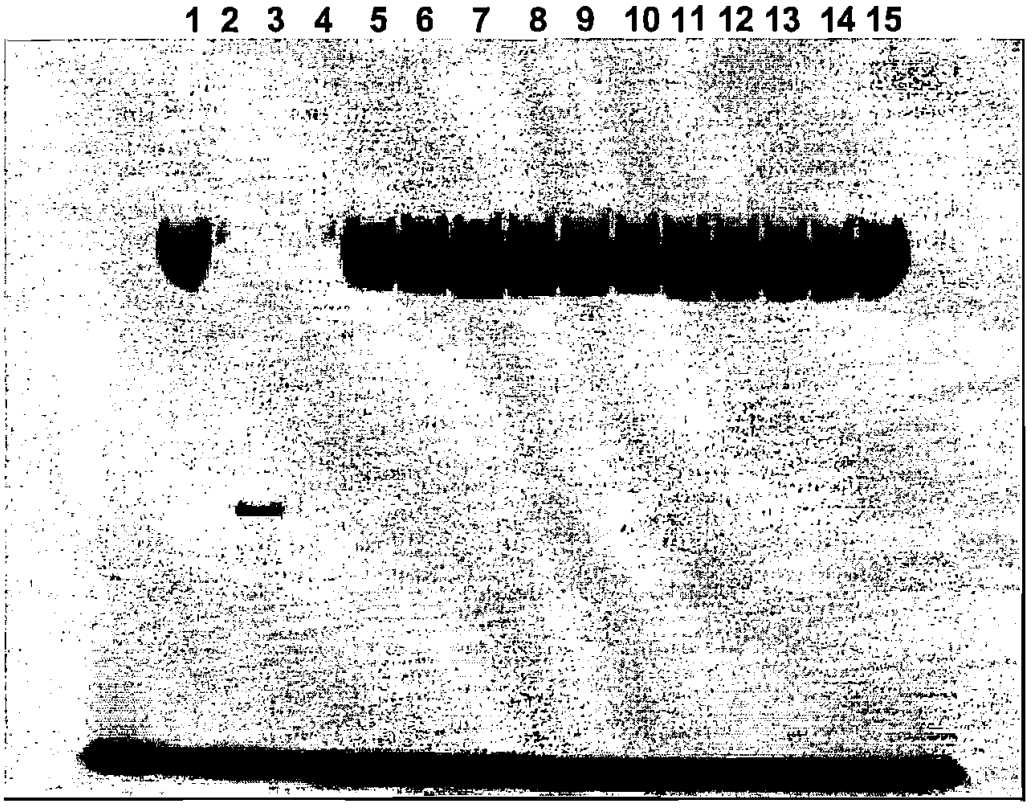


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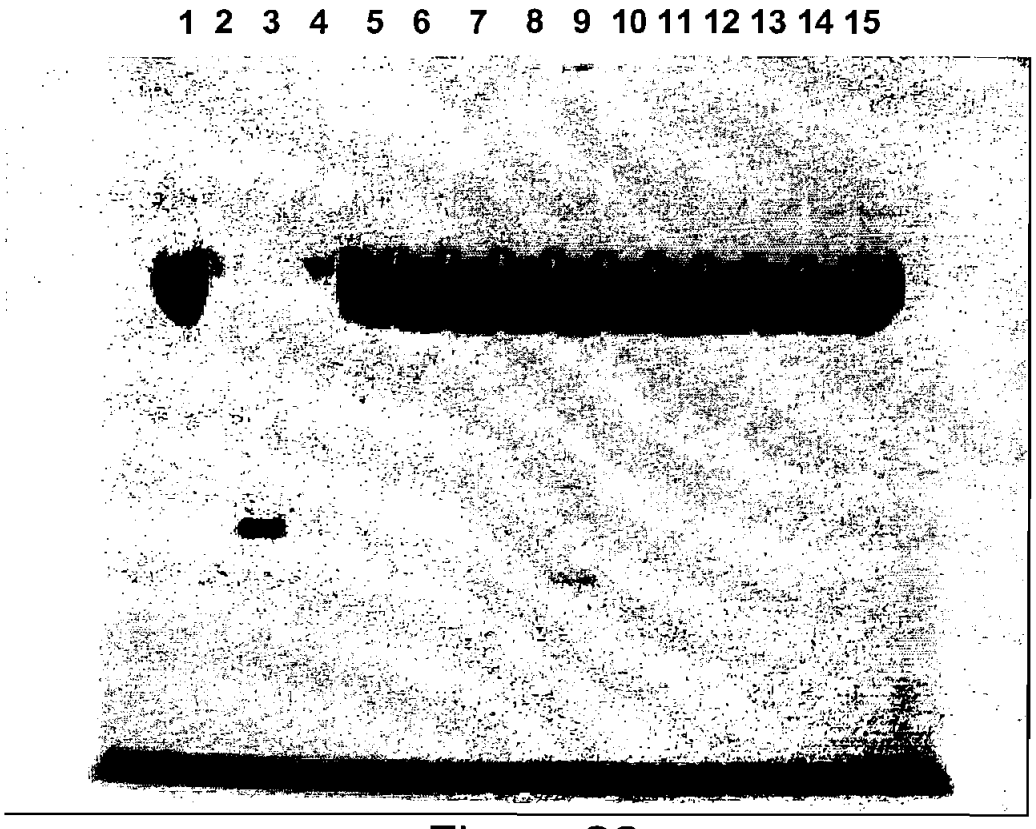


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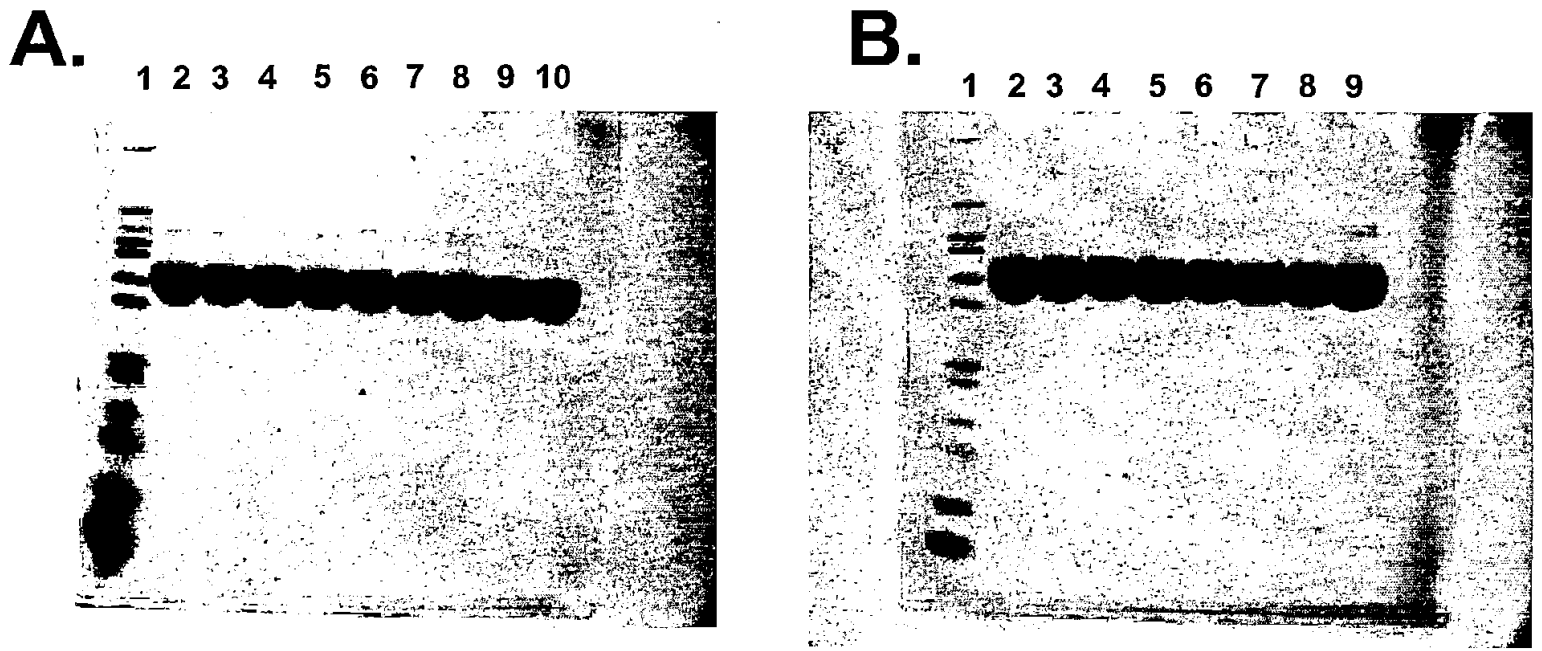


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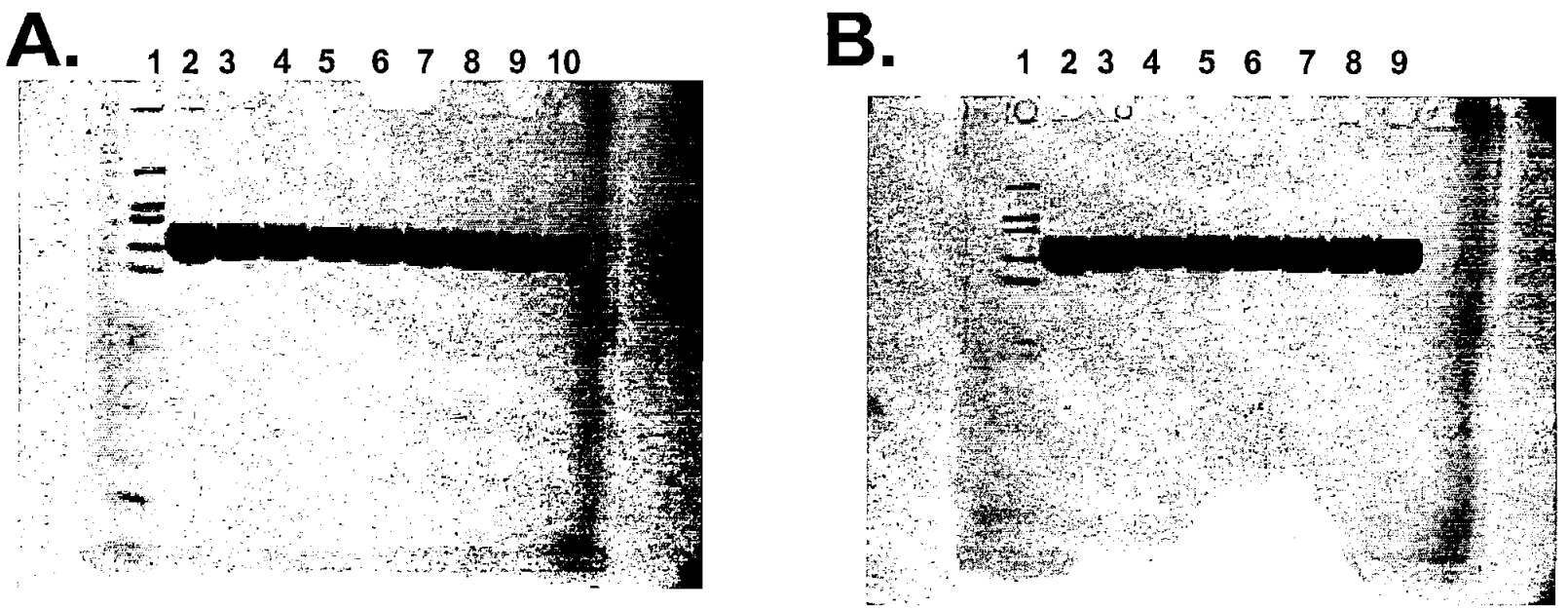


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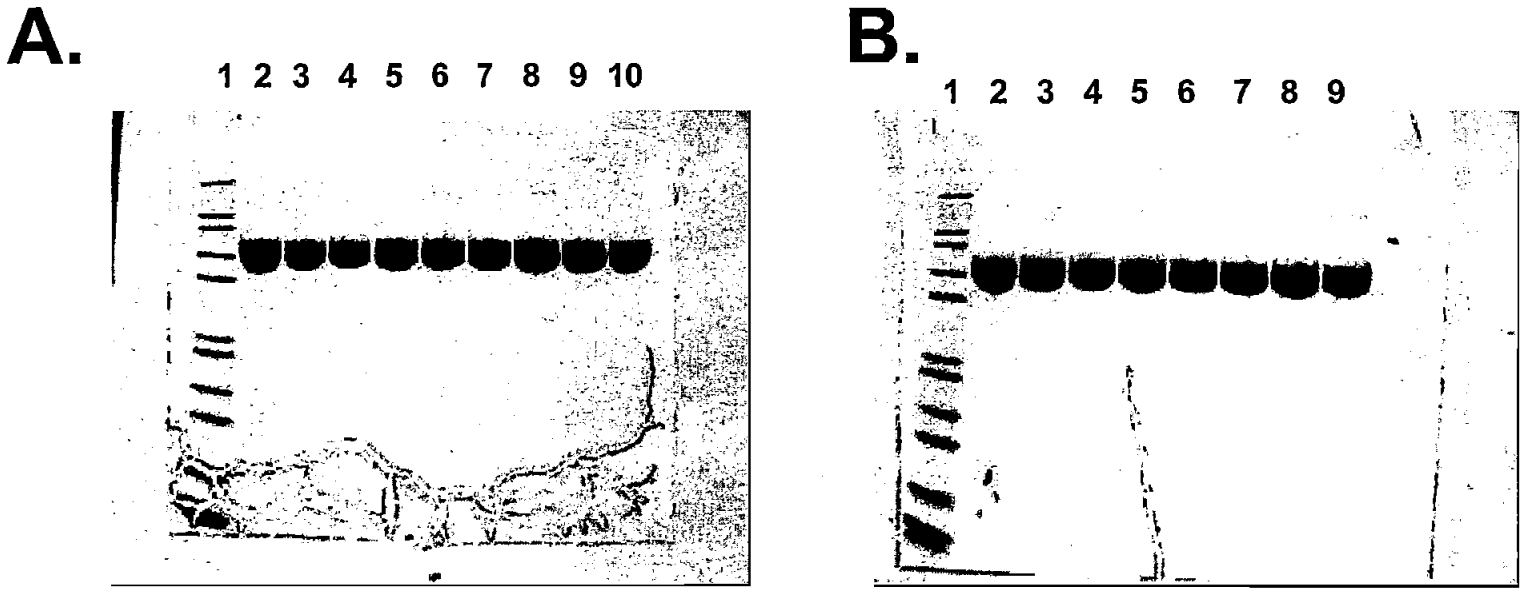


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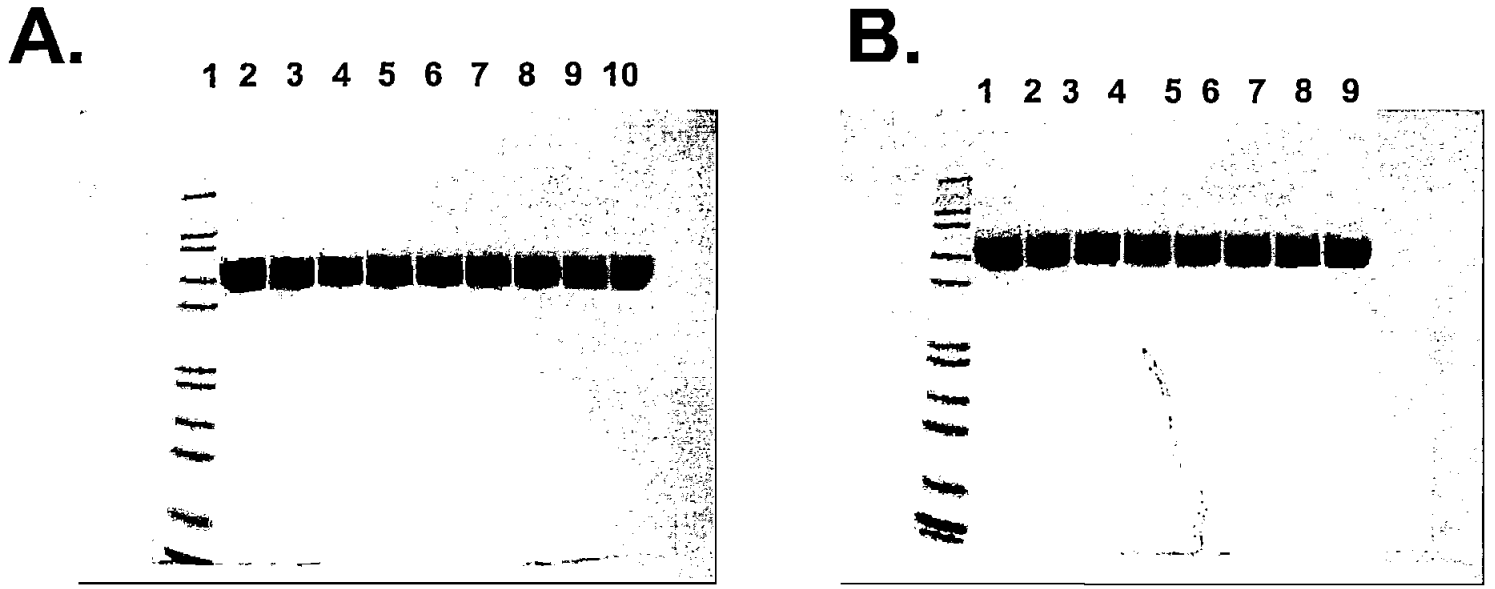


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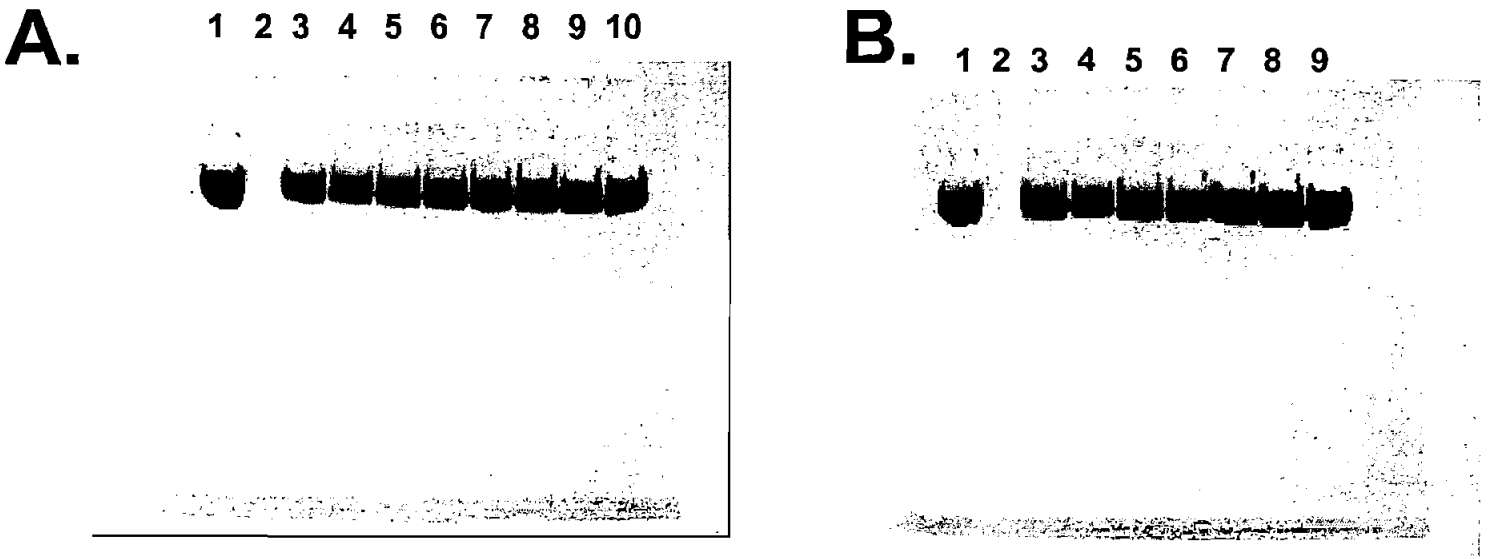


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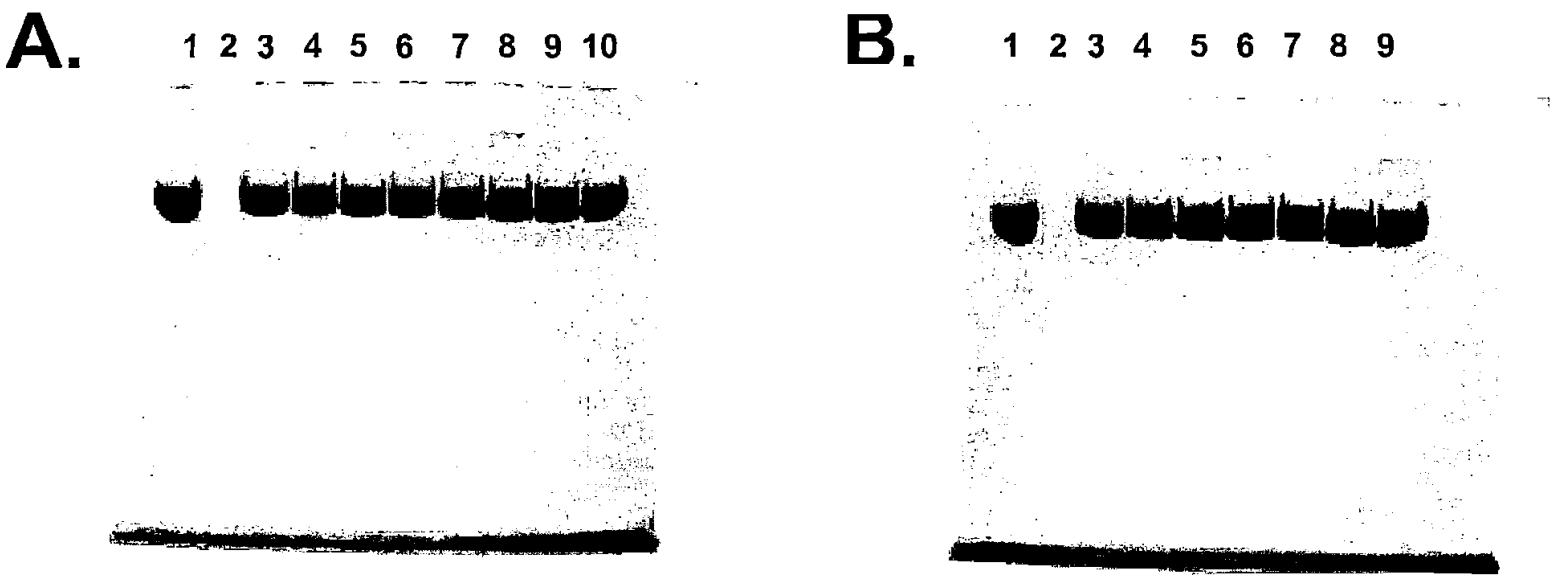


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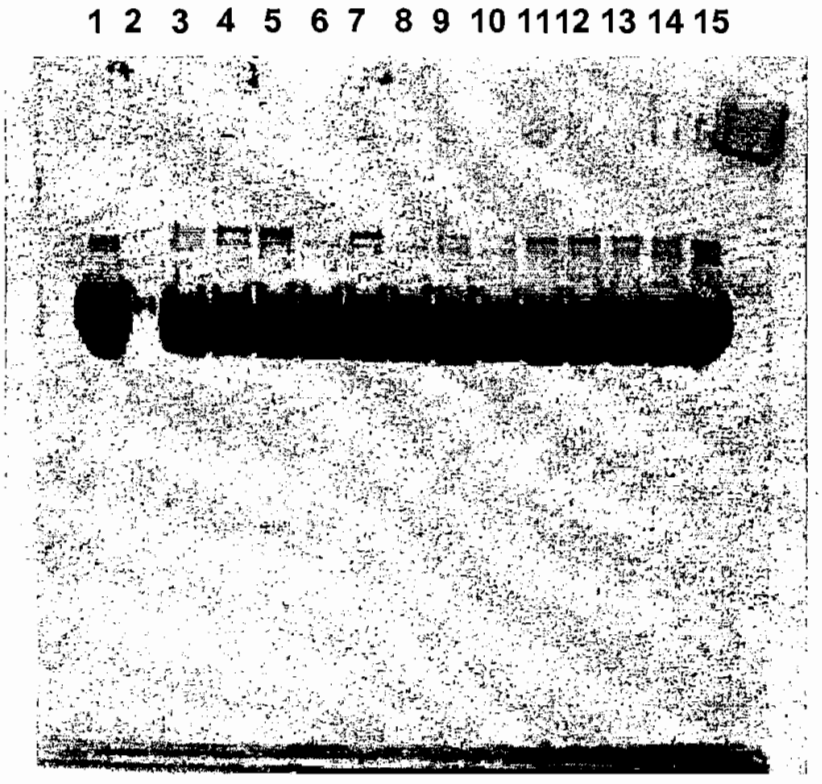


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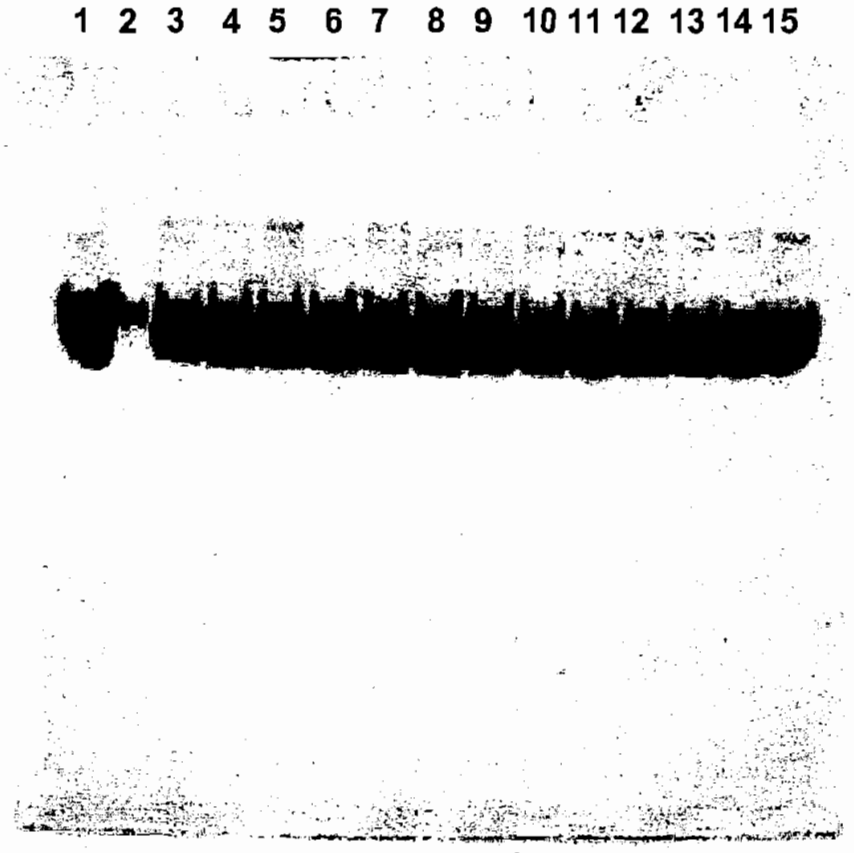


Figure 44

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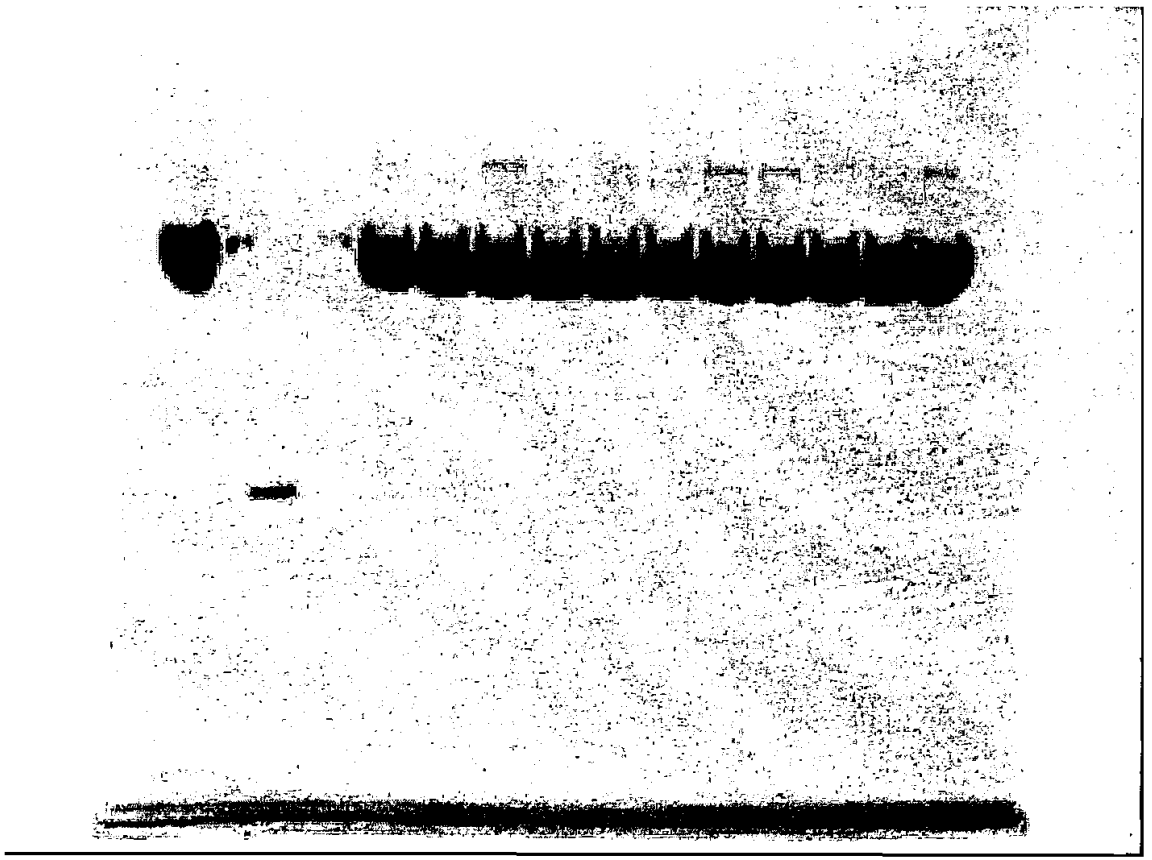


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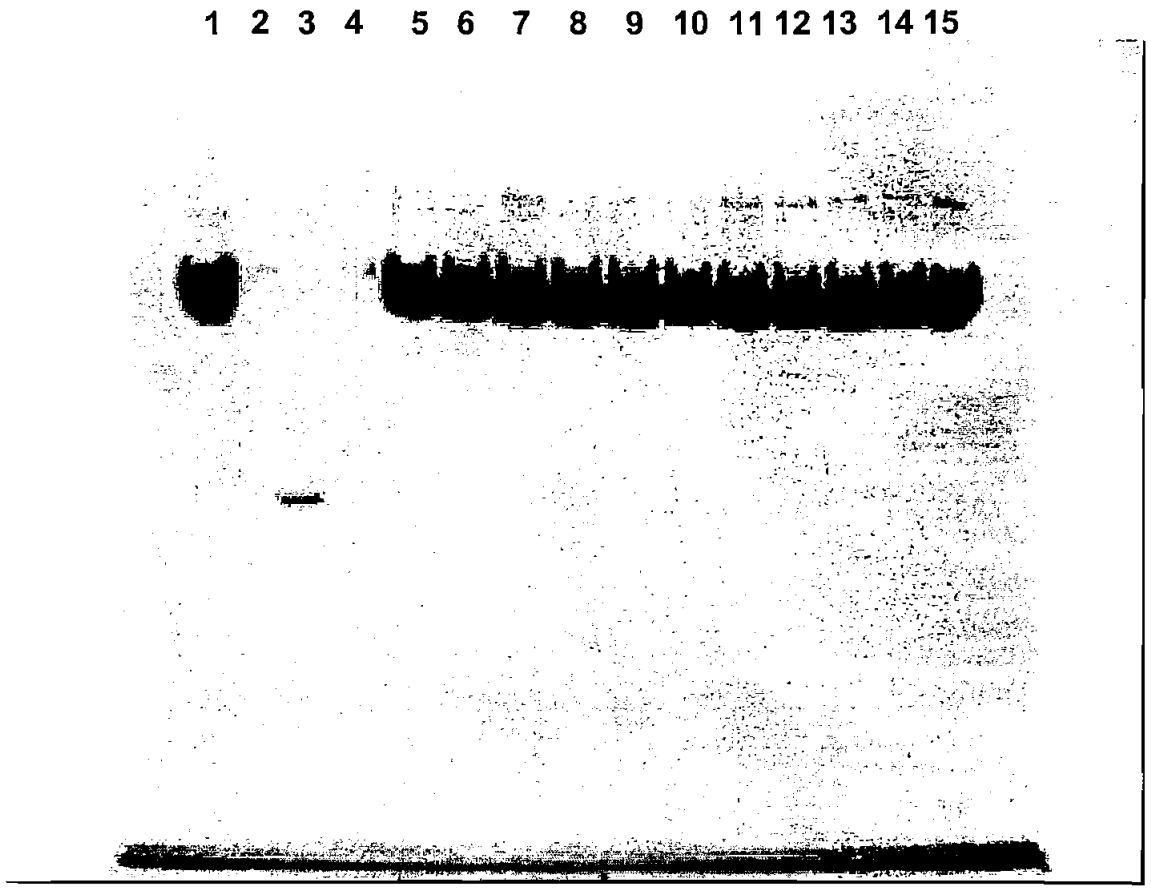


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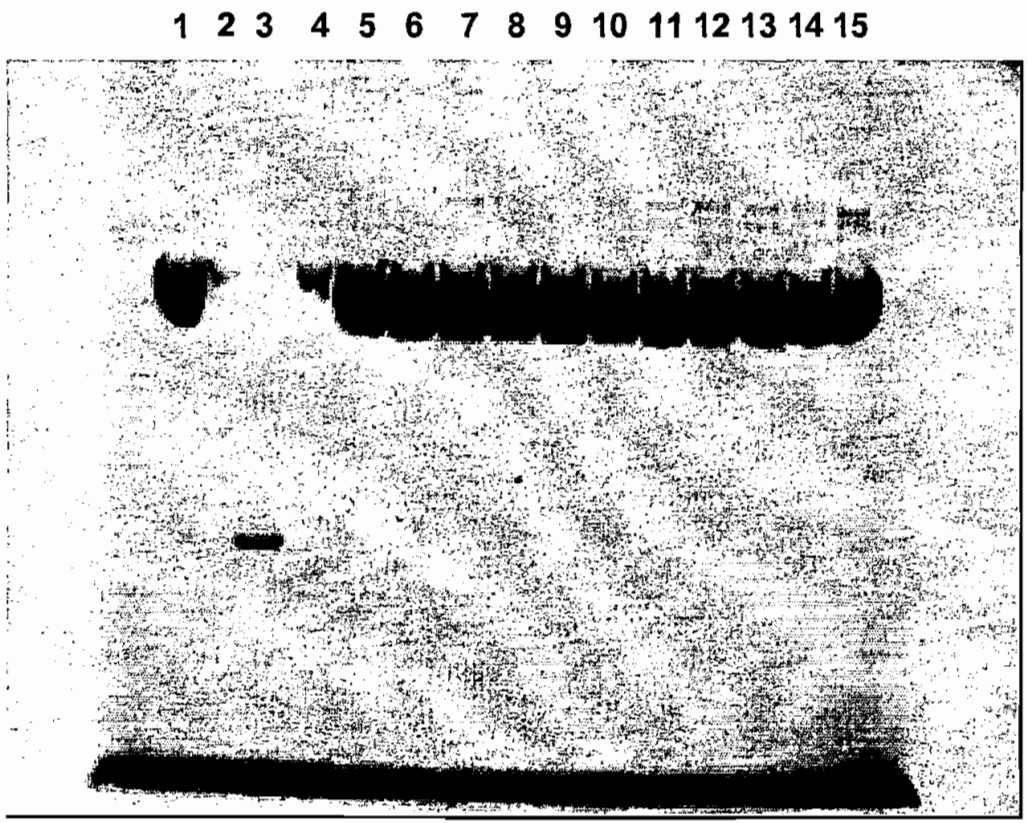


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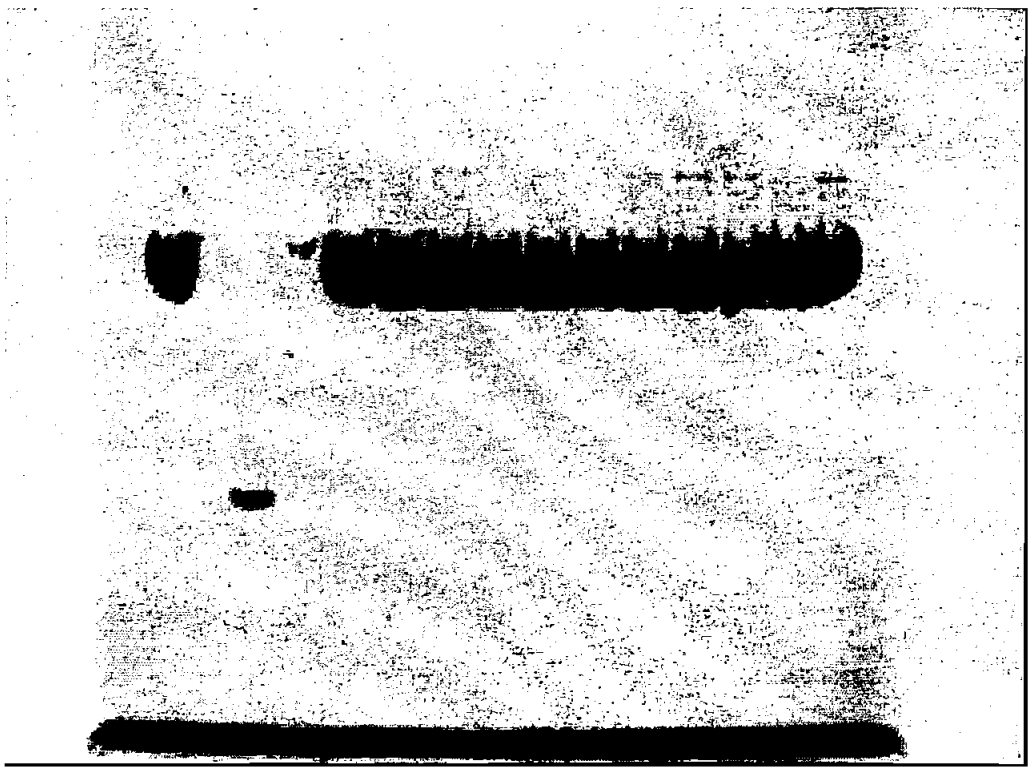


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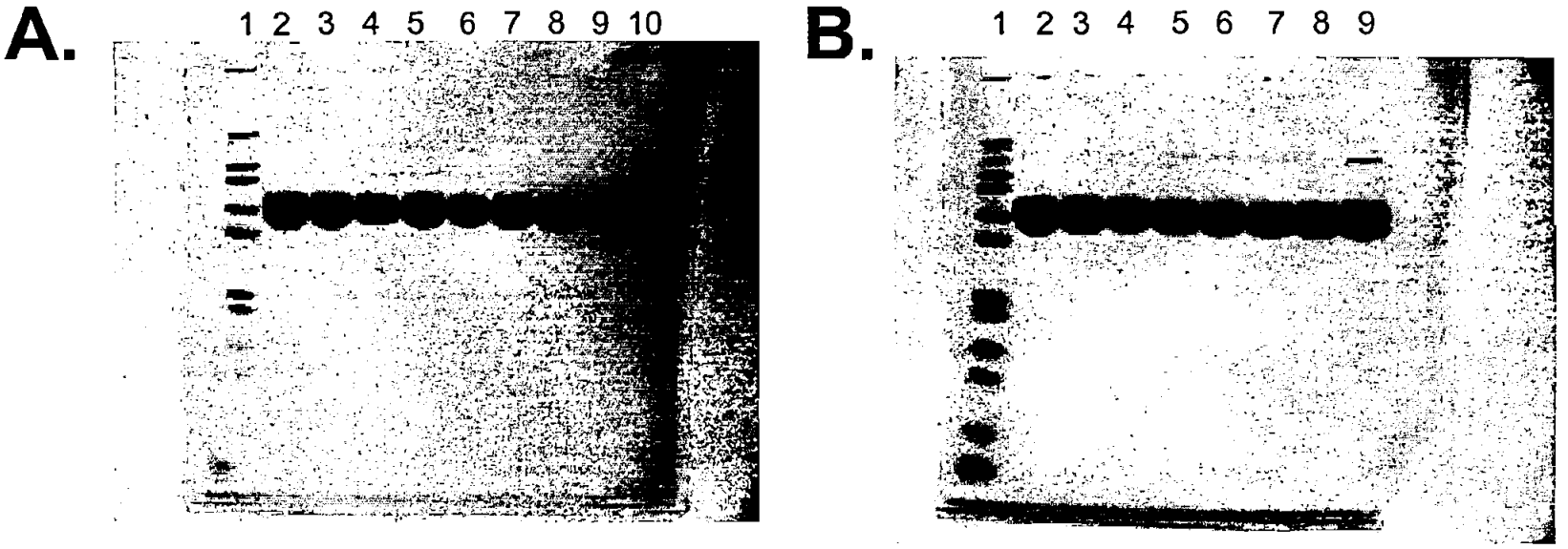


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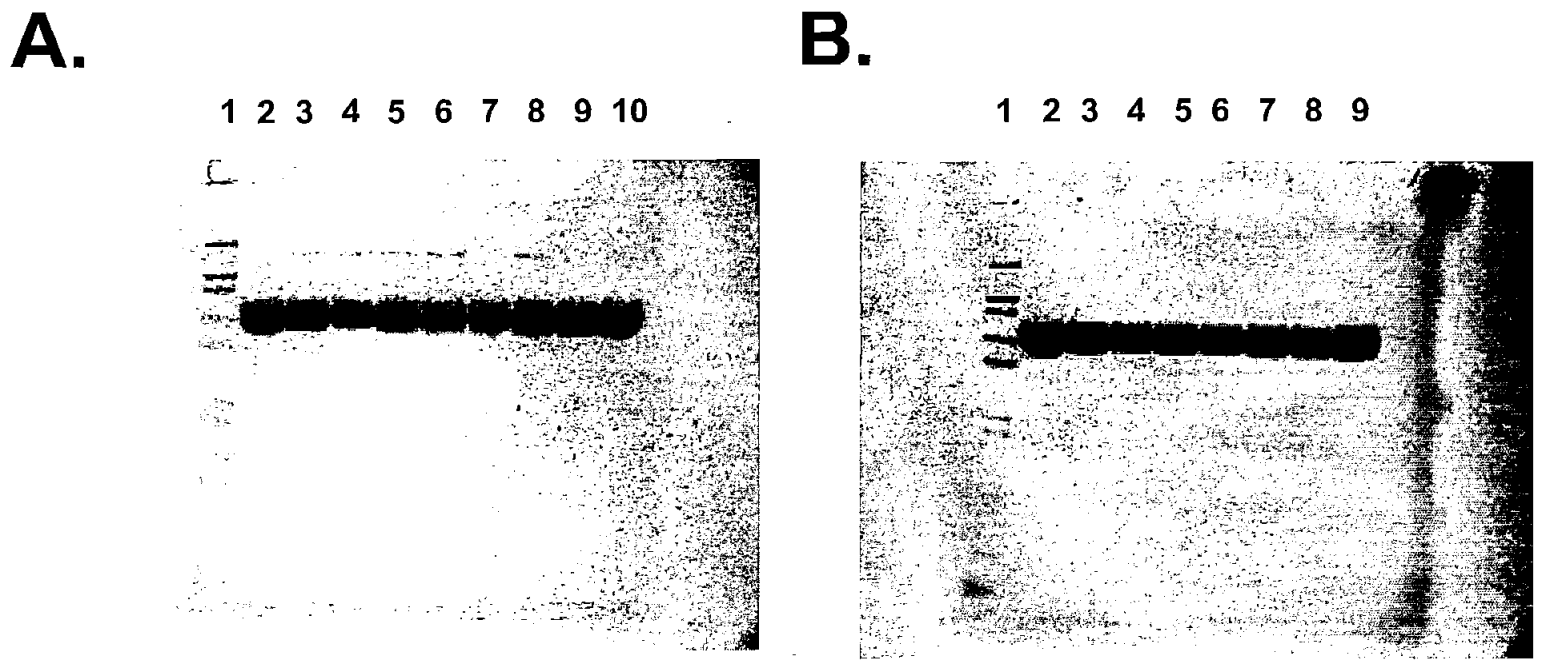


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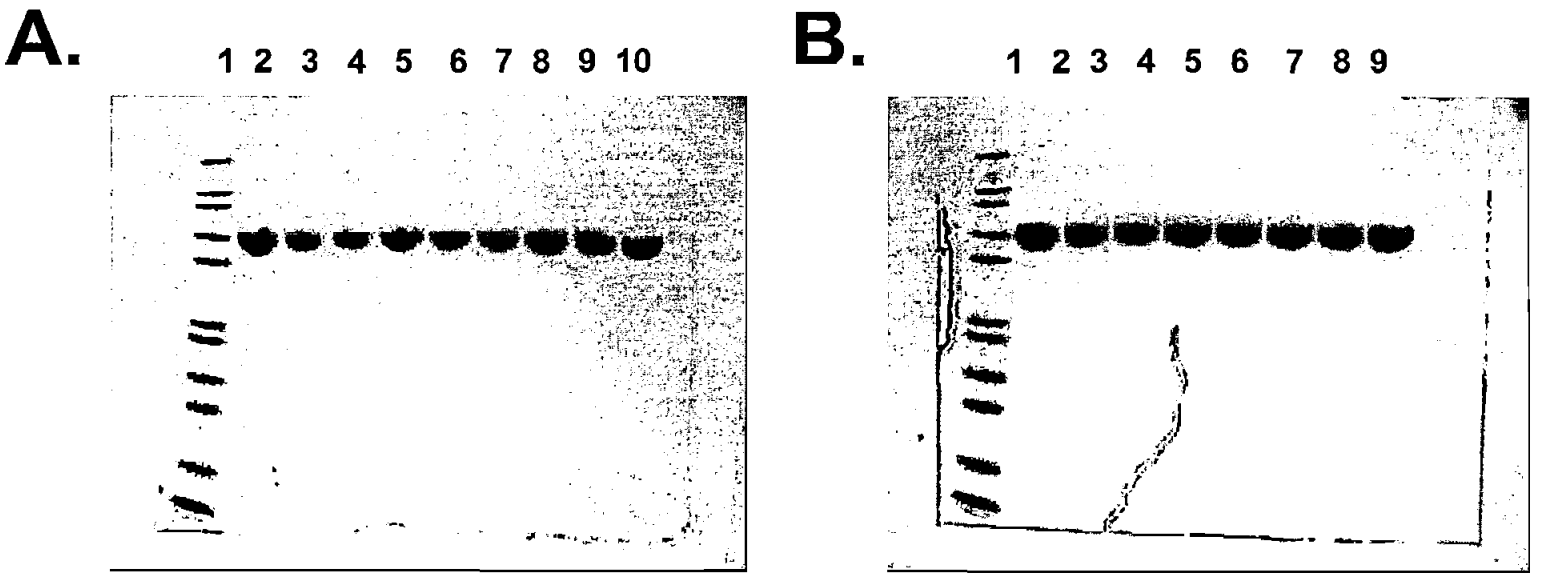


Figure 51

Title: Formulations of Human Growth Hormone Comprising a Non-Naturally Encoded Amino Acid		
Inventors: Anna-Maria Hays et al.	Appl. No.: Not Assigned	
Docket Number: AMBX-0077.00PCT	Atty: John W. Wallen, III	Telephone: (858) 875-2403

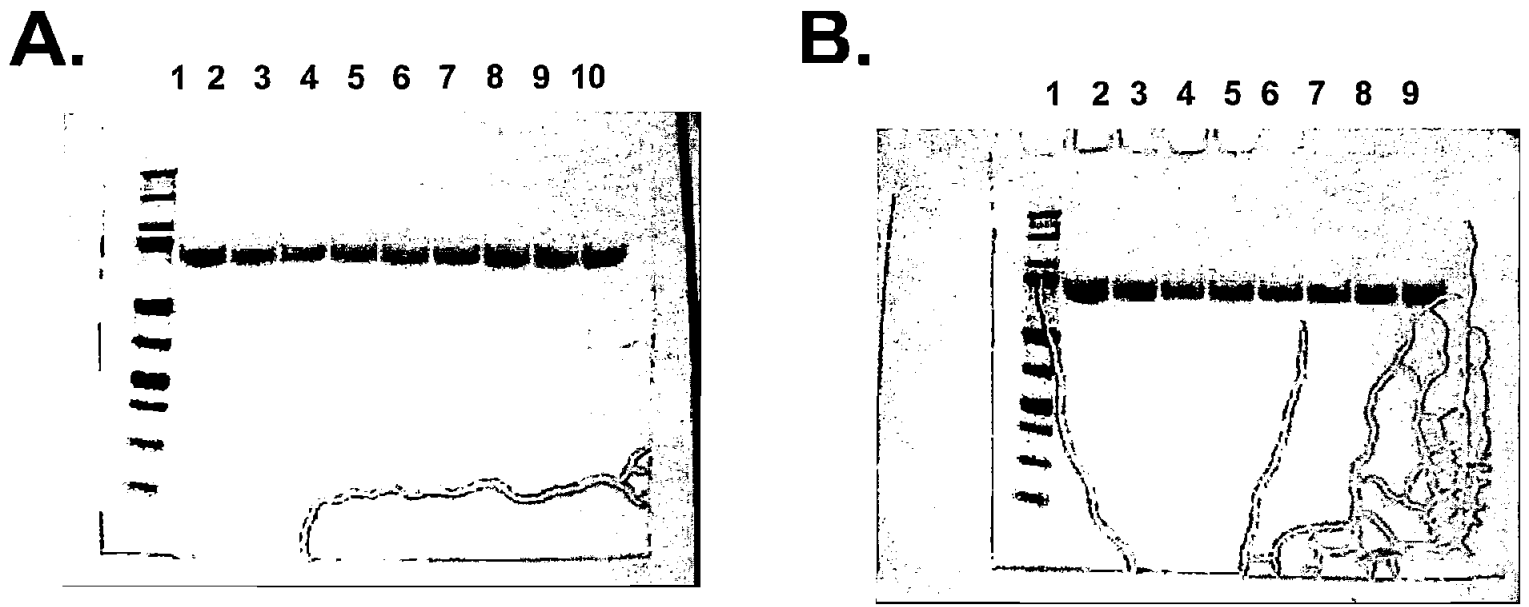


Figure 52

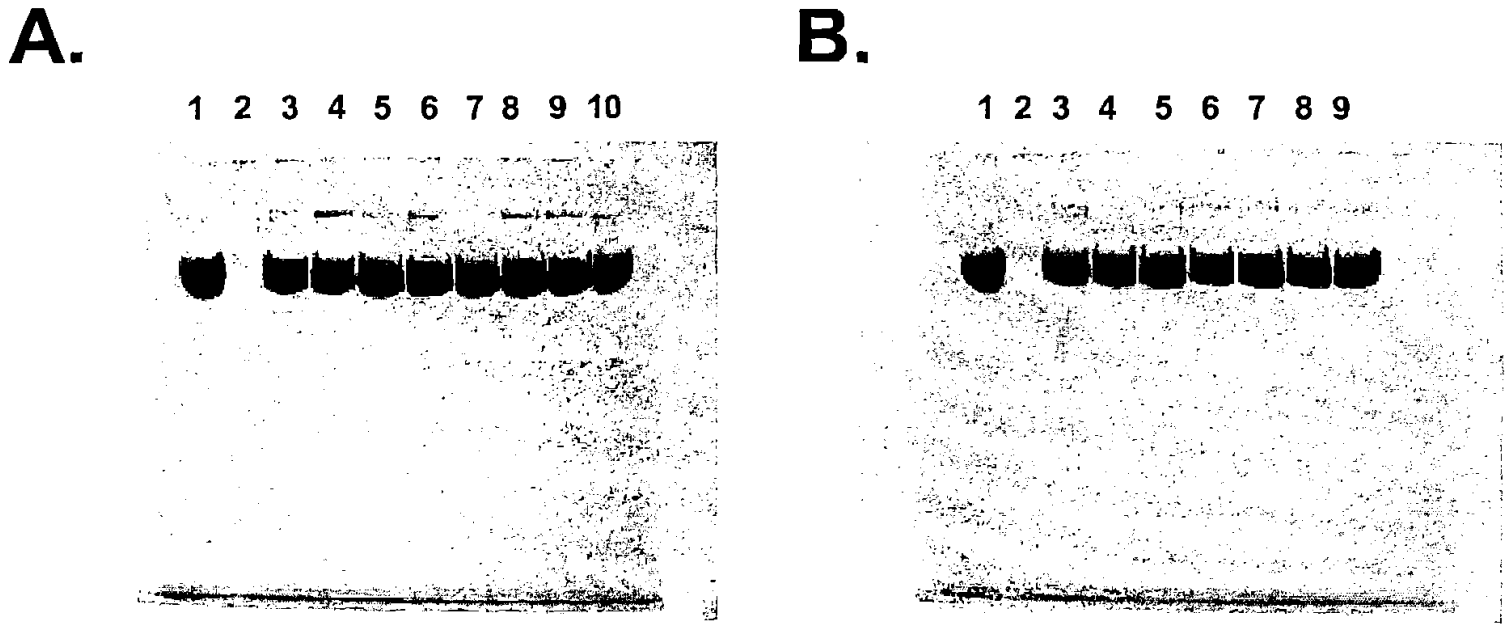


Figure 53

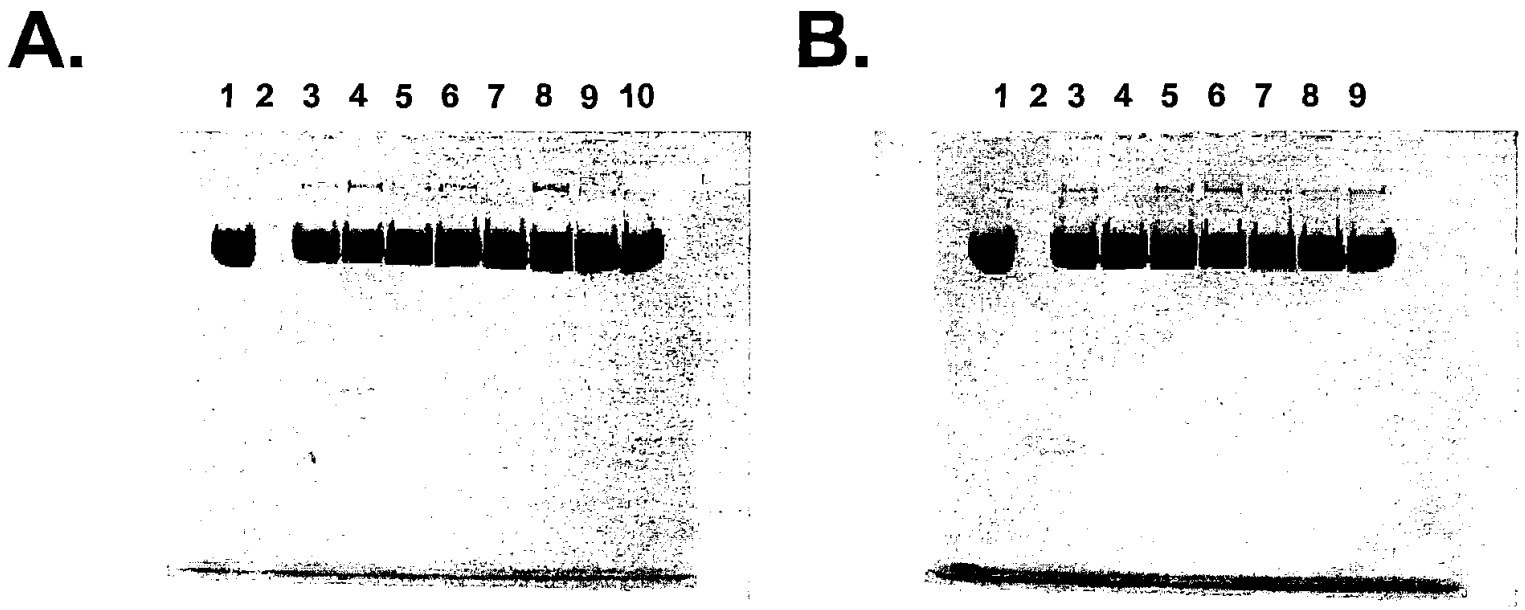


Figure 54

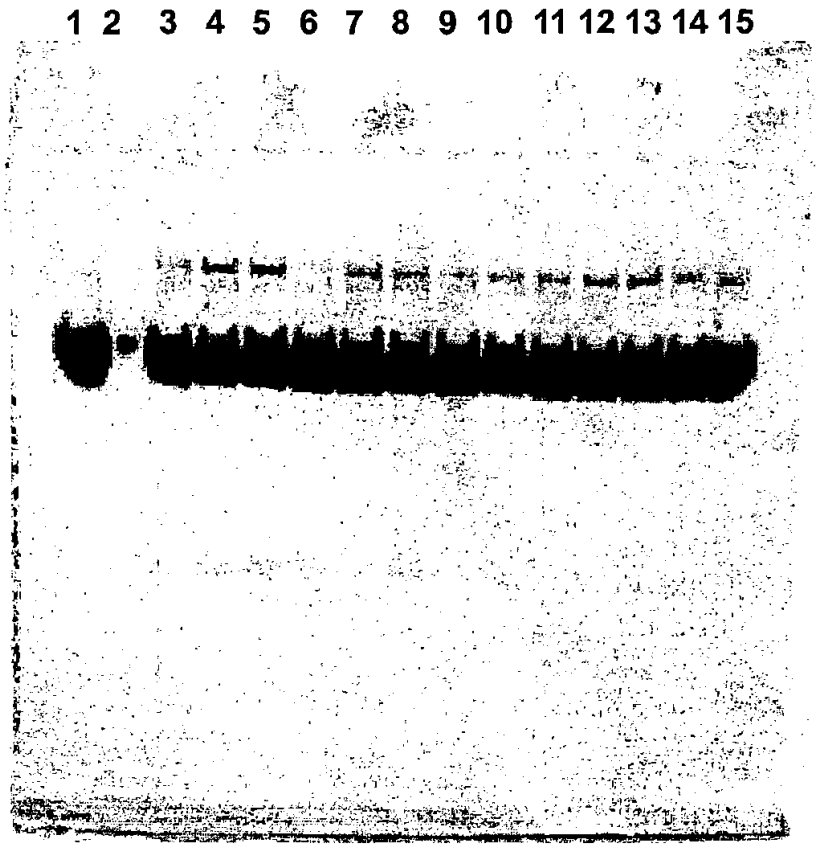


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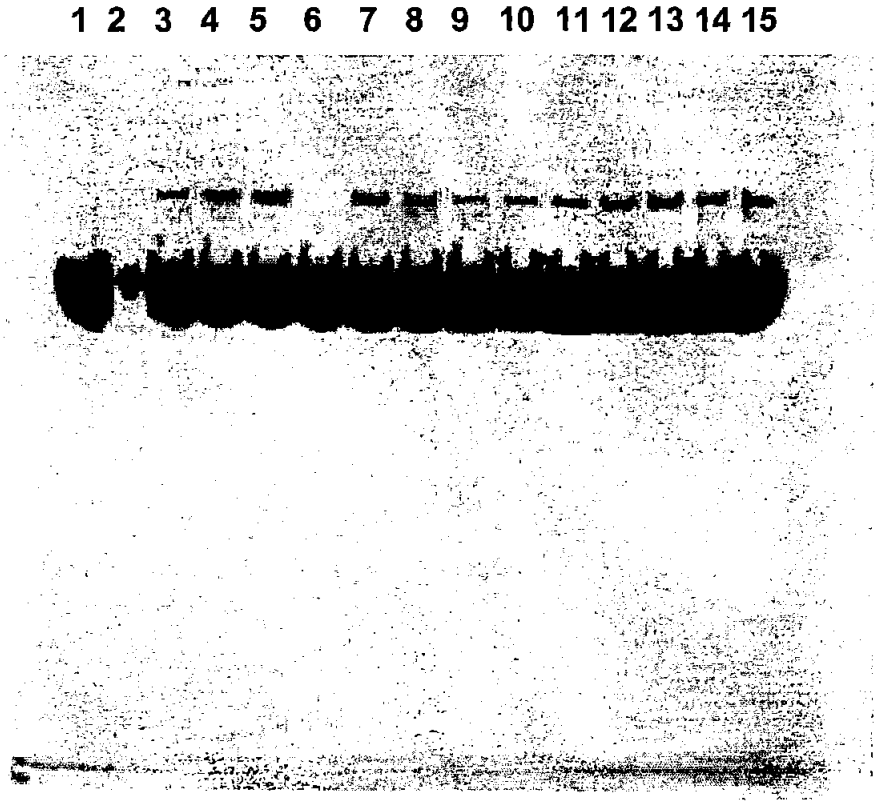


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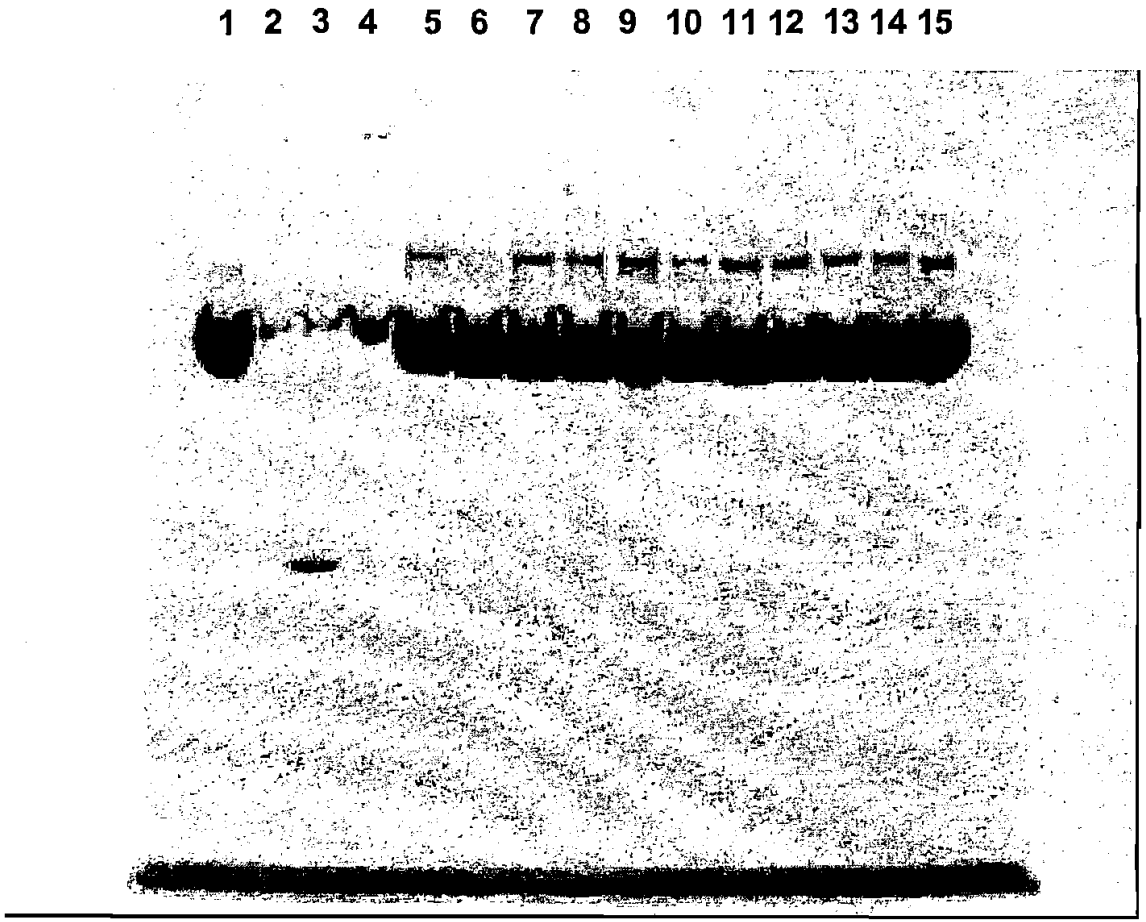


Figure 57

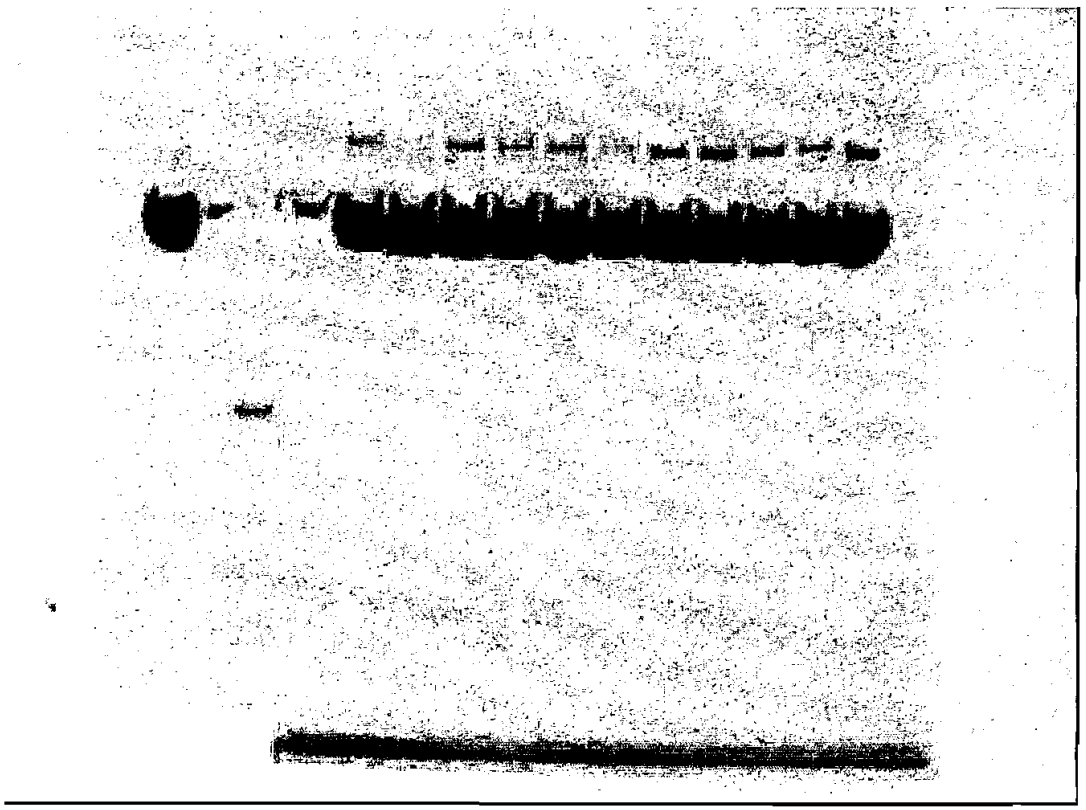


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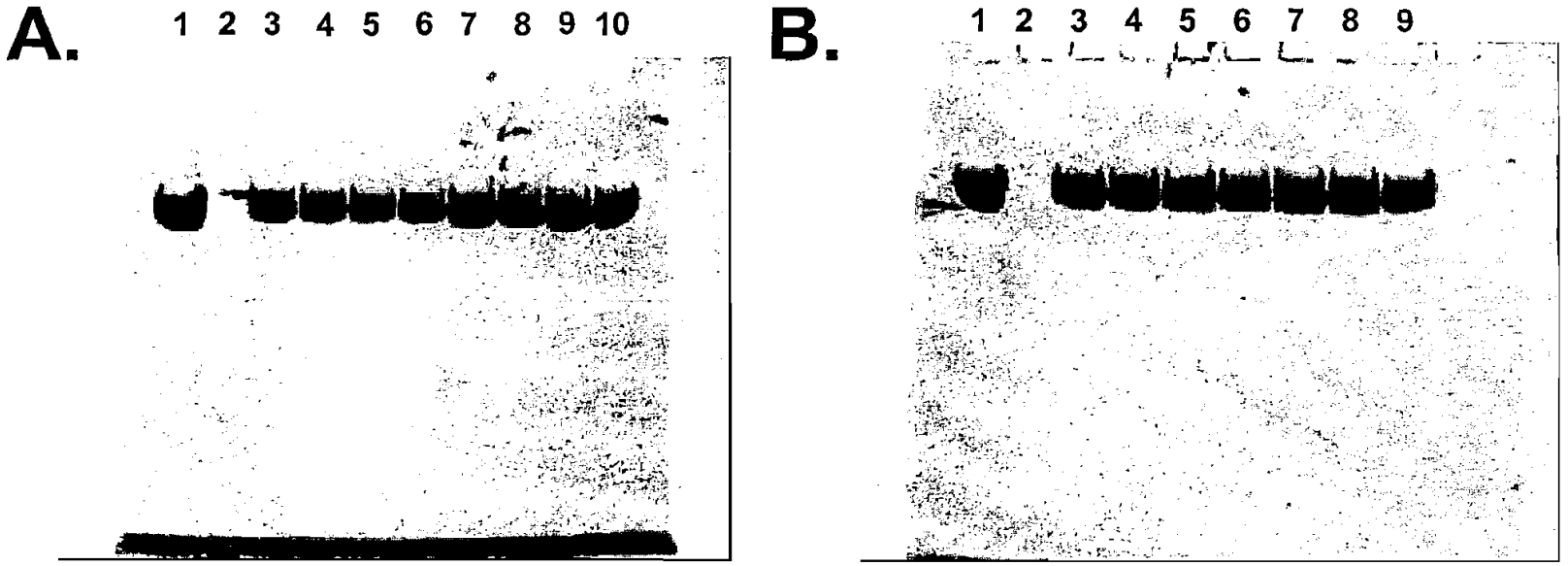


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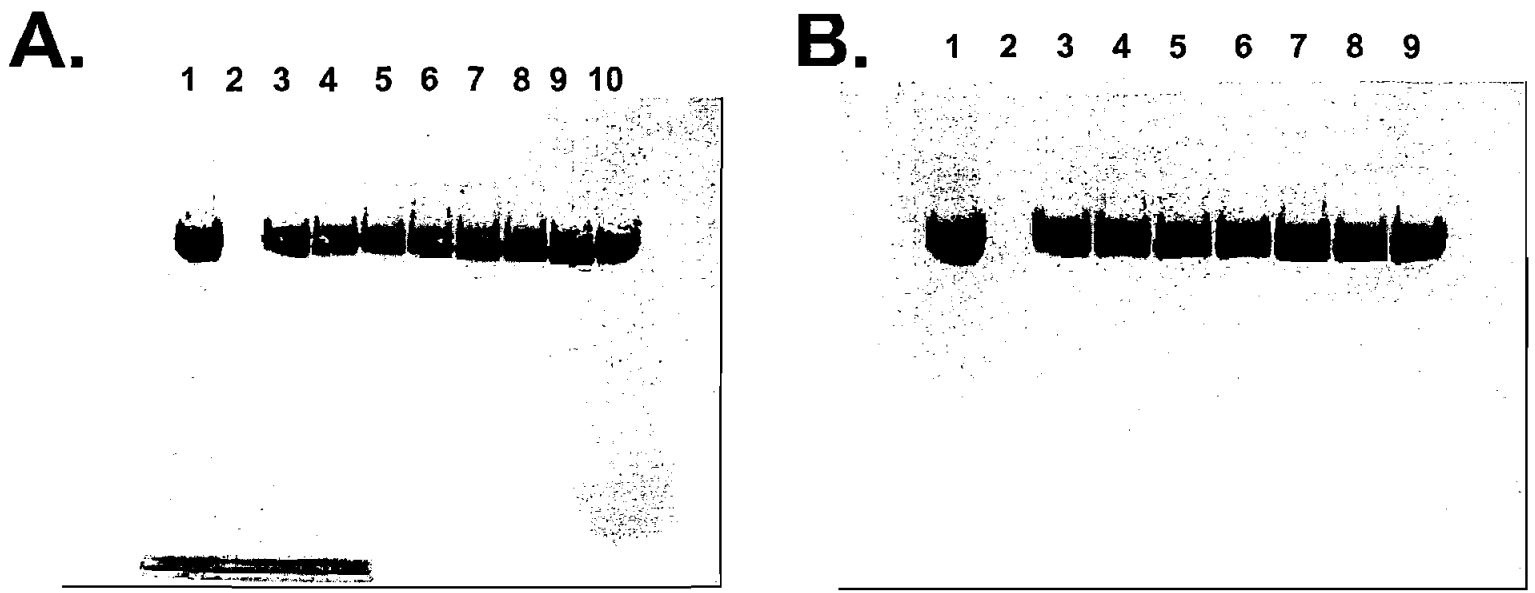


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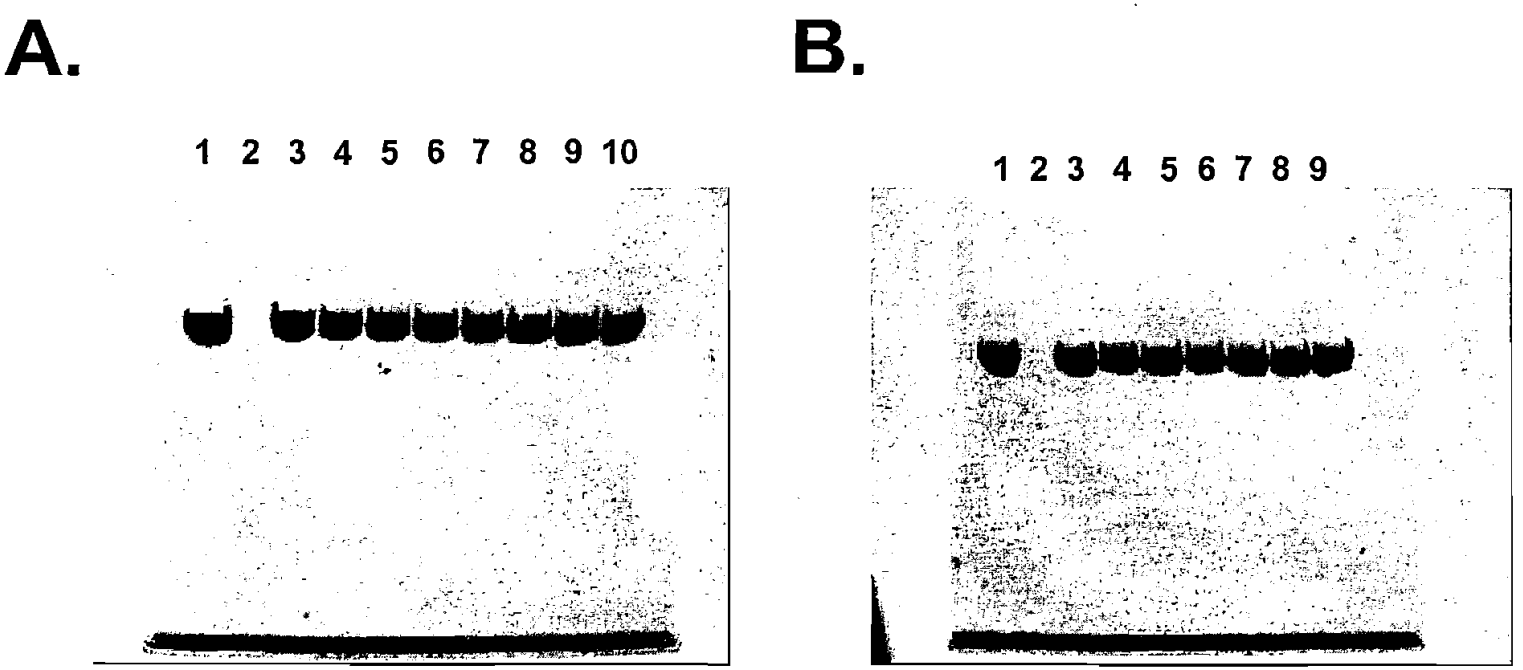


Figure 61

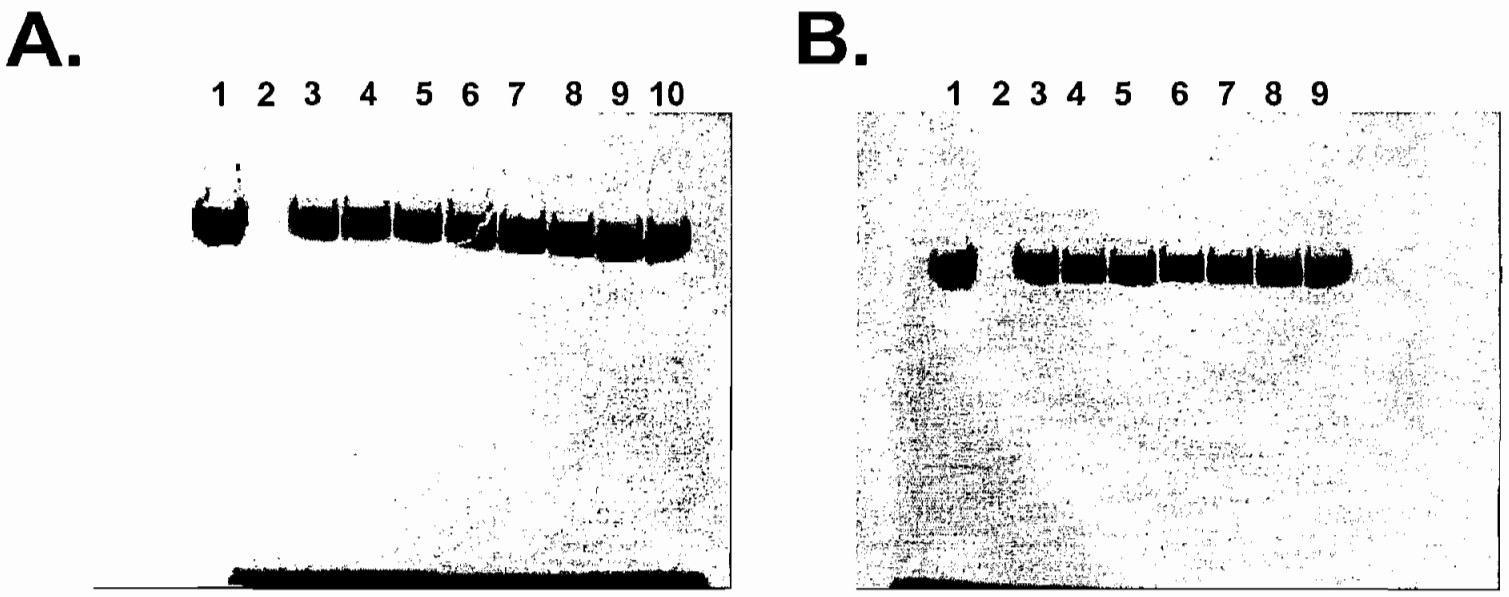


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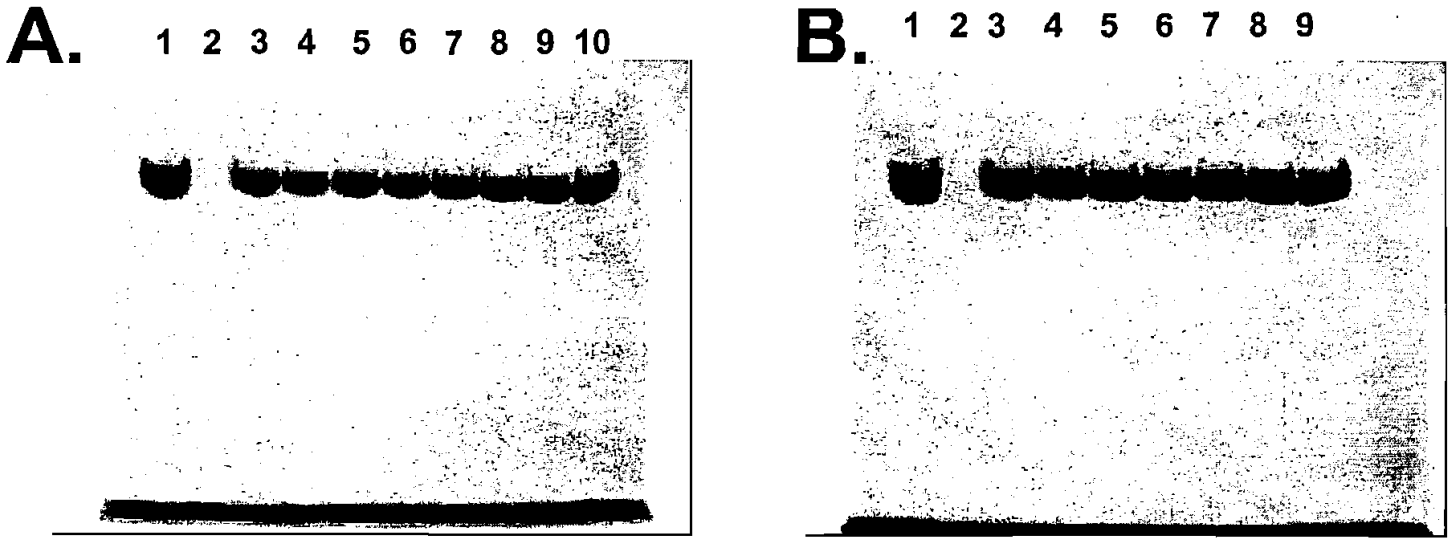


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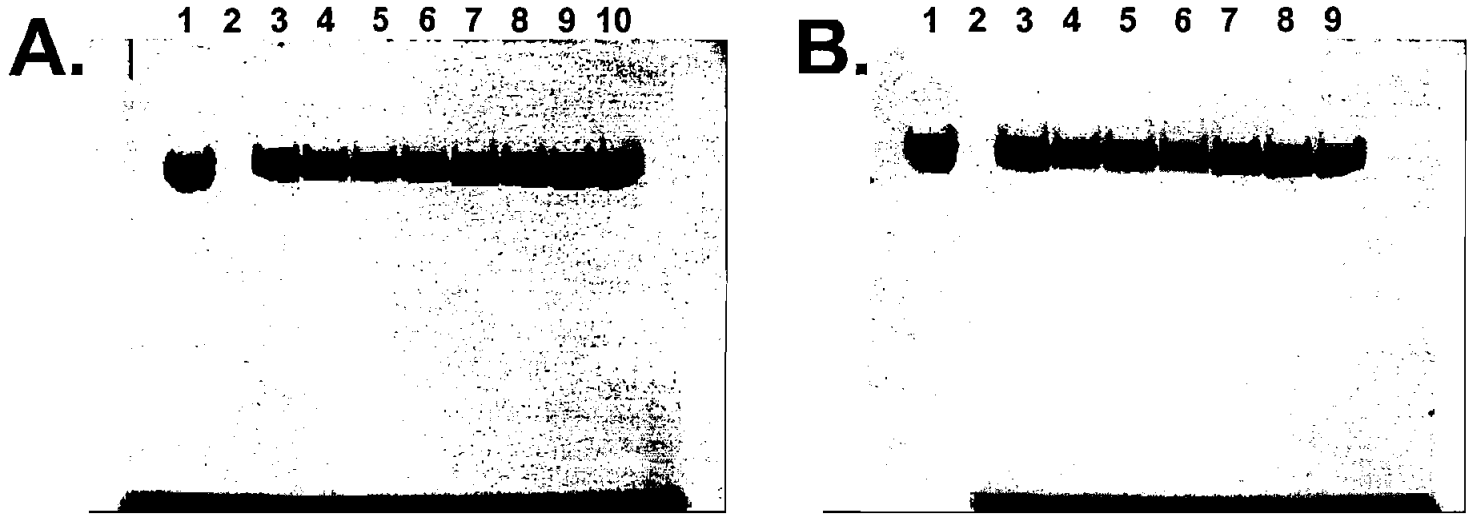


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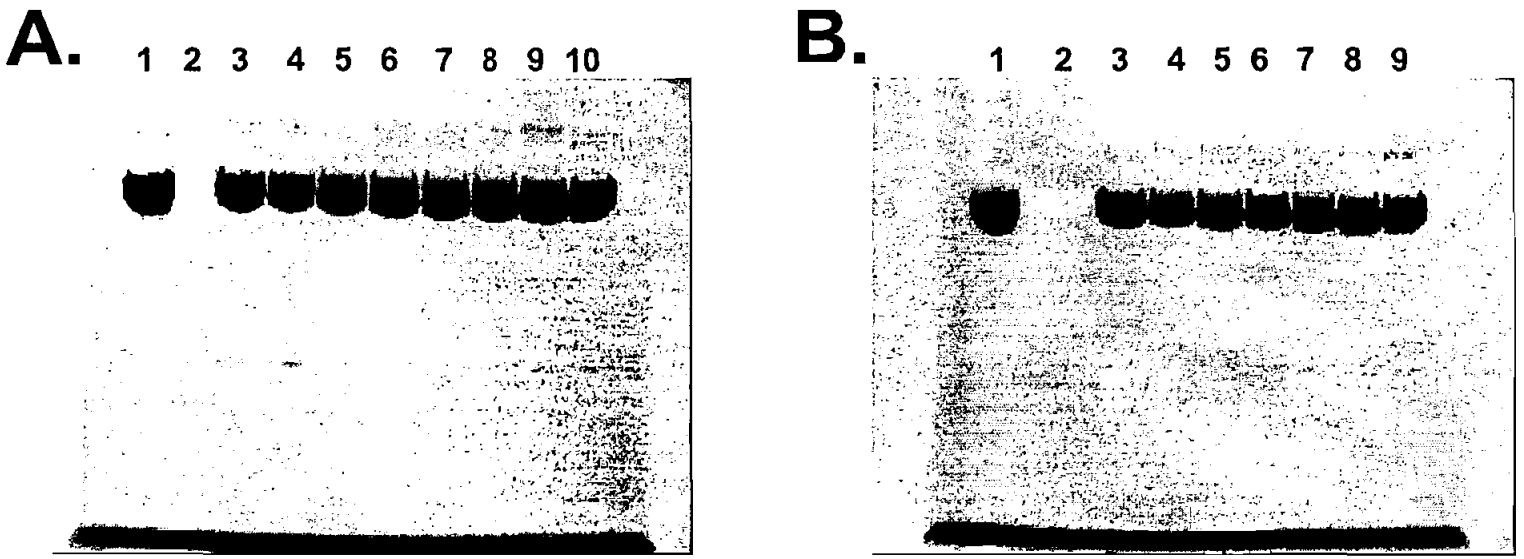


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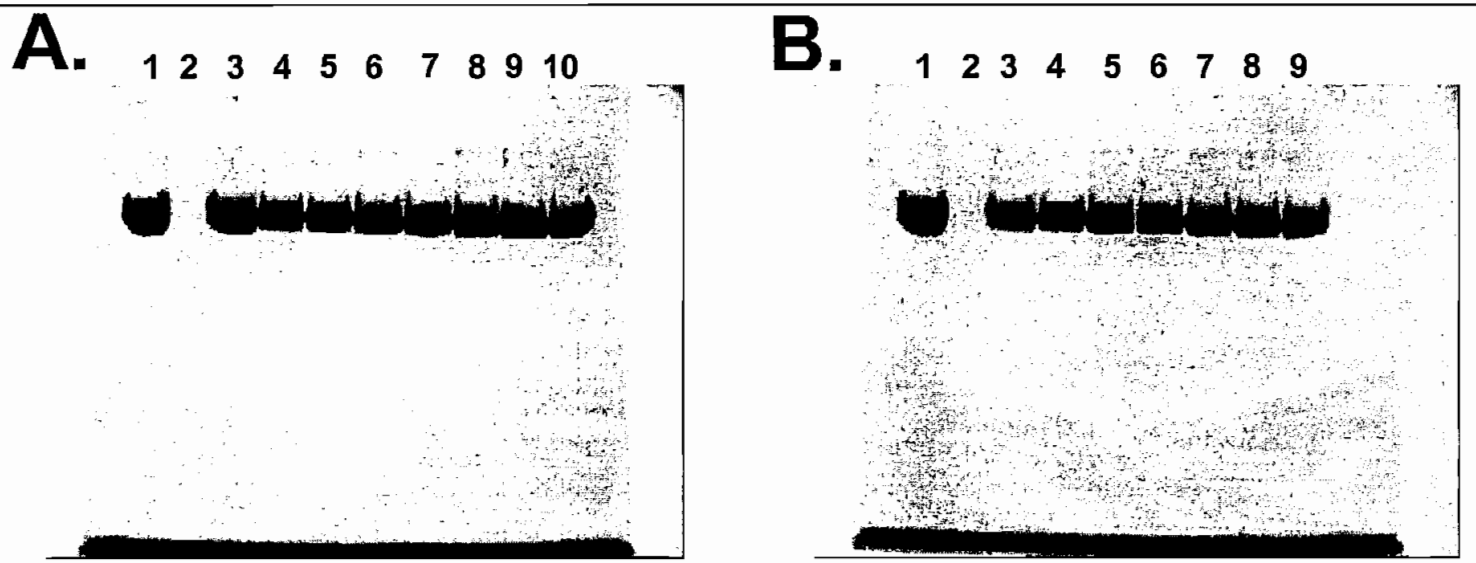


Figure 66

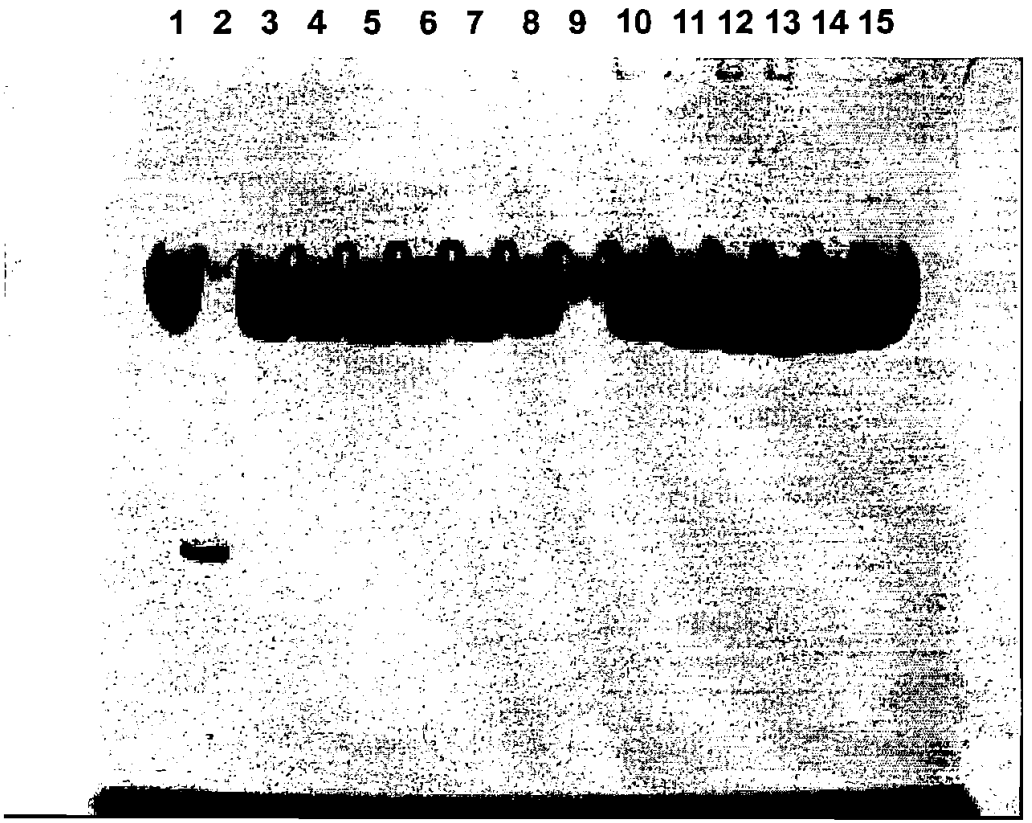


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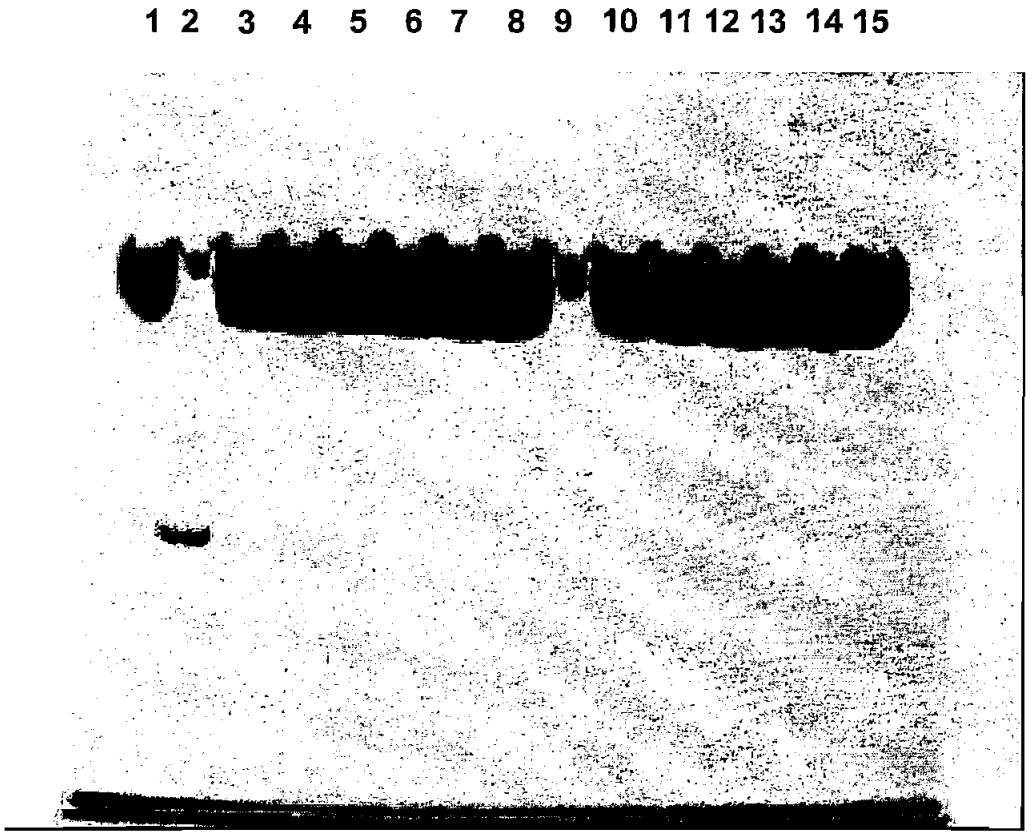


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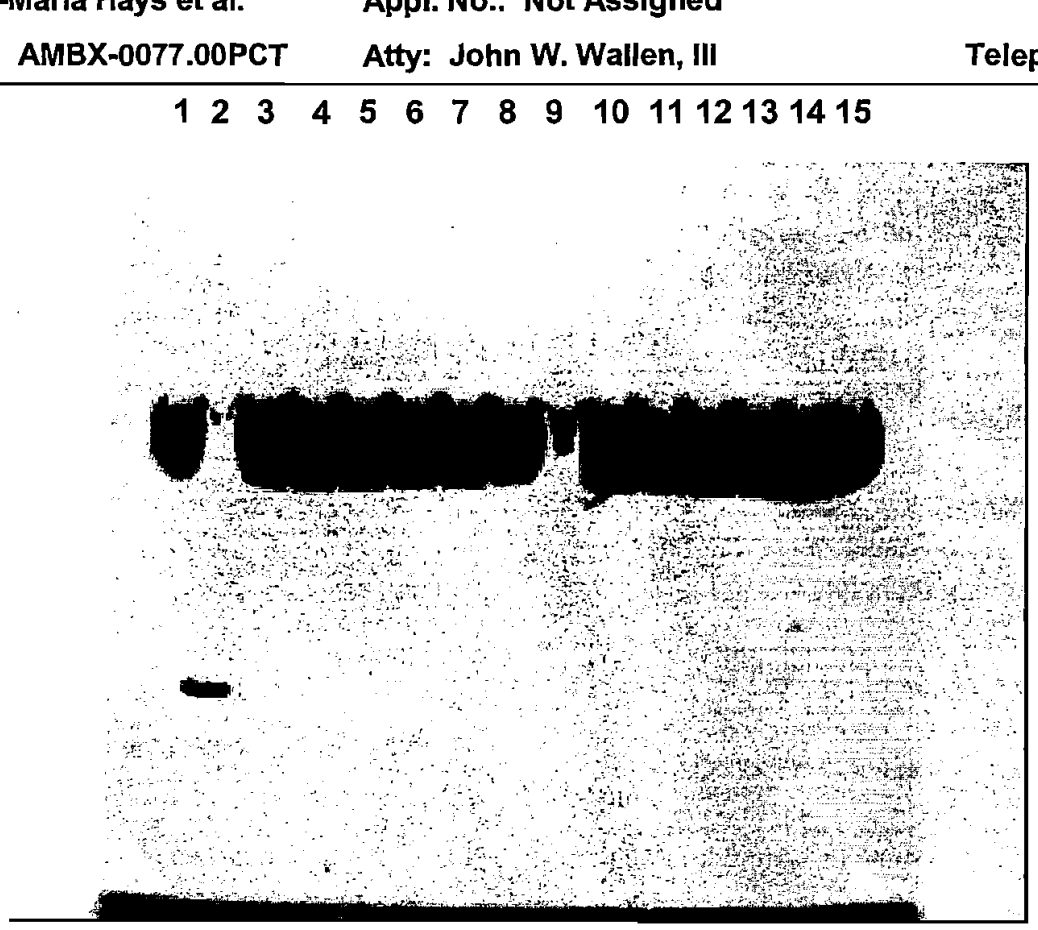


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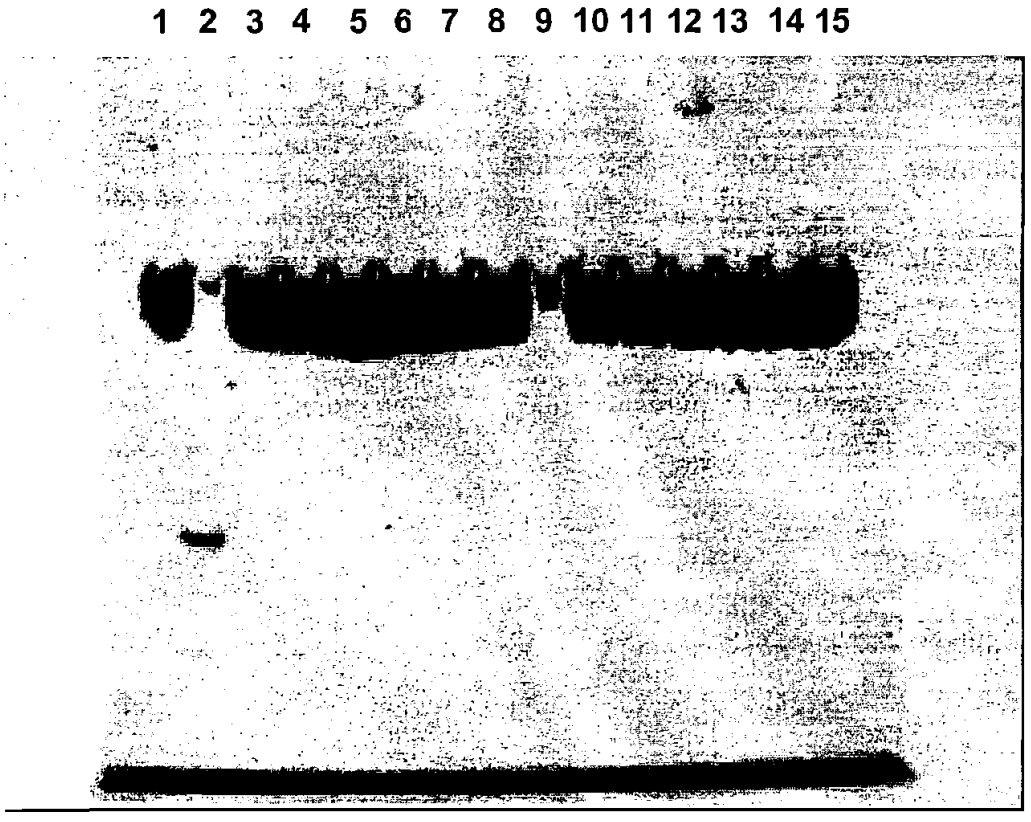


Figure 70

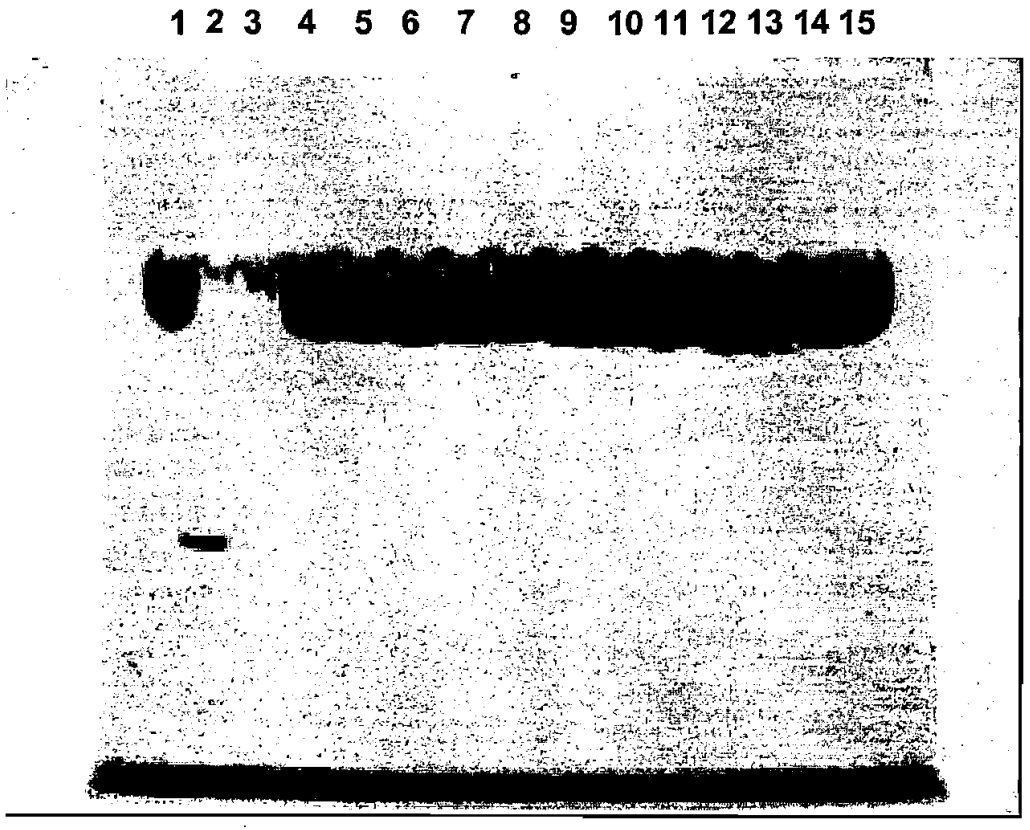


Figure 71

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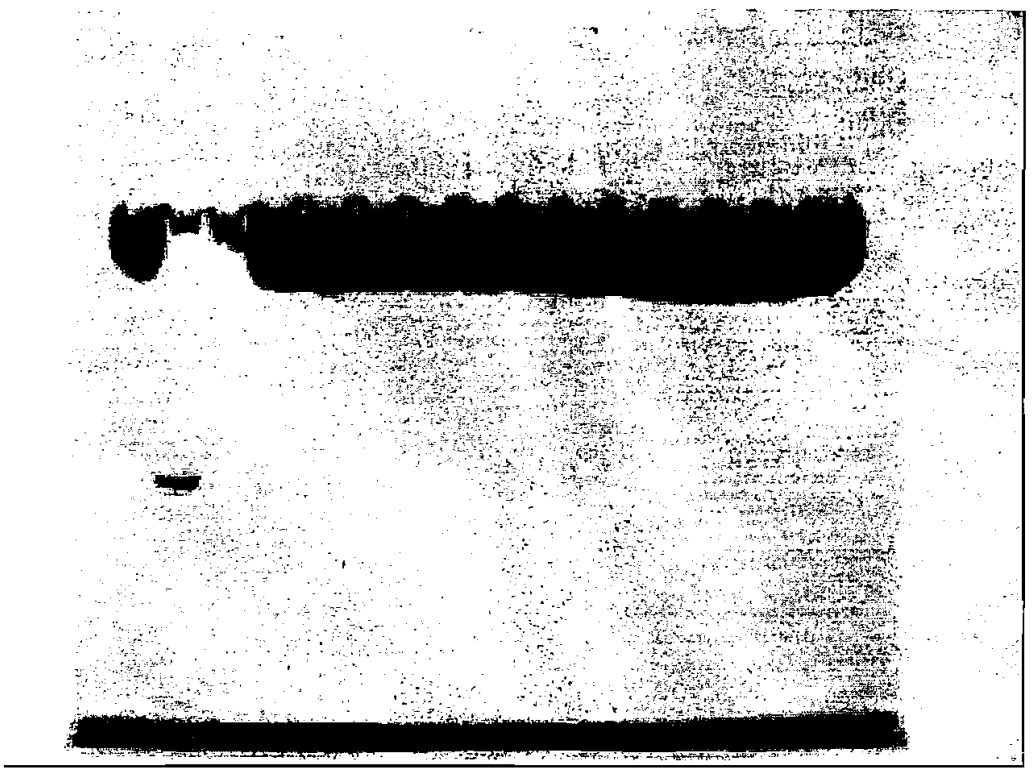


Figure 72

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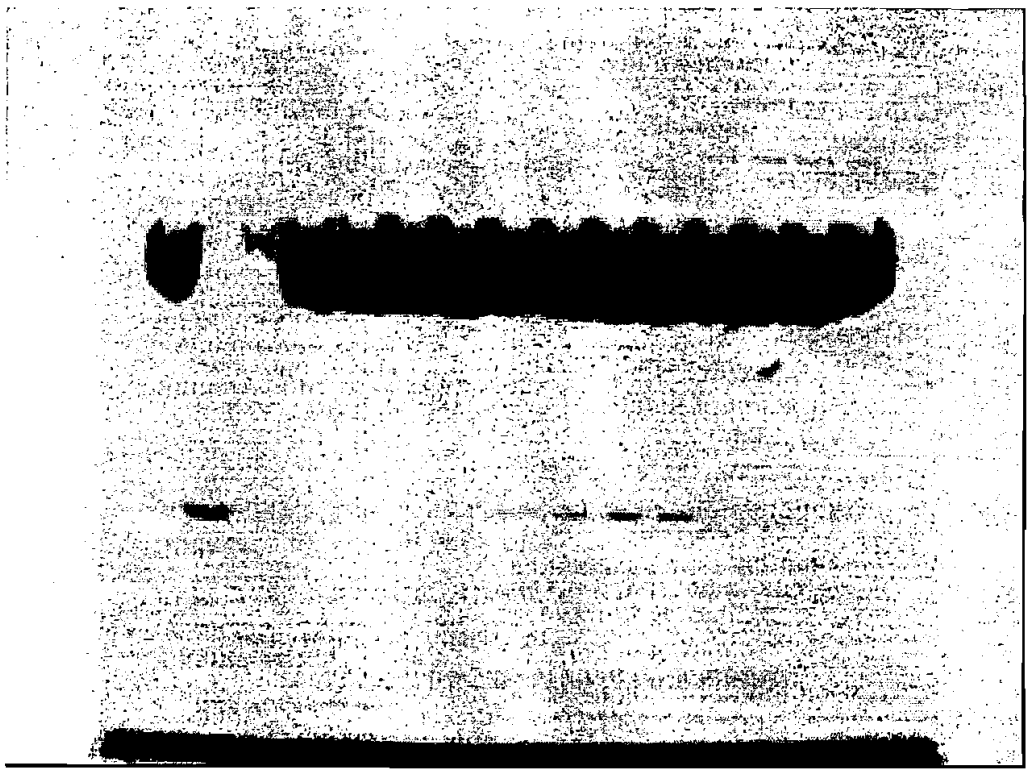


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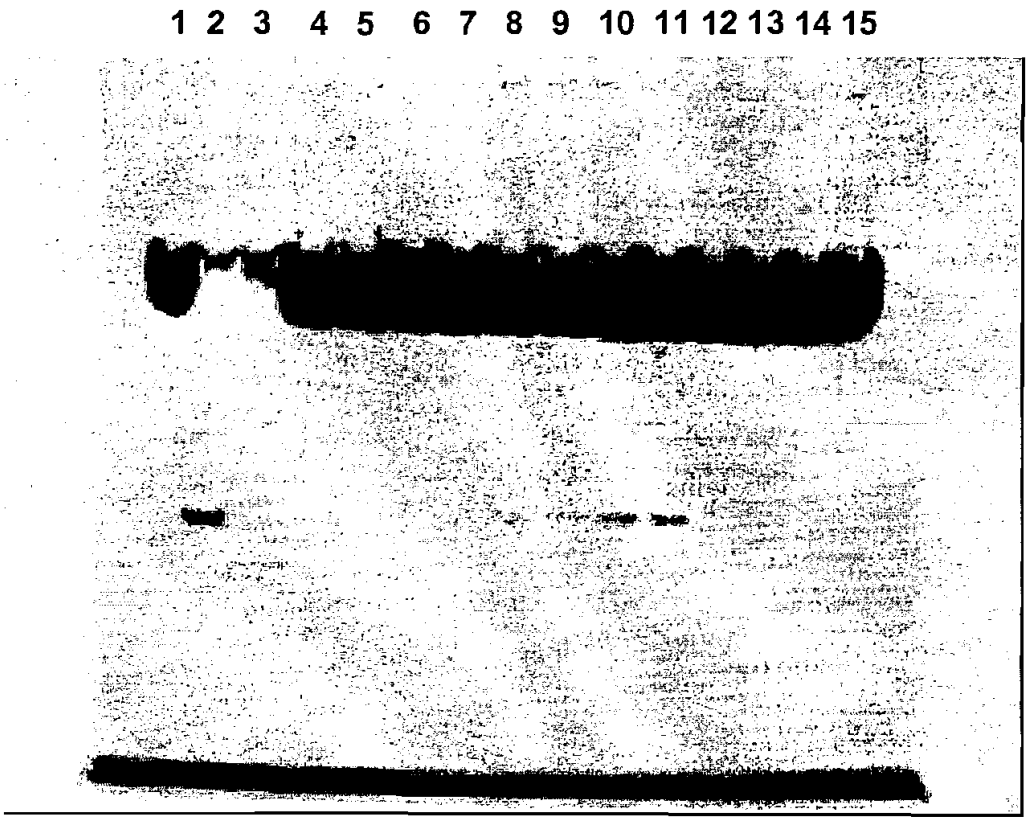


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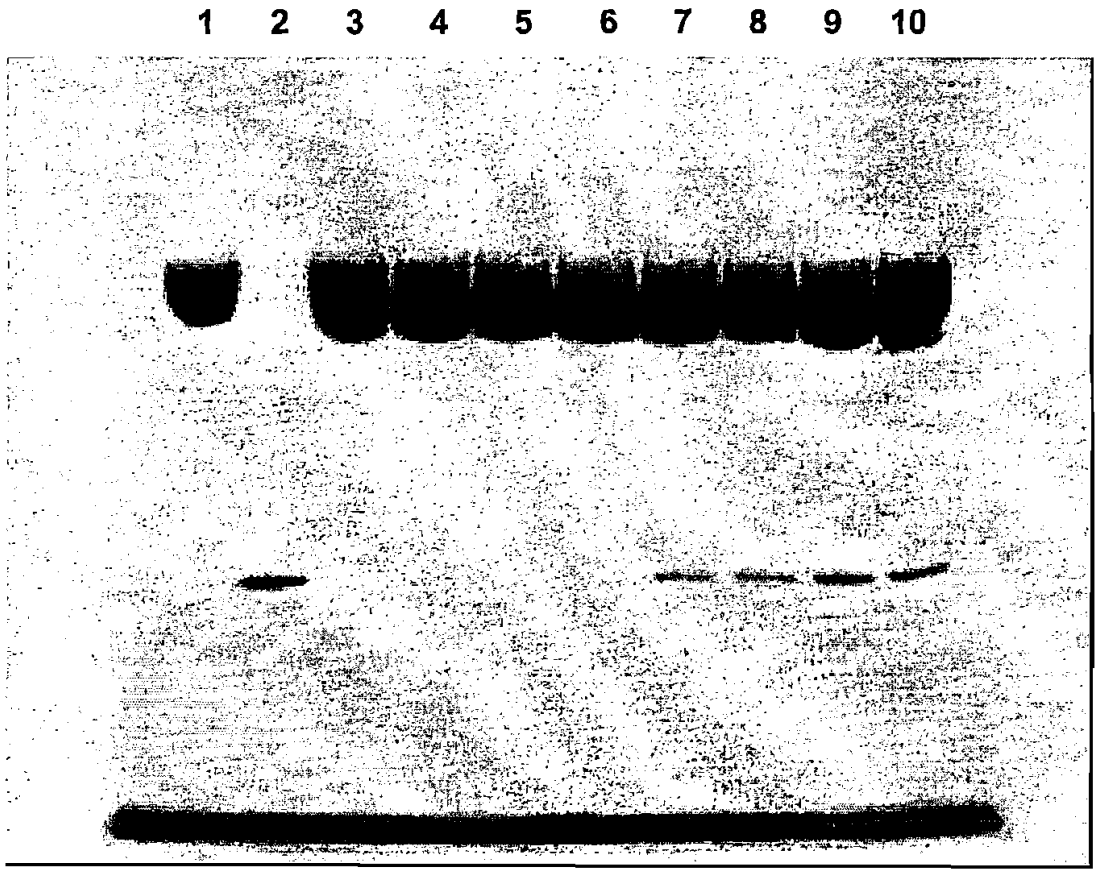


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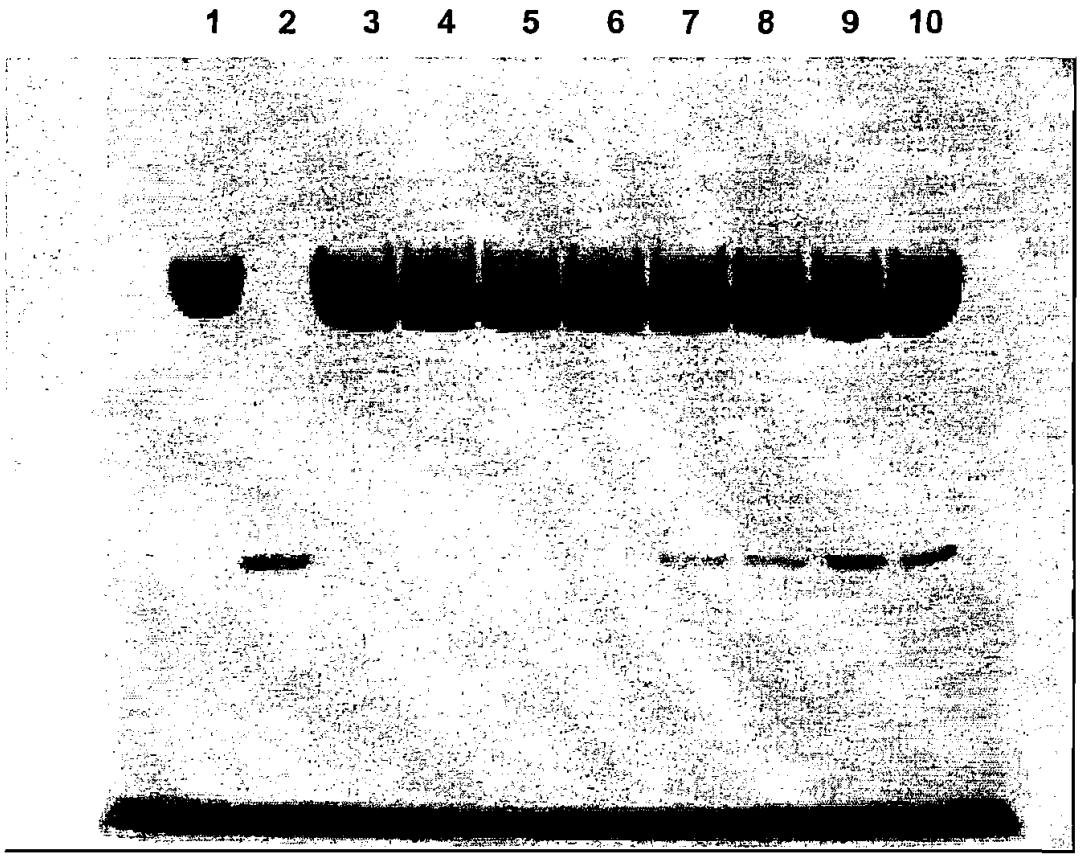


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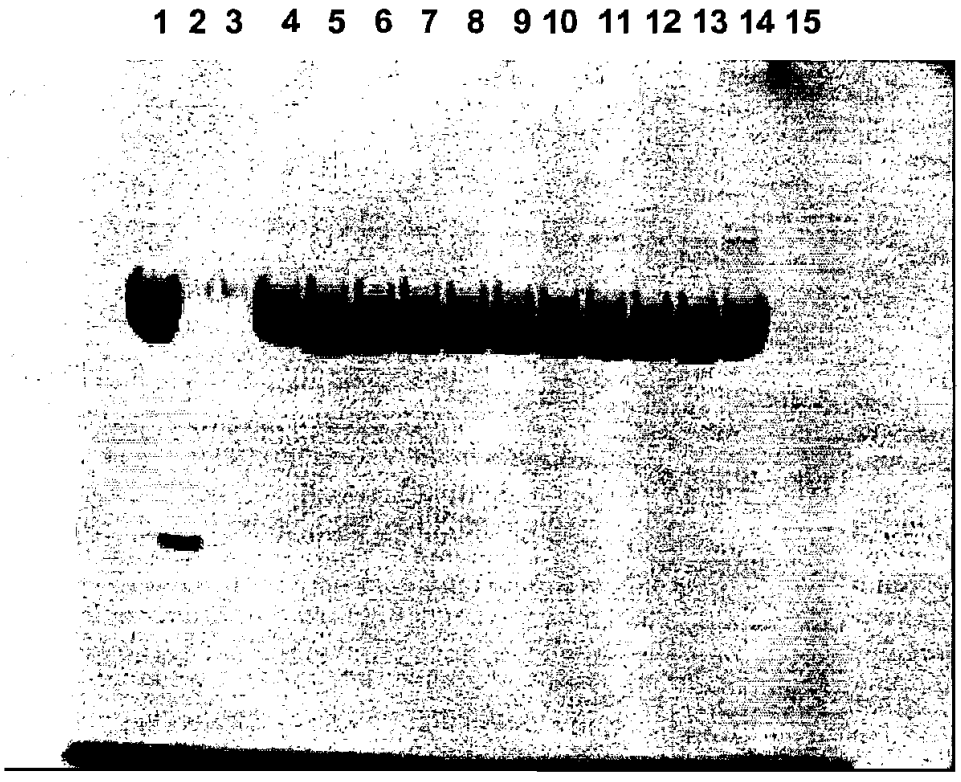


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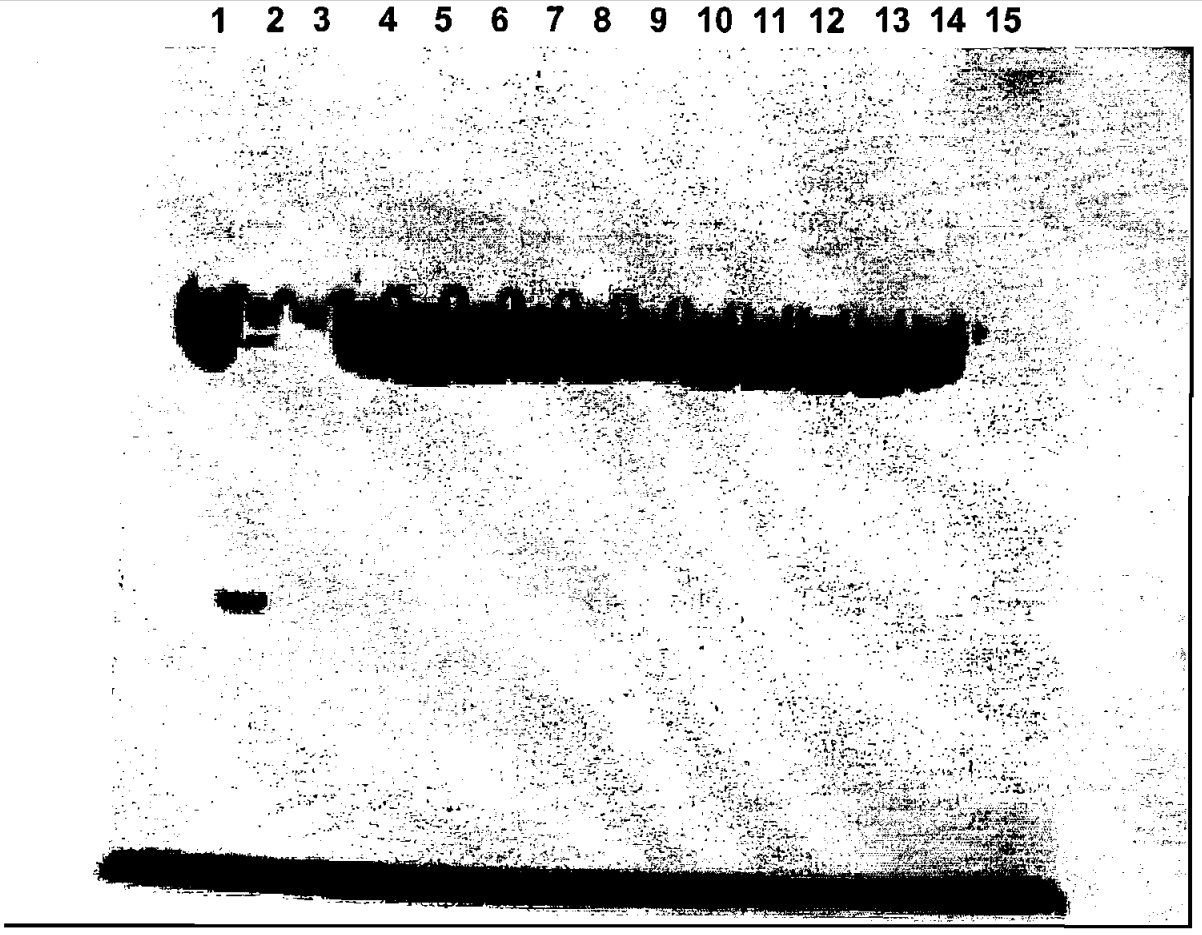


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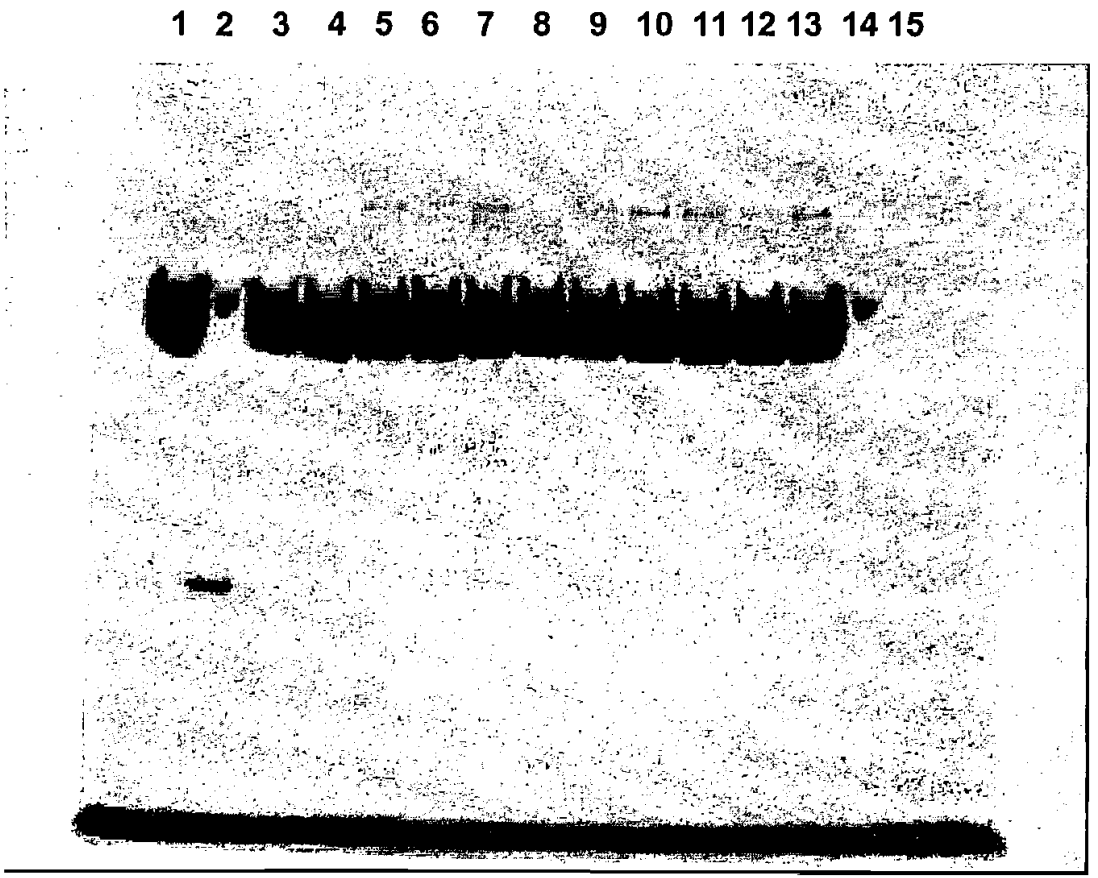


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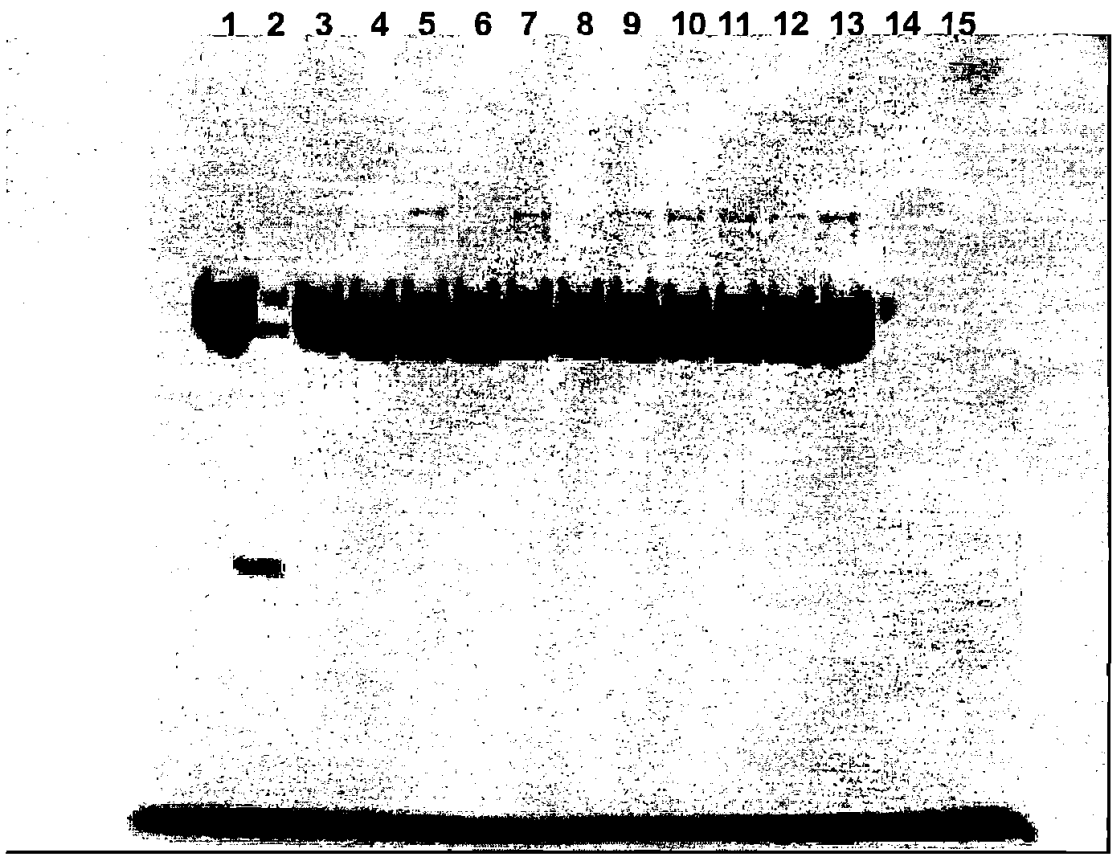


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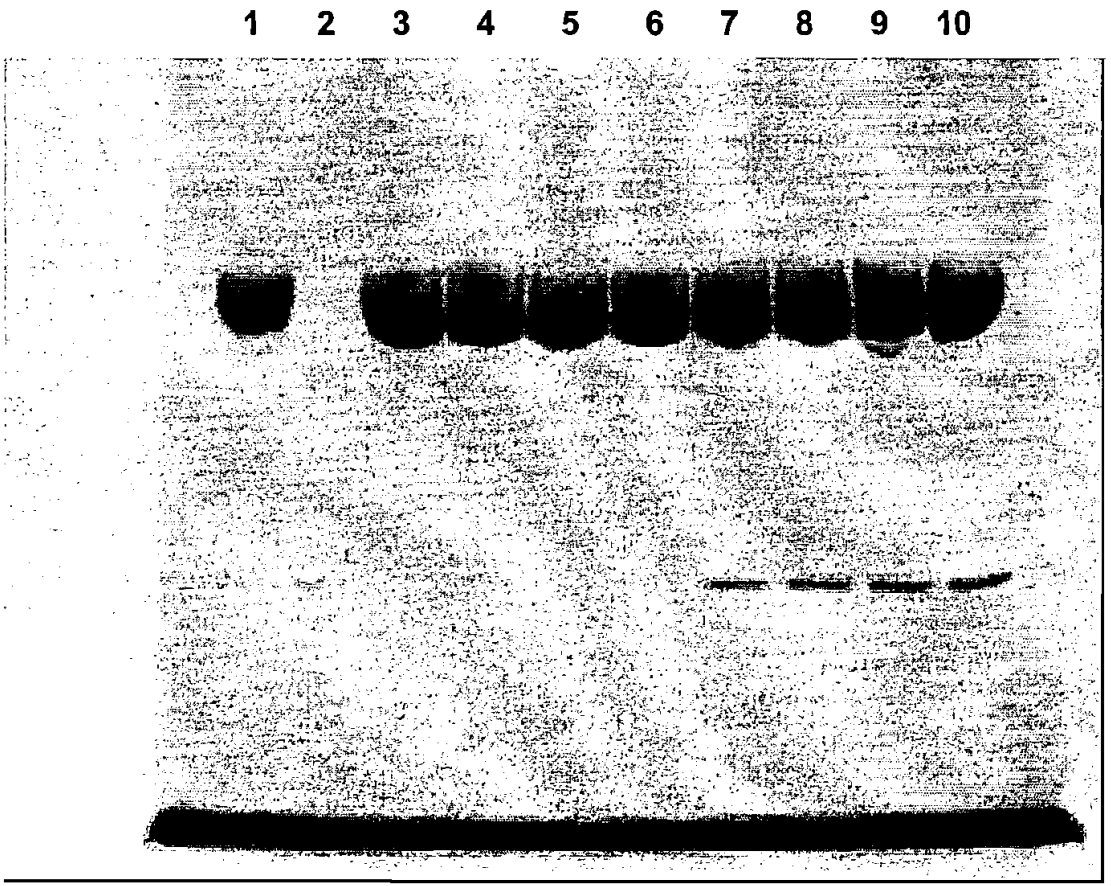


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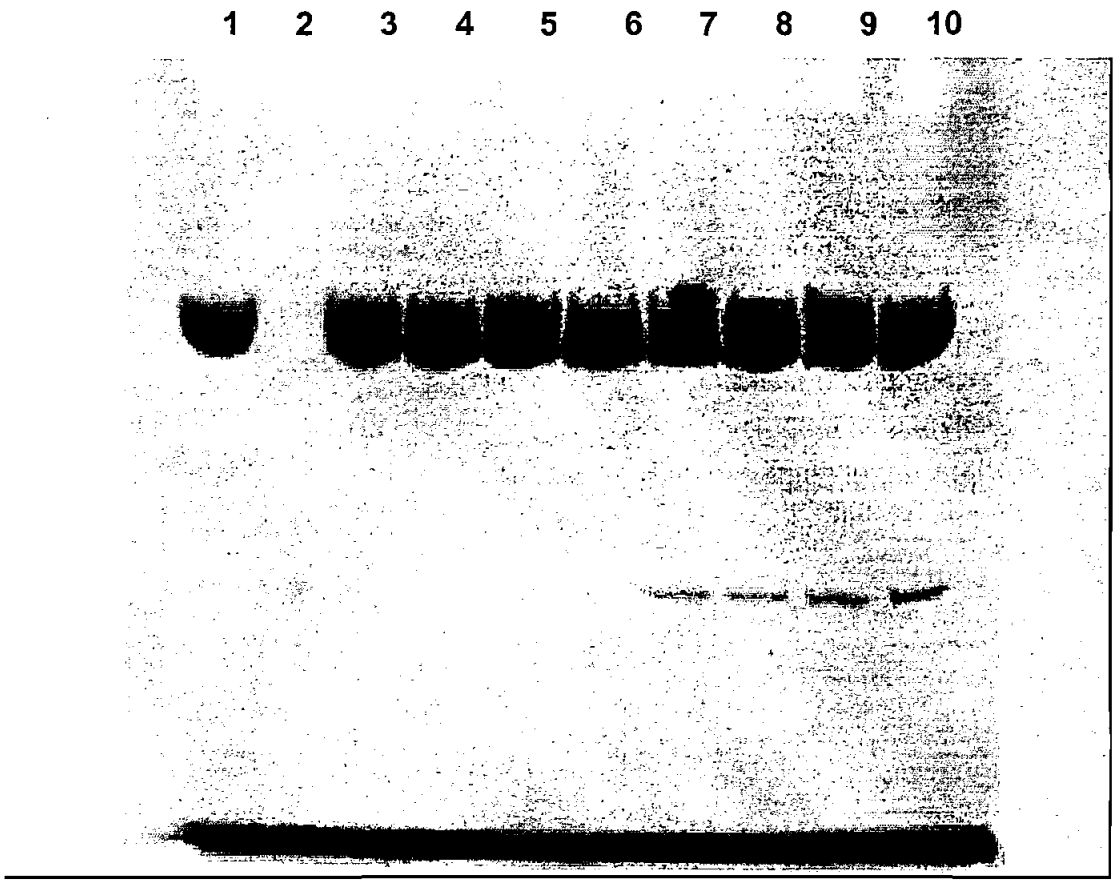


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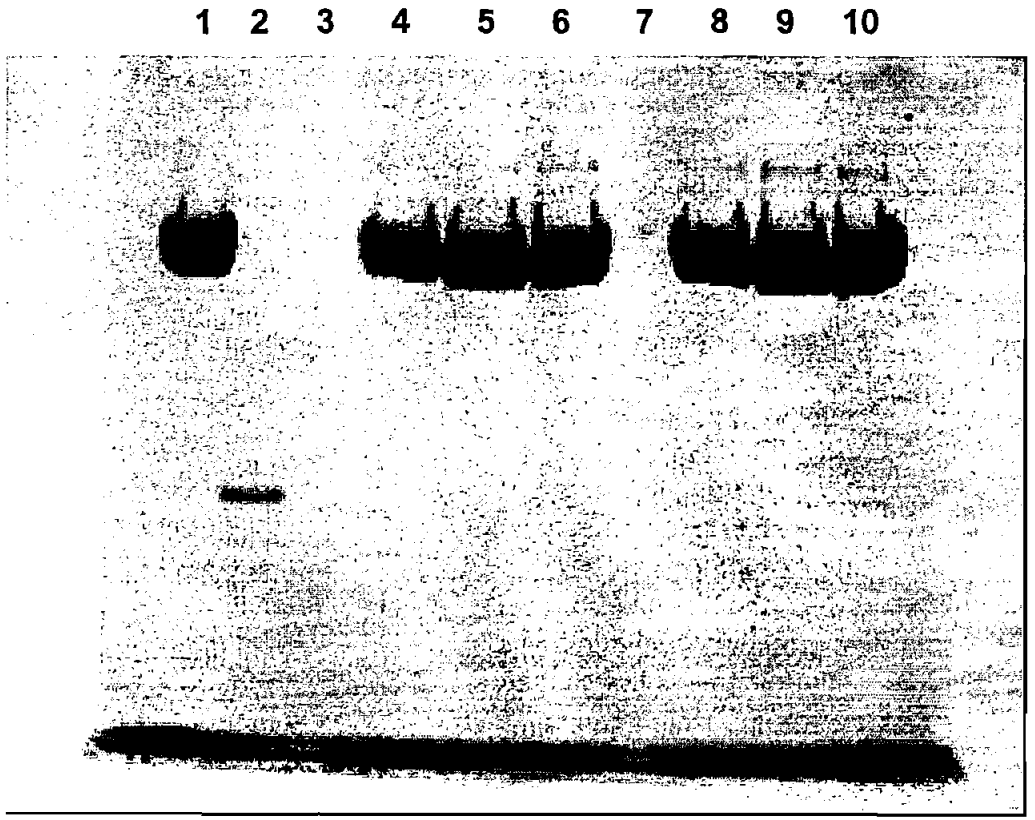


Figure 83