```
MSIVMTQAAPSIPVTPGESVSISCR      25
SSKSLLNSNGNTYLYWFLQRPGQSP      50
QLLIYRMSNLASGVPDRFSGSGSGT      75
AFTLRISRVEAEDVGVYYCMQHLEY      100
PLTFGCGTKLEIKRGGGGSGGGGSG      125
GGGSGGGGSQVQLQQSGPELIKPGA      150
SVKMSCKASGYTFTSYVMHWVKQKP      175
GQCLEWIGYINPYNDGTKYNEKFKG      200
KATLTSDKSSSTAYMELSSLTSEDS      225
AVYYCARGTYYYGSRVFDYWGQGTT      250
LTVTVSSASGAGTGTCYDYKDHDGK      275
DHDIDYKDDDDK*SR                290
```

FIG. 1

Exotoxin A domain III



FIG. 2

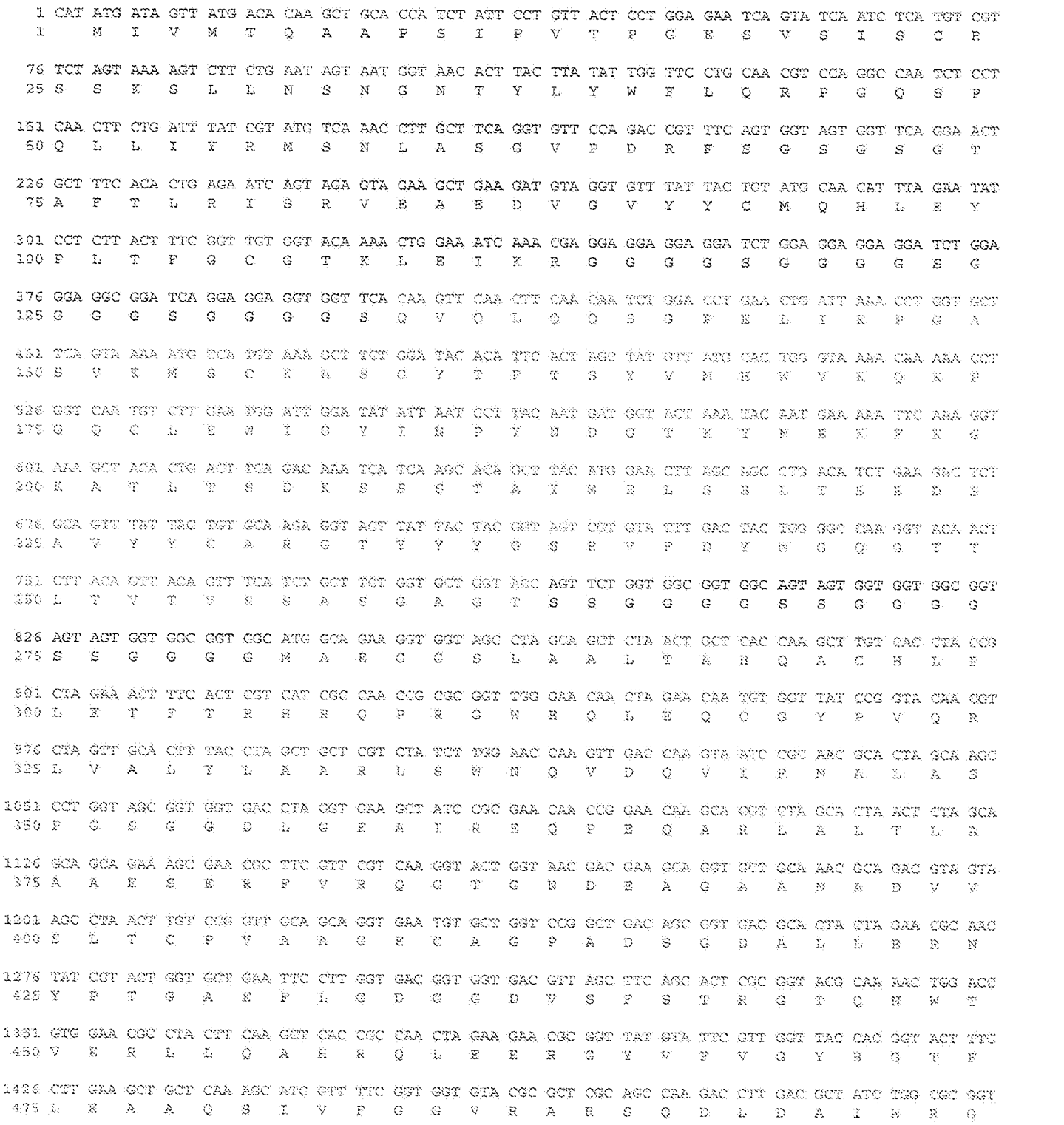


FIG. 3

```
1501 TTC TAT ATC GCA GGT GAT CCG GCT CTA GCA TAC GGT TAC GCA CAA GAC CAA GAA CCT GAC GCA CGC GGT CGT ATC
 500 F   Y   I   A   G   D   P   A   L   A   Y   G   Y   A   Q   D   Q   E   P   D   A   R   G   R   I

1576 CGC AAC GGT GCA CTA CTA CGT GTT TAT GTA CCG CGC TCT AGC CTA CCG GGT TTC TAC CGC ACT AGC CTA ACT CTA
 525 R   N   G   A   L   L   R   V   Y   V   P   R   S   S   L   P   G   F   Y   R   T   S   L   T   L

1651 GCA GCT CCG GAA GCT GCT GGT GAA GTT GAA CGT CTA ATC GGT CAT CCG CTA CCG CTA CGC CTA GAC GCA ATC ACT
 550 A   A   P   E   A   A   G   E   V   E   R   L   I   G   H   P   L   P   L   R   L   D   A   I   T

1726 GGT CCT GAA GAA GAA GGT GGT CGC CTA GAA ACT ATT CTT GGT TGG CCG CTA GCA GAA CGC ACT GTA GTA ATT CCT
 575 G   P   E   E   E   G   G   R   L   E   T   I   L   G   W   P   L   A   E   R   T   V   V   I   P

1801 TCT GCT ATC CCT ACT GAC CCG CGC AAC GTT GGT GGT GAC CTT GAC CCG TCA AGC ATC CCT GAC AAG GAA CAA GCT
 600 S   A   I   P   T   D   P   R   N   V   G   G   D   L   D   P   S   S   I   P   D   K   E   Q   A

1876 ATC AGC GCA CTA CCG GAC TAC GCA AGC CAA CCT GGT AAA CCG CCG CGC GAA GAC CTA AAG GGT ACA TGT TAC GAT
 625 I   S   A   L   P   D   Y   A   S   Q   P   G   K   P   P   R   E   D   L   K   G   T   C   Y   D

1951 TAC AAA GAT CAC GAT GGT GAT TAC AAA GAT CAC GAT ATT GAT TAT AAA GAT GAT GAT GAT AAA TAA TCTAGA
 650 Y   K   D   H   D   G   D   Y   K   D   H   D   I   D   Y   K   D   D   D   D   K   *
```

FIG. 3 (Cont'd.)

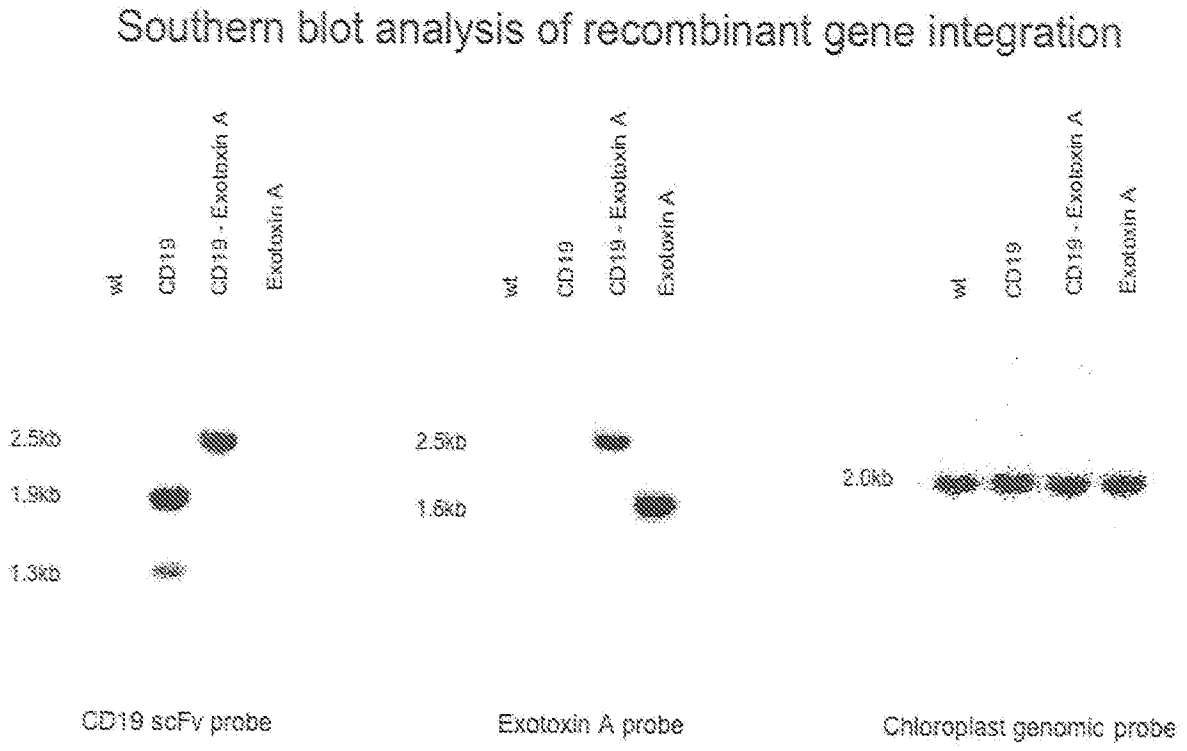


FIG. 4

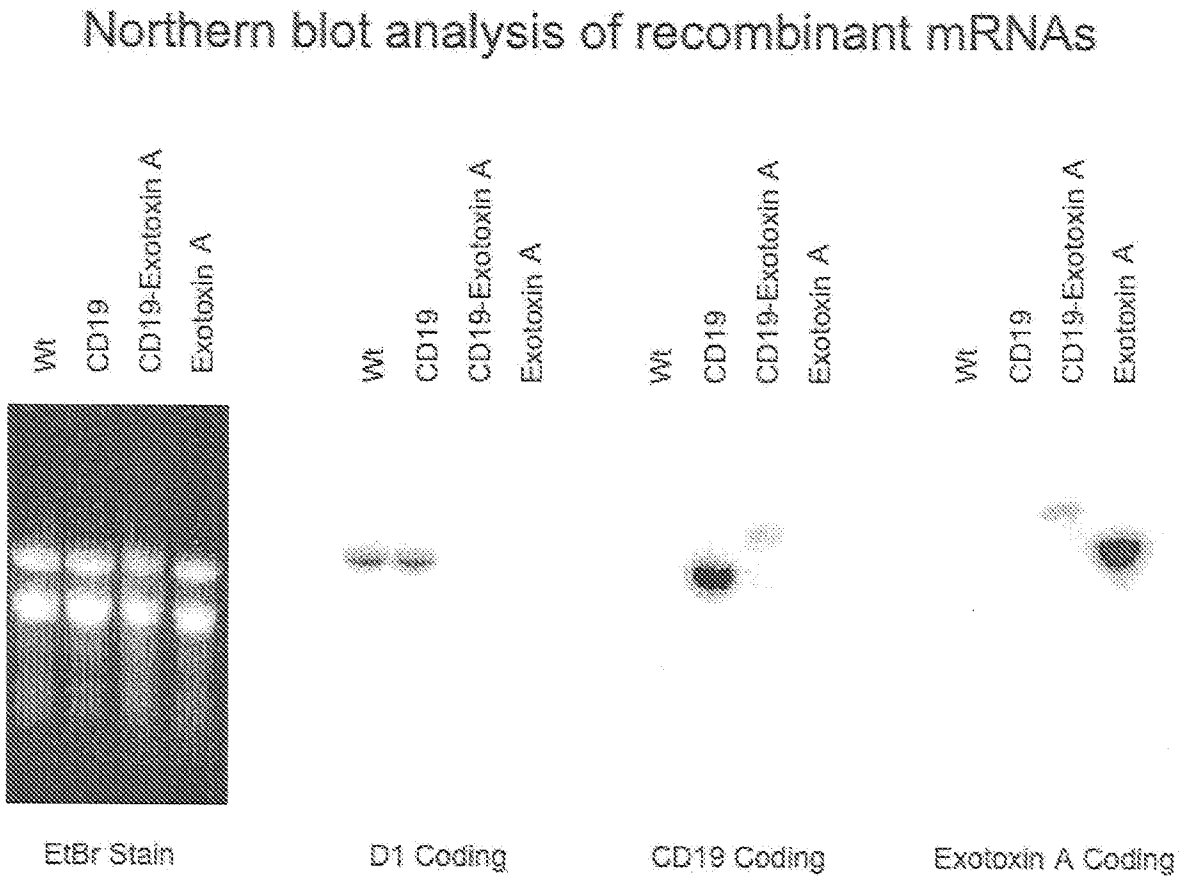

Northern blot analysis of recombinant mRNAs
Wt
CD19
CD19-Exotoxin A
Exotoxin A
EtBr Stain
D1 Coding
CD19 Coding
Exotoxin A Coding

FIG. 5

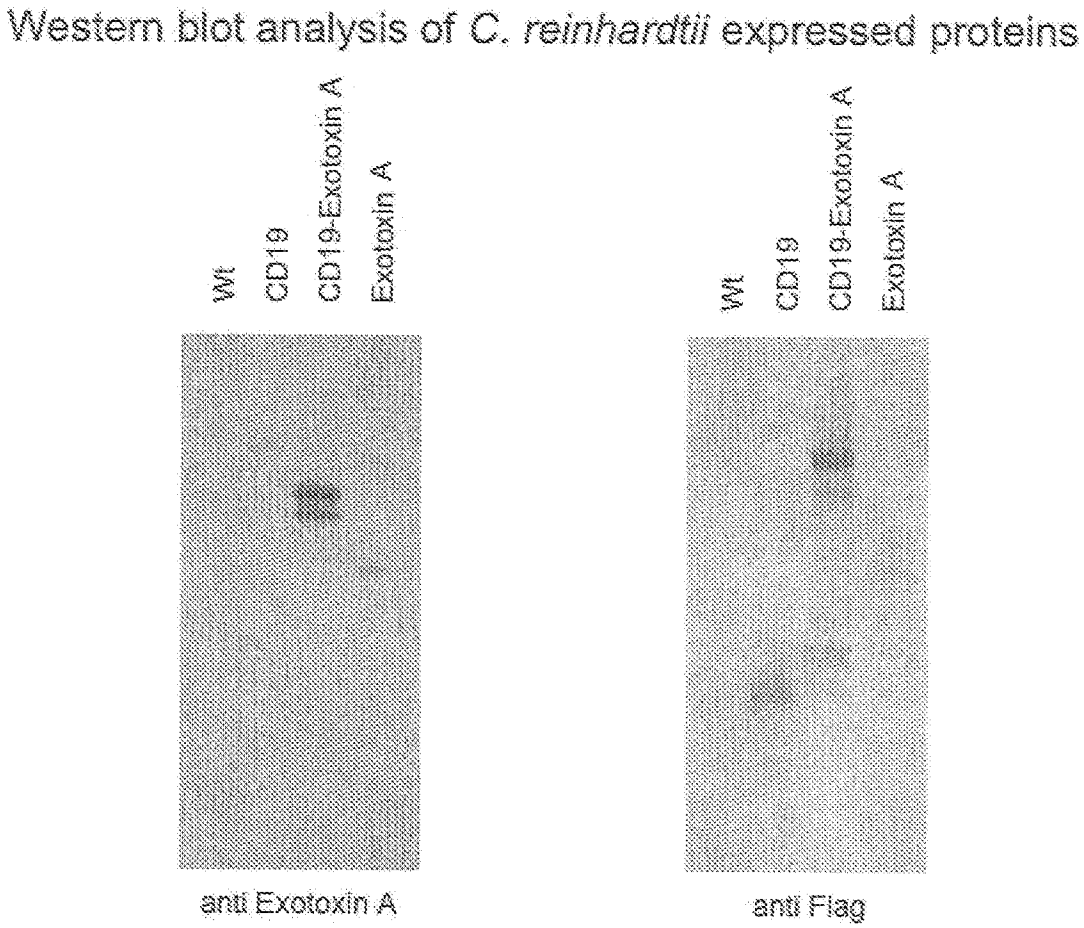

Western blot analysis of C. reinhardtii expressed proteins
Wt
CD19
CD19-Exotoxin A
Exotoxin A
anti Exotoxin A
Wt
CD19
CD19-Exotoxin A
Exotoxin A
anti Flag

FIG. 6

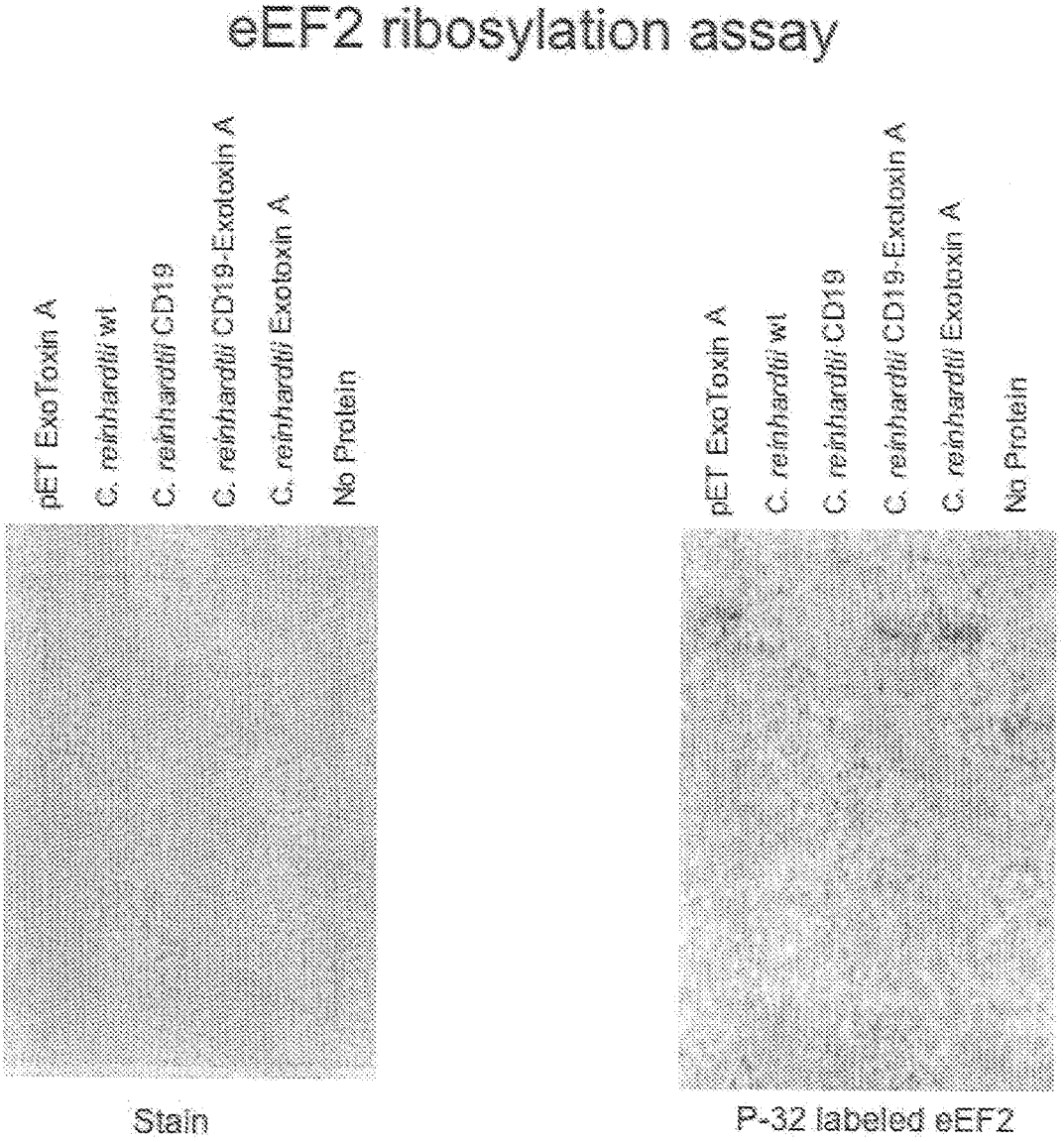

eEF2 ribosylation assay
pET ExoToxin A
C. reinhardtii wt
C. reinhardtii CD19
C. reinhardtii CD19-Exotoxin A
C. reinhardtii Exotoxin A
No Protein
Stain
P-32 labeled eEF2

FIG. 7

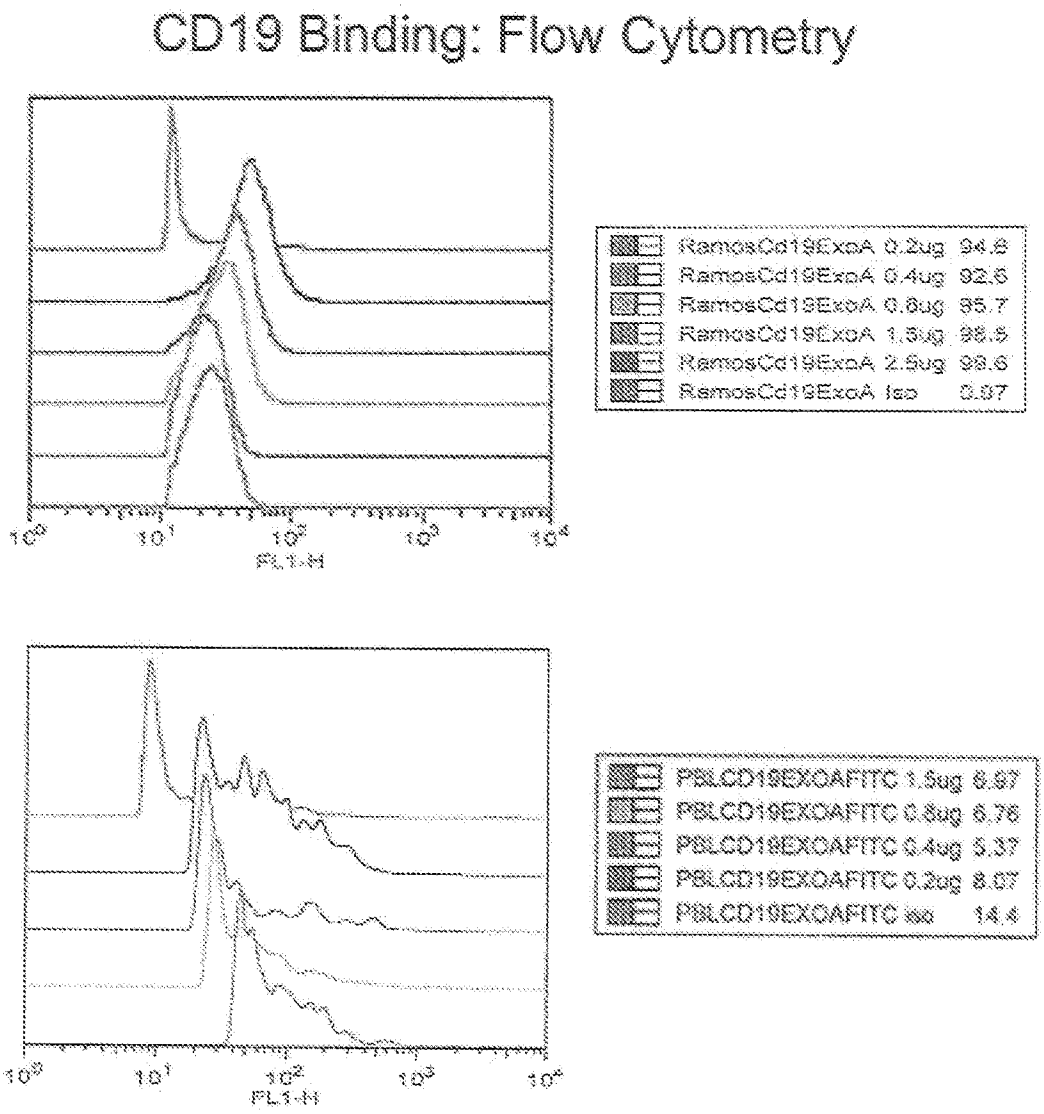

CD19 Binding: Flow Cytometry
RamosCd19ExoA 0.2ug 94.8
RamosCd19ExoA 0.4ug 92.6
RamosCd19ExoA 0.8ug 95.7
RamosCd19ExoA 1.5ug 98.5
RamosCd19ExoA 2.5ug 98.6
RamosCd19ExoA iso 0.87
FL1-H
PBLCD19EXOAFITC 1.5ug 6.87
PBLCD19EXOAFITC 0.8ug 6.76
PBLCD19EXOAFITC 0.4ug 5.37
PBLCD19EXOAFITC 0.2ug 8.07
PBLCD19EXOAFITC iso 14.4
FL1-H

FIG. 8

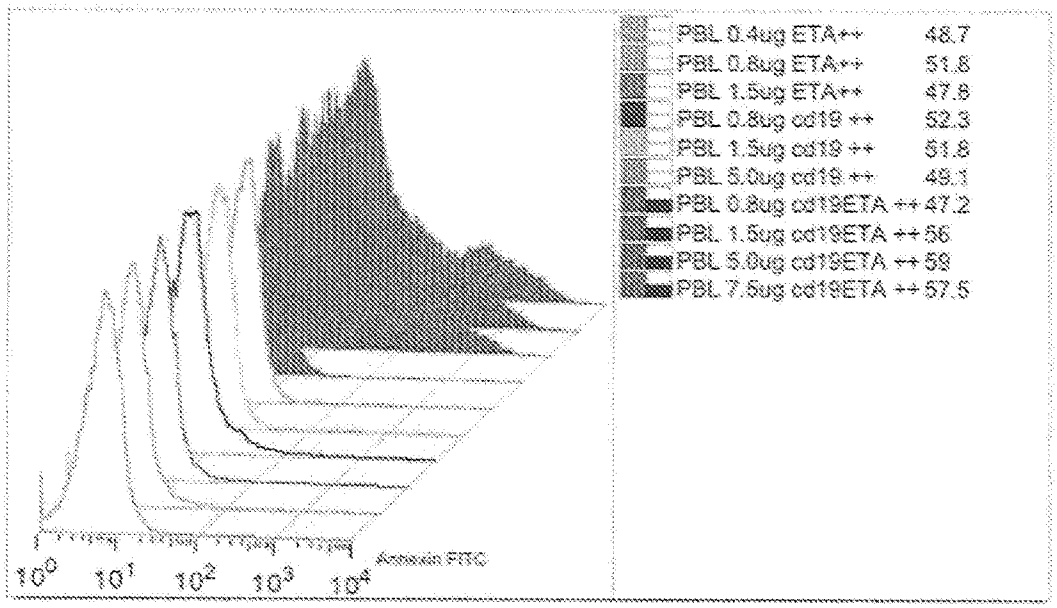

CD19-exotoxin A PBL cell killing
PBL 0.4ug ETA++ 48.7
PBL 0.8ug ETA++ 51.8
PBL 1.5ug ETA++ 47.8
PBL 0.8ug cd19 ++ 52.3
PBL 1.5ug cd19 ++ 51.8
PBL 5.0ug cd19 ++ 48.1
PBL 0.8ug cd19ETA ++ 47.2
PBL 1.5ug cd19ETA ++ 56
PBL 5.0ug cd19ETA ++ 59
PBL 7.5ug cd19ETA ++ 57.5
Annexin FITC
10^0 10^1 10^2 10^3 10^4

FIG. 9

SAA3-Gelonin Sequence

```
   1 CAT ATG TGG GGT ACA TTC CTT AAA GAA GCT GGT CAA GGT GCT AAA GAC ATG TGG AGA GCT 60
   1 H   M   W   G   T   F   L   K   E   A   G   Q   G   A   K   D   M   W   R   A  20

  61 TAC CAA GAC ATG AAA GAA GCT AAC TAC CGT GGT GCA GAC AAA TAC TTC CAC GCT CGT GGT 120
  21 Y   Q   D   M   K   E   A   N   Y   R   G   A   D   K   Y   F   H   A   R   G  40

 121 AAC TAT GAC GCT GCT CGA CGT GGT CCT GGT GGT GCT TGG GCT GCT AAA GTA ATC AGT AAC 180
  41 N   Y   D   A   A   R   R   G   P   G   G   A   W   A   A   K   V   I   S   N  60

 181 GCT AGA GAA ACT ATT CAA GGT ATC ACA GAC CCT CTT TTT AAA GGT ATG ACA CGT GAC CAA 240
  61 A   R   E   T   I   Q   G   I   T   D   P   L   F   K   G   M   T   R   D   Q  80

 241 GTA CGT GAA GAT TCT AAA GCT GAC CAA TTT GCT AAC GAA TGG GGT CGT AGC GGT AAA GAC 300
  81 V   R   E   D   S   K   A   D   Q   F   A   N   E   W   G   R   S   G   K   D 100

 301 CCT AAC CAC TTC AGA CCT GCT GGT CTT CCT GAC AAA TAC TCA GGT GGT GGT GGT TCA CCA 360   G4S2 Linker
 101 P   N   H   F   R   P   A   G   L   P   D   K   Y   S   G   G   G   G   S   P  120

 361 TGG GAA AAT TTA TAT TTT CAA TCA GGT TTA GAT ACA GTT TCA TTT TCA ACA AAA GGT GCT 420   Tev Cleavage Site
 121 W   E   N   L   Y   F   Q   S   G   L   D   T   V   S   F   S   T   K   G   A  140

 421 ACA TAT ATT ACA TAC GTT AAC TTT TTA AAT GAA TTA CGT GTT AAA TTA AAA CCA GAA GGT 480
 141 T   Y   I   T   Y   V   N   F   L   N   E   L   R   V   K   L   K   P   E   G  160

 481 AAT TCA CAT GGT ATT CCA TTA TTA CGT AAA AAA TGT GAT GAT CCA GGT AAA TGT TTT GTT 540
 161 N   S   H   G   I   P   L   L   R   K   K   C   D   D   P   G   K   C   F   V  180

 541 TTA GTT GCT TTA TCA AAT GAT AAT GGT CAA TTA GCT GAA ATT GCT ATT GAT GTT ACA TCA 600
 181 L   V   A   L   S   N   D   N   G   Q   L   A   E   I   A   I   D   V   T   S  200

 601 GTT TAT GTT GTT GGT TAT CAA GTT CGT AAT CGT TCA TAT TTT TTT AAA GAT GCT CCA GAT 660
 201 V   Y   V   V   G   Y   Q   V   R   N   R   S   Y   F   F   K   D   A   P   D  220

 661 GCT GCT TAT GAA GGT TTA TTT AAA AAT ACA ATT AAA ACA CGT TTA CAT TTT GGT GGT TCA 720
 221 A   A   Y   E   G   L   F   K   N   T   I   K   T   R   L   H   F   G   G   S  240   Gelonin

 721 TAT CCA TCA TTA GAA GGT GAA AAA GCT TAT CGT GAA ACA ACA GAT CTT GGT ATT GAA CCA 780
 241 Y   P   S   L   E   G   E   K   A   Y   R   E   T   T   D   L   G   I   E   P  260

 781 CTT CGT ATC GGC ATC AAA AAA CTT GAC GAA AAC GCG ATC GAC AAC TAC AAA CCA ACA GAA 840
 261 L   R   I   G   I   K   K   L   D   E   N   A   I   D   N   Y   K   P   T   E  280

 841 ATC GCG AGC TCT CTT CTT GTT GTA ATC CAA ATG GTA AGC GAA GCG GCA CGT TTC ACA TTC 900
 281 I   A   S   S   L   L   V   V   I   Q   M   V   S   E   A   A   R   F   T   F  300

 901 ATC GAA AAC CAA ATT CGT AAC AAC TTC CAA CAA CGT ATC CGT CCA GCG AAC AAC ACA ATC 960
 301 I   E   N   Q   I   R   N   N   F   Q   Q   R   I   R   P   A   N   N   T   I  320

 961 TCT CTT GAA AAC AAA TGG GGC AAA CTT AGC TTC CAA ATC CGT ACA AGC GGT GCG AAC GGT 1020
 321 S   L   E   N   K   W   G   K   L   S   F   Q   I   R   T   S   G   A   N   G  340

1021 ATG TTC AGC GAA GCG GTA GAA CTT GAA CGC GCG AAC GGC AAA AAA TAC TAC GTA ACT GCG 1080
 341 M   F   S   E   A   V   E   L   E   R   A   N   G   K   K   Y   Y   V   T   A  360

1081 GTA GAT CAA GTA AAA CCA AAA ATC GCA CTT CTT AAA TTC GTA GAC AAA GAC CCA GAA GGT 1140
 361 V   D   Q   V   K   P   K   I   A   L   L   K   F   V   D   K   D   P   E   G  380

1141 ACC TGT TAC GAT TAT AAA GAT CAC GAT GGT GAT TAC AAA GAT CAC GAT ATT GAT TAT AAA 1200   3x Flag
 381 T   C   Y   D   Y   K   D   H   D   G   D   Y   K   D   H   D   I   D   Y   K  400

1201 GAT GAT GAT GAT AAA 1215
 401 D   D   D   D   K   405
```

FIG. 10

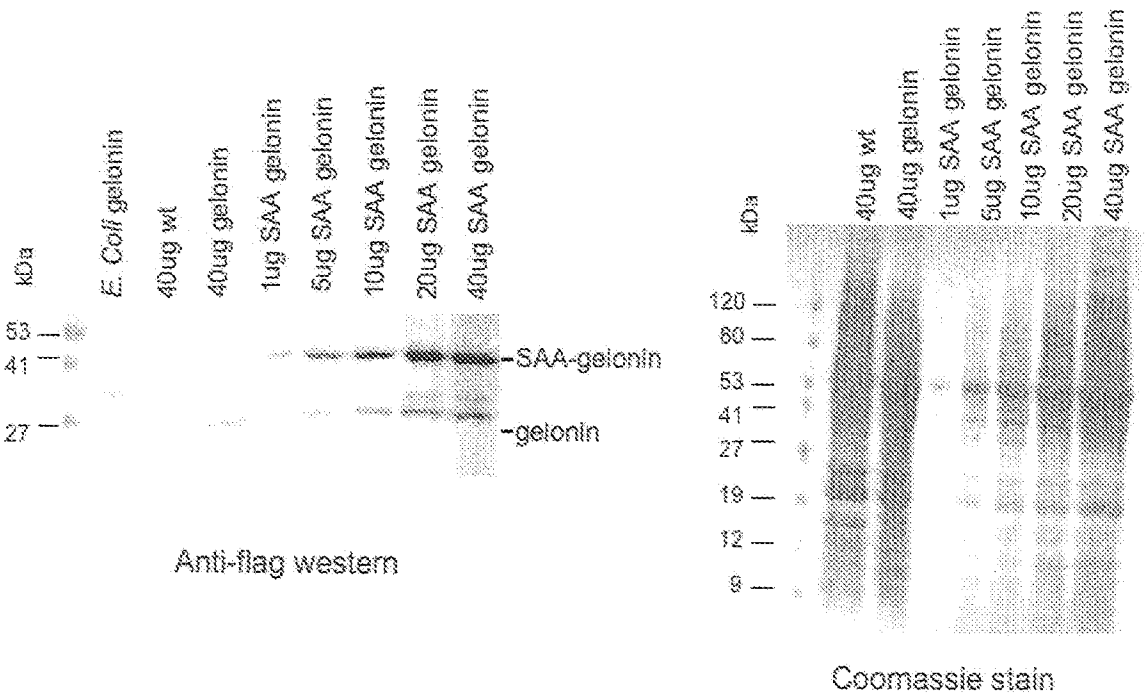

kDa
E. Coli gelonin
40ug wt
40ug gelonin
1ug SAA gelonin
5ug SAA gelonin
10ug SAA gelonin
20ug SAA gelonin
40ug SAA gelonin
53
41
27
SAA-gelonin
gelonin
Anti-flag western
kDa
40ug wt
40ug gelonin
1ug SAA gelonin
5ug SAA gelonin
10ug SAA gelonin
20ug SAA gelonin
40ug SAA gelonin
120
80
53
41
27
19
12
9
Coomassie stain

FIG. 11

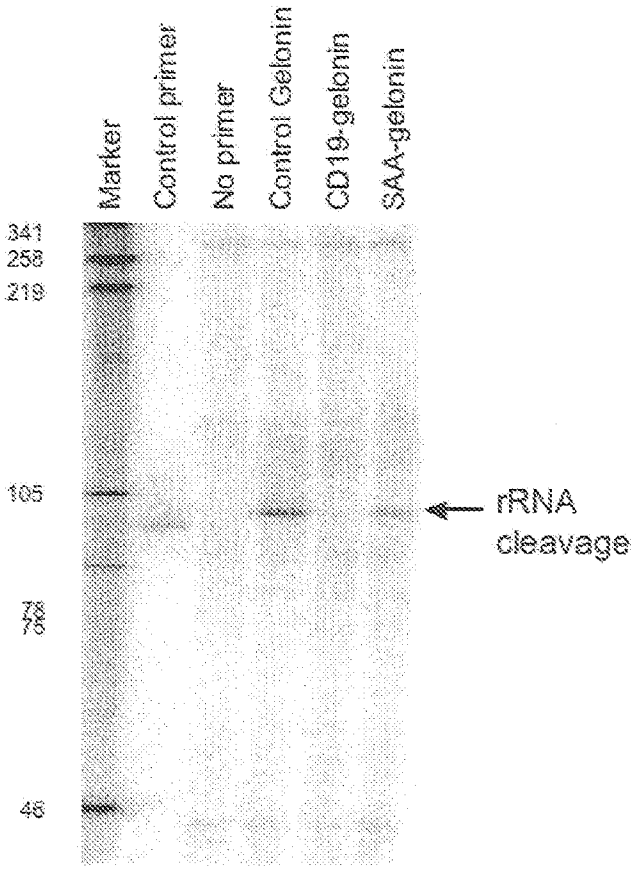

Marker
Control primer
No primer
Control Gelonin
CD19-gelonin
SAA-gelonin
341
258
219
105
78
75
46
rRNA
cleavage

FIG. 12

Gelonin Sequence

```
   1 GAT ATG GGT TTA GAT GCA GTT TCA TTT TTA ATA AAA GGT GCT GCT ATG TAT ATT ATA TAT GTT AAA TTT TTA AAT GAA TTA CCT GTT AAA TTA 90
   1  M   G   L   D   T   V   S   F   S   T   K   G   A   T   Y   I   T   Y   V   M   F   L   N   E   L   R   V   K   L  30

  91 AAA TCA GAA GAT ATT TCA CAT GGT ATT CTA TTA TTA CCT AAA AAA TAT AAT GAT TCA GGT AAA TGT TTT GTT TTA GCT GCT TTA TCA AAT 180
  31 K   P   E   G   M   S   H   G   I   P   L   L   P   K   K   Y   N   D   P   G   K   C   F   V   L   A   L   S   M   N  60

 181 GAT AAT CGT CAA TTA GCT GAA ATT GCT ATT GAT ATT TCA GTT ATA TAT GTT TAT GCA TAT GAT CAA GTT CGT AAT TGT TTA TAT TTT AAA 270
  61  D   N   G   Q   L   A   E   I   A   I   D   V   S   V   Y   V   Y   A   Y   D   Q   V   R   N   C   L   Y   F   K  90

 271 GAT GCT TCA GAT gac GCT TAT GAA GGT TTA TTT AAA AAT ACA ACA GTT TTA CAT TTT GCT GGT TCA TCA TAT GTA TTA GAA GGT GCT 360
  90  D   A   F   D   A   Y   E   G   L   F   K   N   T   T   V   L   H   F   G   G   S   S   Y   V   L   E   G   A  120

 361 GAA AAA GCT TAT GAT GCA ATA GAT CTT GAT ATC GAA CCA CTT TAC AGT GAA ATT AAA AAA CTT GAC GAC AAT GTA GAT AAT TAT 450
 121  E   K   A   Y   D   A   I   D   L   D   I   E   P   L   Y   S   E   I   K   K   L   D   D   N   V   D   N   Y  150

 451 AAA TCA GCA AAT AAG GAA ATC TCT CTT GTT GTA ATC CAA ATG GTA AGC GAA GCG GCT TTT AAA TTC GAA AAC CAA ATT CGT ATA AGC 540
 151  K   P   T   E   I   A   S   L   V   V   I   Q   M   V   S   E   A   A   R   F   T   F   K   N   Q   I   R   I   S  180

 541 AAC AAC TTC CAA CAA GGT ATC CGT TTA GCG AAC AGC ATC TCT CTT AAA GAA TGG GGC AAA CTT AGC GAT GCA GTA AAA GGT AGC 630
 181  N   N   F   Q   Q   G   K   I   R   F   A   N   S   I   S   L   K   E   W   G   K   L   S   D   A   V   K   G   S  210

 631 GGT GCG AAC GGT ATT TTC AGC GAA GCG GTA GAA CTT GAA CGC AAC GCG AAA AAA TAC TAC GTA ACT GCG GTA GAT CAA GTA AAA TCA 720
 211  G   A   N   G   M   F   S   E   A   V   E   L   E   R   A   N   A   K   K   Y   Y   V   T   A   V   D   Q   V   K   S  240

 721 AAA ATC GCA CTT CTT AAA TTC GTA GAC AAA GAC TCA GAC GGT ATC TGT TAC GAT AAA GAT TAT AAA GAT GCT GAT GAC GAT GAT 810
 241  K   I   A   L   L   K   F   V   D   K   D   S   D   G   I   C   Y   D   K   D   Y   K   D   A   D   D   D   K 270

 811 ATT GAT TGT AAA GAT GAT GAT GAT TAA TTT AGA 841
 271  I   D   C   K   D   D   D   D   K   *   *   R 281
```

Gelonin

3x Flag

FIG. 13

CD19 scFv-Gelonin Sequence

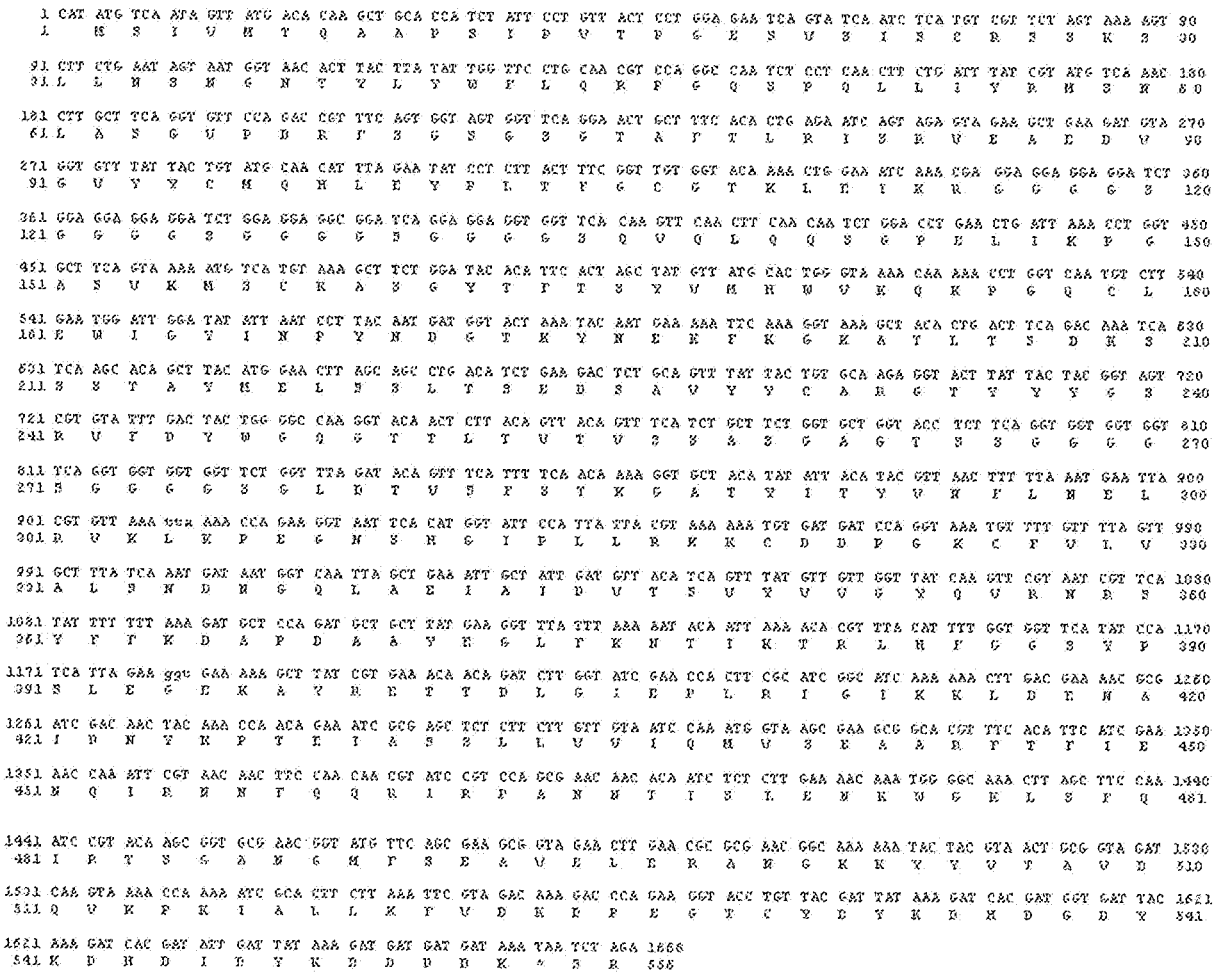


FIG. 14

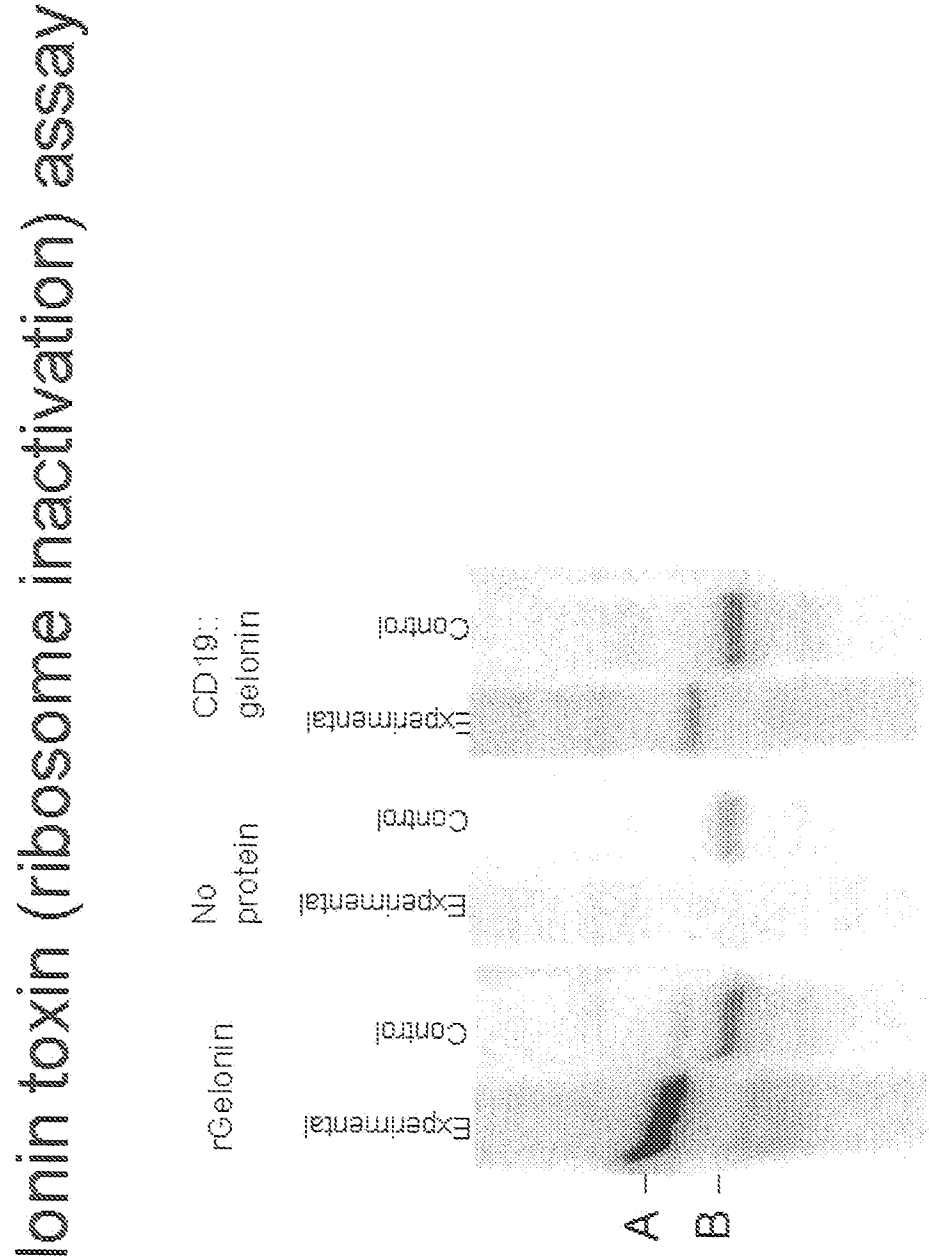

Anti-CD19-gelonin toxin (ribosome inactivation) assay
rGelonin
No protein
CD19::gelonin
Experimental
Control
Experimental
Control
Experimental
Control
A
B

FIG. 15

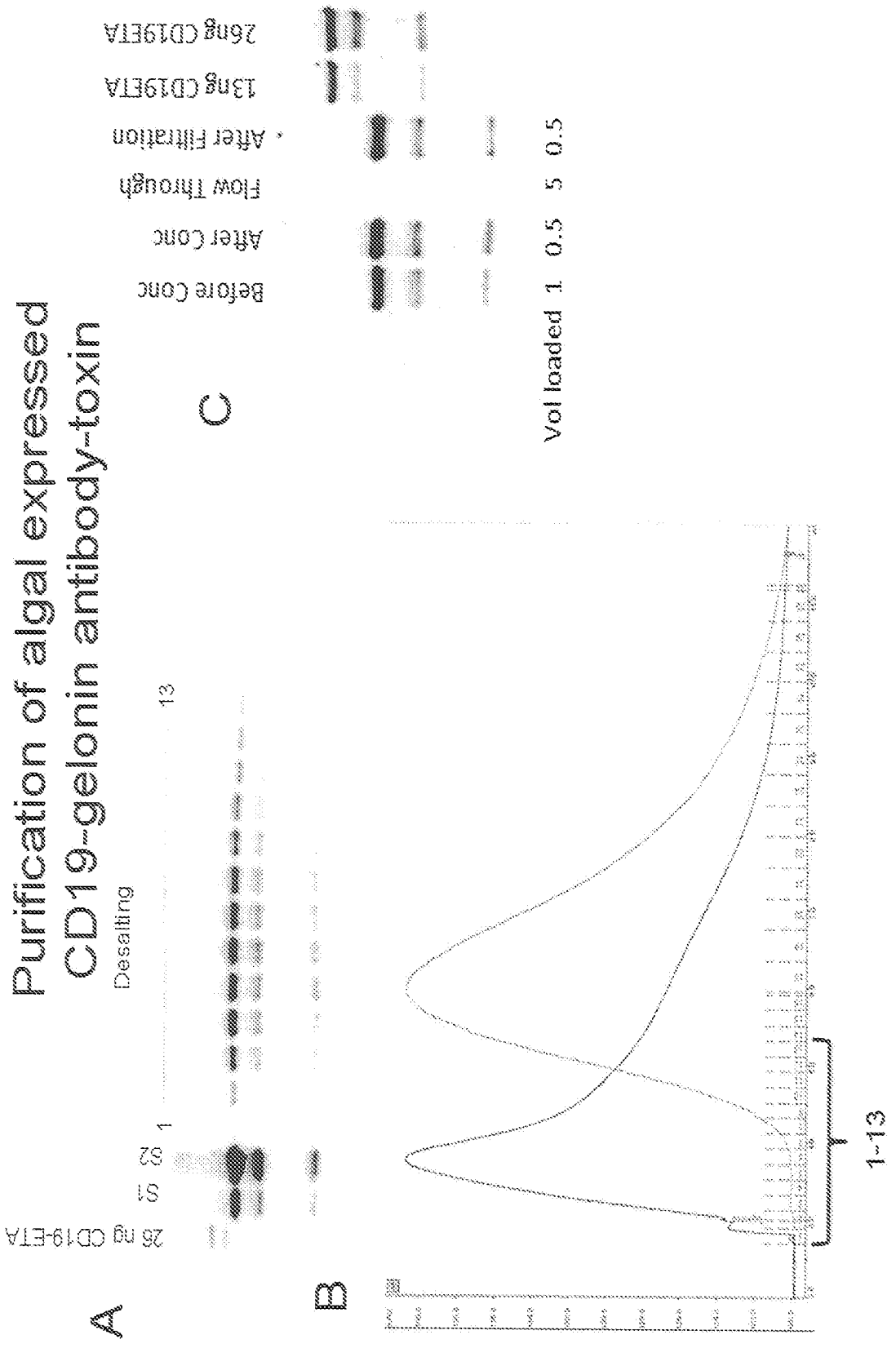

Purification of algal expressed
CD19-gelonin antibody-toxin
A
25 ng CD19-ETA
S1
S2
1
13
Desalting
B
1-13
C
Before Conc
After Conc
Flow Through
After Filtration
13ng CD19ETA
26ng CD19ETA
Vol loaded 1 0.5 5 0.5

FIG. 16

CD19 scFv-CH2-ETA sequence

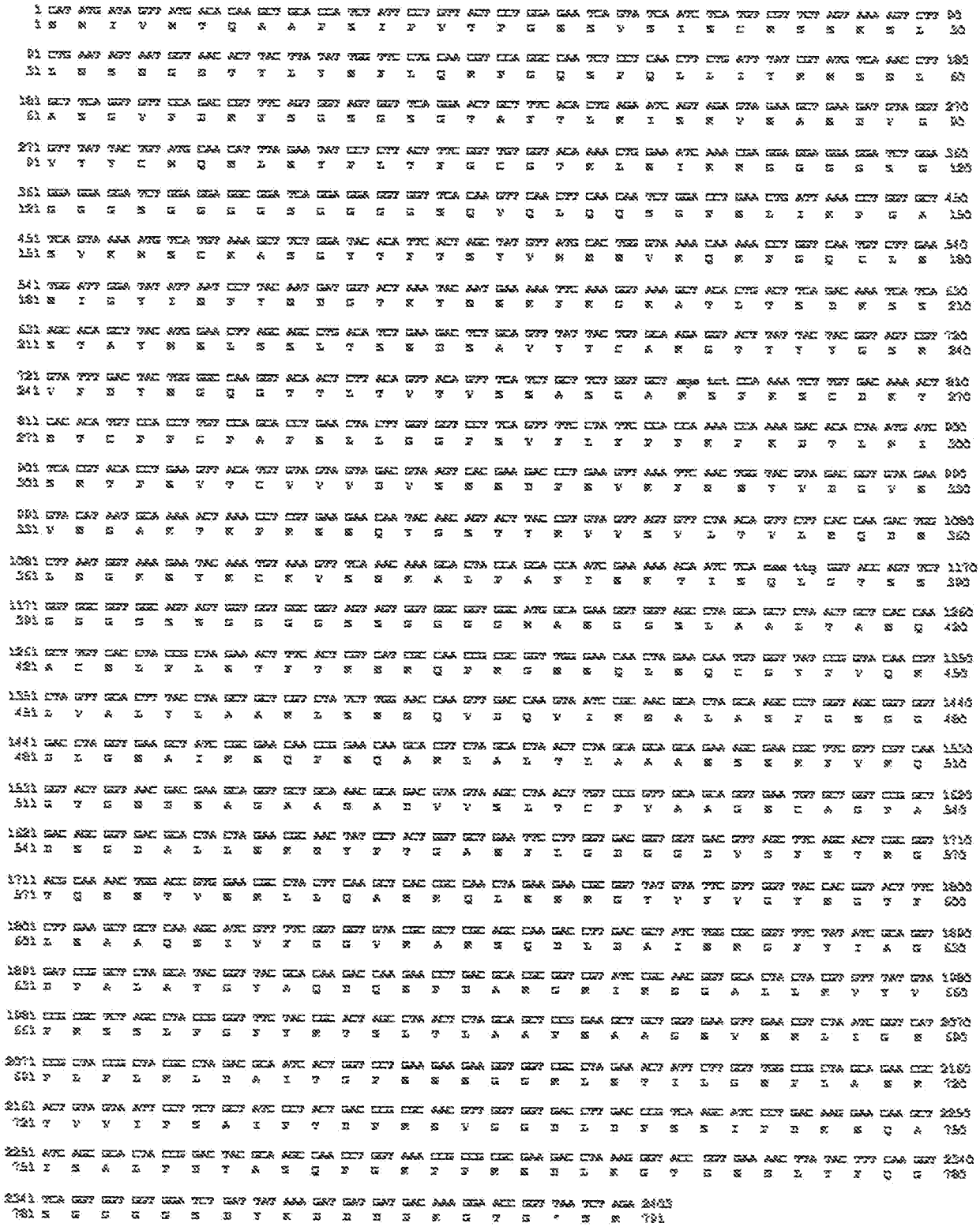


FIG. 17

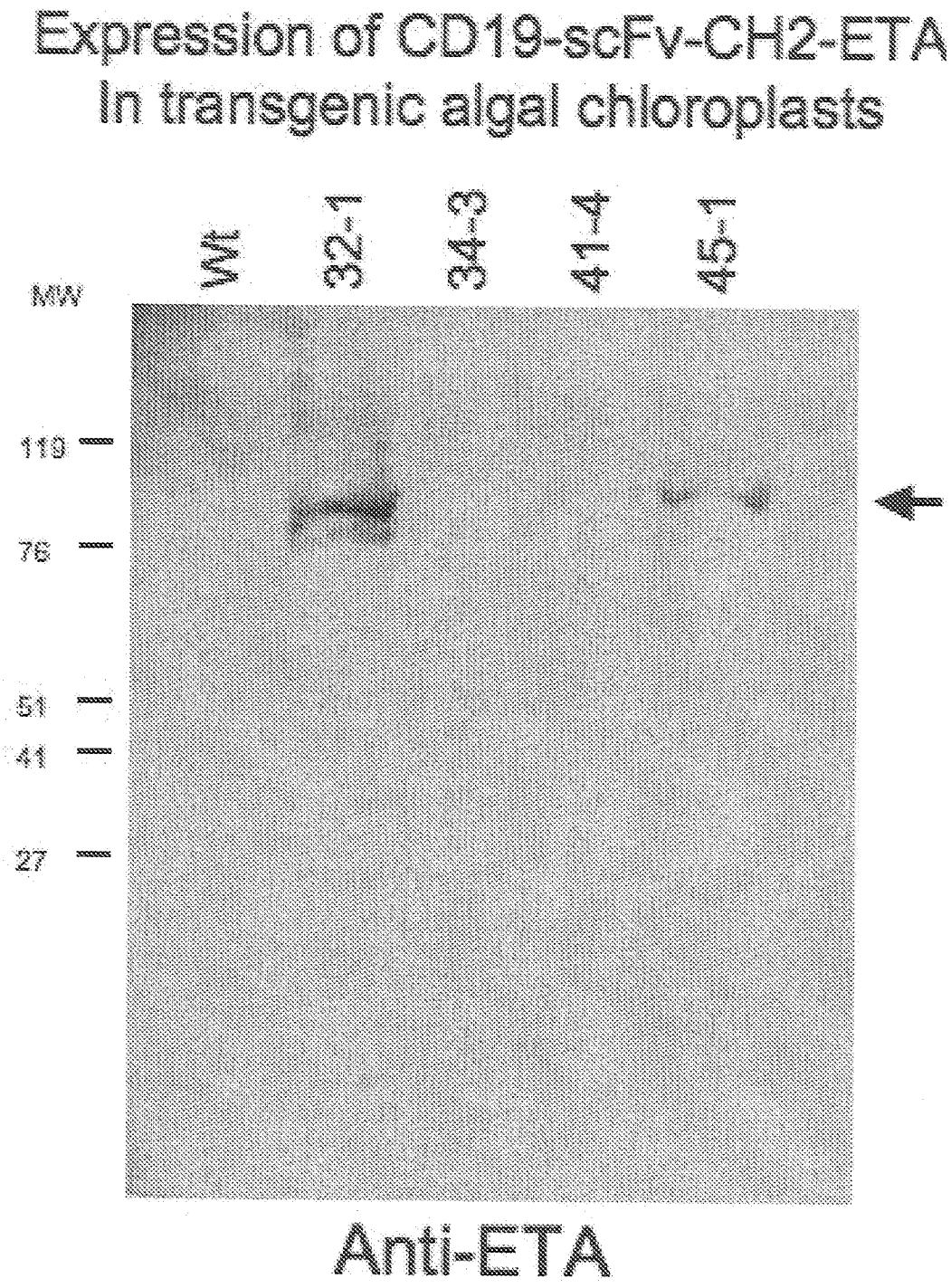

Expression of CD19-scFv-CH2-ETA
In transgenic algal chloroplasts
Wt
32-1
34-3
41-4
45-1
MW
119
76
51
41
27
Anti-ETA

FIG. 18