

# SEQUENCE LISTING

<110> IPSEN BIOPHARM LIMITED

<120> NON-NEURONAL SNARE-CLEAVING BOTULINUM NEUROTOXINS

<130> P48382WO

<160> 11

<170> PatentIn version 3.5

<210> 1

<211> 438

<212> PRT

<213> Clostridium botulinum

<400> 1

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
1 5 10 15

Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
20 25 30

Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
35 40 45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu  
50 55 60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
65 70 75 80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu  
85 90 95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val  
100 105 110

Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys  
115 120 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr  
130 135 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile  
145 150 155 160

Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr  
165 170 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe  
180 185 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu  
195 200 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu  
210 215 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn  
225 230 235 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu  
245 250 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys  
260 265 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn  
275 280 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val  
290 295 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys  
305 310 315 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu  
325 330 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp  
340 345 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn

355

360

365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr  
 370 375 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn  
 385 390 395 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu  
 405 410 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg  
 420 425 430

Gly Ile Ile Thr Ser Lys  
 435

<210> 2  
 <211> 211  
 <212> PRT  
 <213> Homo sapiens

<400> 2

Met Asp Asn Leu Ser Ser Glu Glu Ile Gln Gln Arg Ala His Gln Ile  
 1 5 10 15

Thr Asp Glu Ser Leu Glu Ser Thr Arg Arg Ile Leu Gly Leu Ala Ile  
 20 25 30

Glu Ser Gln Asp Ala Gly Ile Lys Thr Ile Thr Met Leu Asp Glu Gln  
 35 40 45

Lys Glu Gln Leu Asn Arg Ile Glu Glu Gly Leu Asp Gln Ile Asn Lys  
 50 55 60

Asp Met Arg Glu Thr Glu Lys Thr Leu Thr Glu Leu Asn Lys Cys Cys  
 65 70 75 80

Gly Leu Cys Val Cys Pro Cys Asn Arg Thr Lys Asn Phe Glu Ser Gly  
 85 90 95

Lys Ala Tyr Lys Thr Thr Trp Gly Asp Gly Gly Glu Asn Ser Pro Cys  
100 105 110

Asn Val Val Ser Lys Gln Pro Gly Pro Val Thr Asn Gly Gln Leu Gln  
115 120 125

Gln Pro Thr Thr Gly Ala Ala Ser Gly Gly Tyr Ile Lys Arg Ile Thr  
130 135 140

Asn Asp Ala Arg Glu Asp Glu Met Glu Glu Asn Leu Thr Gln Val Gly  
145 150 155 160

Ser Ile Leu Gly Asn Leu Lys Asp Met Ala Leu Asn Ile Gly Asn Glu  
165 170 175

Ile Asp Ala Gln Asn Pro Gln Ile Lys Arg Ile Thr Asp Lys Ala Asp  
180 185 190

Thr Asn Arg Asp Arg Ile Asp Ile Ala Asn Ala Arg Ala Lys Lys Leu  
195 200 205

Ile Asp Ser  
210

<210> 3  
<211> 206  
<212> PRT  
<213> Homo sapiens

<400> 3

Met Ala Glu Asp Ala Asp Met Arg Asn Glu Leu Glu Glu Met Gln Arg  
1 5 10 15

Arg Ala Asp Gln Leu Ala Asp Glu Ser Leu Glu Ser Thr Arg Arg Met  
20 25 30

Leu Gln Leu Val Glu Glu Ser Lys Asp Ala Gly Ile Arg Thr Leu Val  
35 40 45

Met Leu Asp Glu Gln Gly Glu Gln Leu Glu Arg Ile Glu Glu Gly Met  
50 55 60

Asp Gln Ile Asn Lys Asp Met Lys Glu Ala Glu Lys Asn Leu Thr Asp  
65 70 75 80

Leu Gly Lys Phe Cys Gly Leu Cys Val Cys Pro Cys Asn Lys Leu Lys  
85 90 95

Ser Ser Asp Ala Tyr Lys Lys Ala Trp Gly Asn Asn Gln Asp Gly Val  
100 105 110

Val Ala Ser Gln Pro Ala Arg Val Val Asp Glu Arg Glu Gln Met Ala  
115 120 125

Ile Ser Gly Gly Phe Ile Arg Arg Val Thr Asn Asp Ala Arg Glu Asn  
130 135 140

Glu Met Asp Glu Asn Leu Glu Gln Val Ser Gly Ile Ile Gly Asn Leu  
145 150 155 160

Arg His Met Ala Leu Asp Met Gly Asn Glu Ile Asp Thr Gln Asn Arg  
165 170 175

Gln Ile Asp Arg Ile Met Glu Lys Ala Asp Ser Asn Lys Thr Arg Ile  
180 185 190

Asp Glu Ala Asn Gln Arg Ala Thr Lys Met Leu Gly Ser Gly  
195 200 205

<210> 4  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> IgA-protease site His6Tag

<400> 4

Pro Pro Thr Pro Gly His His His His His His  
1 5 10

<210> 5  
<211> 35  
<212> PRT

<213> Artificial Sequence

<220>

<223> Twin Strep Tag

<400> 5

Met Ala Ser Trp Ser His Pro Gln Phe Glu Lys Gly Gly Gly Ser Gly  
1 5 10 15

Gly Gly Ser Gly Gly Gly Ser Trp Ser His Pro Gln Phe Glu Lys Gly  
20 25 30

Ala Gly Ser  
35

<210> 6

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> His6 Tag

<400> 6

Gly His His His His His His  
1 5

<210> 7

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> V-IgA-protease site His6Tag

<400> 7

Val Pro Pro Thr Pro Gly His His His His His His  
1 5 10

<210> 8

<211> 1296

<212> PRT

<213> Clostridium botulinum

<400> 8

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
1 5 10 15

Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
20 25 30

Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
35 40 45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu  
50 55 60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
65 70 75 80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu  
85 90 95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val  
100 105 110

Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys  
115 120 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr  
130 135 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile  
145 150 155 160

Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr  
165 170 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe  
180 185 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu  
195 200 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu  
210 215 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn  
225 230 235 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu  
245 250 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys  
260 265 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn  
275 280 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val  
290 295 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys  
305 310 315 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu  
325 330 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp  
340 345 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn  
355 360 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr  
370 375 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn  
385 390 395 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu  
405 410 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg  
420 425 430

Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys  
435 440 445

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe  
450 455 460

Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu  
465 470 475 480

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu  
485 490 495

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro  
500 505 510

Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu  
515 520 525

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu  
530 535 540

Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu  
545 550 555 560

His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu  
565 570 575

Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys  
580 585 590

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu  
595 600 605

Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr  
610 615 620

Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala  
625 630 635 640

Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu  
645 650 655

Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala  
660 665 670

Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys  
675 680 685

Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu  
690 695 700

Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys  
705 710 715 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu  
725 730 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn  
740 745 750

Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp  
755 760 765

Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile  
770 775 780

Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met  
785 790 795 800

Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys  
805 810 815

Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly  
820 825 830

Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp  
835 840 845

Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser  
850 855 860

Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn  
865 870 875 880

Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser  
885 890 895

Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn  
900 905 910

Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu  
915 920 925

Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser  
930 935 940

Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn  
945 950 955 960

Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val  
965 970 975

Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu  
980 985 990

Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser  
995 1000 1005

Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg  
1010 1015 1020

Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln  
1025 1030 1035

Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile  
1040 1045 1050

Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp  
1055 1060 1065

Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu  
1070 1075 1080

Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys  
1085 1090 1095

Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met  
1100 1105 1110

Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val  
1115 1120 1125

Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val  
1130 1135 1140

Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr  
1145 1150 1155

Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile  
1160 1165 1170

Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn  
1175 1180 1185

Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu  
1190 1195 1200

Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser  
1205 1210 1215

Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn  
1220 1225 1230

Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly  
1235 1240 1245

Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala  
1250 1255 1260

Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu  
1265 1270 1275

Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu  
1280 1285 1290

Arg Pro Leu  
1295

<210> 9  
<211> 23  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> LHN linker

<400> 9

Val Arg Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr  
1 5 10 15

Asn Lys Ala Leu Asn Asp Leu  
20

<210> 10  
<211> 5  
<212> PRT  
<213> Homo sapiens

<400> 10

Asp Asp Asp Asp Lys  
1 5

<210> 11  
<211> 23  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Modified BoNT/A1 activation loop

<400> 11

Val Asp Gly Ile Ile Thr Ser Lys Thr Lys Ser Asp Asp Asp Asp Lys  
1 5 10 15

Asn Lys Ala Leu Asn Leu Gln  
20

