

LISTAGEM DE SEQUÊNCIAS

<110> FUNDAÇÃO OSWALDO CRUZ

<120> "POLIPEPTÍDEO COM ATIVIDADE ASPARAGINASE, POLINUCLEOTÍDEO, CASSETE DE EXPRESSÃO,

VETOR DE EXPRESSÃO, CÉLULA HOSPEDEIRA, COMPOSIÇÃO FARMACÊUTICA, MÉTODOS PARA PRODUZIR UM POLIPEPTÍDEO COM ATIVIDADE ASPARAGINASE E PARA PREVENIR OU TRATAR NEOPLASIAS, E, USO DE UM POLIPEPTÍDEO"

<130> R000607

<160> 12

<170> PatentIn version 3.5

<210> 1

<211> 326

<212> PRT

<213> Escherichia coli

<400> 1

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Gly Asp Ser Ala Thr Lys Ser Asn Tyr Thr Ala Gly Lys Val Gly Val
20 25 30

Glu Asn Leu Val Asn Ala Val Pro Gln Leu Lys Asp Ile Ala Asn Val
35 40 45

Lys Gly Glu Gln Val Val Asn Ile Gly Ser Gln Asp Met Asn Asp Asn
50 55 60

Val Trp Leu Thr Leu Ala Lys Lys Ile Asn Thr Asp Cys Asp Lys Thr
65 70 75 80

Asp Gly Phe Val Ile Thr His Gly Thr Asp Thr Met Glu Glu Thr Ala
85 90 95

Tyr Phe Leu Asp Leu Thr Val Lys Cys Asp Lys Pro Val Val Met Val
100 105 110

Gly Ala Met Arg Pro Ser Thr Ser Met Ser Ala Asp Gly Pro Phe Asn
115 120 125

Leu Tyr Asn Ala Val Val Thr Ala Ala Asp Lys Ala Ser Ala Asn Arg
130 135 140

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

Thr Lys Thr Asn Thr Thr Asp Val Ala Thr Phe Lys Ser Val Asn Tyr
165 170 175

Gly Pro Leu Gly Tyr Ile His Asn Gly Lys Ile Asp Tyr Gln Arg Thr
180 185 190

Pro Ala Arg Lys His Thr Ser Asp Thr Pro Phe Asp Val Ser Lys Leu
195 200 205

Asn Glu Leu Pro Lys Val Gly Ile Val Tyr Asn Tyr Ala Asn Ala Ser
210 215 220

Asp Leu Pro Ala Lys Ala Leu Val Asp Ala Gly Tyr Asp Gly Ile Val
225 230 235 240

Ser Ala Gly Val Gly Asn Gly Asn Leu Tyr Lys Ser Val Phe Asp Thr
245 250 255

Leu Ala Thr Ala Ala Lys Asn Gly Thr Ala Val Val Arg Ser Ser Arg
260 265 270

Val Pro Thr Gly Ala Thr Thr Gln Asp Ala Glu Val Asp Asp Ala Lys

275

280

285

Tyr Gly Phe Val Ala Ser Gly Thr Leu Asn Pro Gln Lys Ala Arg Val
290 295 300

Leu Leu Gln Leu Ala Leu Thr Gln Thr Lys Asp Pro Gln Gln Ile Gln
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Gln Ile Phe Asn Gln Tyr
325

<210> 2

<211> 324

<212> PRT

<213> *Erwinia carotovora*

<400> 2

Leu Pro Asn Ile Val Ile Leu Ala Thr Gly Gly Thr Ile Ala Gly Ser
1 5 10 15

Ala Ala Ala Asn Thr Gln Thr Thr Gly Tyr Lys Ala Gly Ala Leu Gly
20 25 30

Val Glu Thr Leu Ile Gln Ala Val Pro Glu Leu Lys Thr Leu Ala Asn
35 40 45

Ile Lys Gly Glu Gln Val Ala Ser Ile Gly Ser Glu Asn Met Thr Ser
50 55 60

Asp Val Leu Leu Thr Leu Ser Lys Arg Val Asn Glu Leu Leu Ala Arg
65 70 75 80

Ser Asp Val Asp Gly Val Val Ile Thr His Gly Thr Asp Thr Leu Asp
85 90 95

Glu Ser Pro Tyr Phe Leu Asn Leu Thr Val Lys Ser Asp Lys Pro Val
100 105 110

Val Phe Val Ala Ala Met Arg Pro Ala Thr Ala Ile Ser Ala Asp Gly
115 120 125

Pro Met Asn Leu Tyr Gly Ala Val Lys Val Ala Ala Asp Lys Asn Ser
130 135 140

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

Arg Phe Ile Ser Lys Thr Asn Ala Ser Thr Leu Asp Thr Phe Lys Ala
165 170 175

Pro Glu Glu Gly Tyr Leu Gly Val Ile Ile Gly Asp Lys Ile Tyr Tyr
180 185 190

Gln Thr Arg Leu Asp Lys Val His Thr Thr Arg Ser Val Phe Asp Val
195 200 205

Thr Asn Val Asp Lys Leu Pro Ala Val Asp Ile Ile Tyr Gly Tyr Gln
210 215 220

Asp Asp Pro Glu Tyr Met Tyr Asp Ala Ser Ile Lys His Gly Val Lys
225 230 235 240

Gly Ile Val Tyr Ala Gly Met Gly Ala Gly Ser Val Ser Lys Arg Gly
245 250 255

Asp Ala Gly Ile Arg Lys Ala Glu Ser Lys Gly Ile Val Val Val Arg
260 265 270

Ser Ser Arg Thr Gly Ser Gly Ile Val Pro Pro Asp Ala Gly Gln Pro
275 280 285

Gly Leu Val Ala Asp Ser Leu Ser Pro Ala Lys Ser Arg Ile Leu Leu
290 295 300

Met Leu Ala Leu Thr Lys Thr Thr Asn Pro Ala Val Ile Gln Asp Tyr
305 310 315 320

Phe His Ala Tyr

<210> 3

<211> 326

<212> PRT

<213> Sequência Artificial

<220>

<223> Sequência de aminoácidos da asparaginase mutada de E. coli

<400> 3

Leu Pro Asn Ile Thr Ile Leu Ala Thr Gly Gly Thr Ile Ala Gly Gly
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Gly Asp Ser Ala Thr Lys Ser Asn Tyr Thr Ala Gly Lys Val Gly Val
20 25 30

Glu Asn Leu Val Asn Ala Val Pro Gln Leu Lys Asp Ile Ala Asn Val
35 40 45

Lys Gly Glu Gln Val Ala Ser Ile Gly Ser Glu Asn Met Thr Ser Asp
50 55 60

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

Asp Gly Phe Val Ile Thr His Gly Thr Asp Thr Met Glu Glu Thr Ala
85 90 95

Tyr Phe Leu Asp Leu Thr Val Lys Cys Asp Lys Pro Val Val Met Val
100 105 110

Gly Ala Met Arg Pro Ser Thr Ser Met Ser Ala Asp Gly Pro Phe Asn
115 120 125

Leu Tyr Asn Ala Val Val Thr Ala Ala Asp Lys Ala Ser Ala Asn Arg
130 135 140

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

Thr Lys Thr Asn Thr Thr Asp Val Ala Thr Phe Lys Ser Val Asn Tyr
165 170 175

Gly Pro Leu Gly Tyr Ile His Asn Gly Lys Ile Asp Tyr Gln Arg Thr
180 185 190

Pro Ala Arg Lys His Thr Ser Asp Thr Pro Phe Asp Val Ser Lys Leu
195 200 205

Asn Glu Leu Pro Lys Val Gly Ile Val Tyr Asn Tyr Ala Asn Ala Ser
210 215 220

Asp Leu Pro Ala Lys Ala Leu Val Asp Ala Gly Tyr Asp Gly Ile Val
225 230 235 240

Ser Ala Gly Val Gly Asn Gly Asn Leu Tyr Lys Ser Val Phe Asp Thr
245 250 255

Leu Ala Thr Ala Ala Lys Asn Gly Thr Ala Val Val Arg Ser Ser Arg
260 265 270

Val Pro Thr Gly Ala Thr Thr Gln Asp Ala Glu Val Asp Asp Ala Lys
275 280 285

Tyr Gly Phe Val Ala Ser Gly Thr Leu Asn Pro Gln Lys Ala Arg Val
290 295 300

Leu Leu Gln Leu Ala Leu Thr Gln Thr Lys Asp Pro Gln Gln Ile Gln
305 310 315 320

Gln Ile Phe Asn Gln Tyr
325

<210> 4

<211> 984

<212> DNA

<213> Sequência Artificial

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<223> Sequência de nucleotídeos da asparagina mutada de E.coli

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gtgccgcaac tgaaggacat tgcgaacggt aaaggcgagc aggtcgcttc gatcggtctc 180

gaaaatatga cctctgatgt tctgctgaaa ctgagtaaac gtgtcaacga agattgcgac 240

aaaaccgatg gttttgtgat taccatggc accgacacga tggaagaaac cgcttacttc 300

ctcgacctga cggtgaaatg cgacaaaccg gtggtgatgg tcggtgcaat gcgtccgtcc 360

acgtctatga gcgcagacgg tccattcaac ctgtataacg cggtagtac tgcagctgat 420

aaagcctccg ctaatcgtgg cgtactggta gtgatgaacg acaccgtgct tgatggccgt 480

gatgtcacca aaaccaacac caccgatgta gcgaccttca agtctgttaa ctacggctct 540

ctgggttaca ttcacaacgg taagattgac taccaacgta ccccggcacg taagcacacc 600

agcgacacgc cgttc gatgt ctctaagctg aatgaactgc cgaaagtcgg cattgtttat 660

aactacgcta acgcatccga tcttccggct aaagcactgg tagatgcggg ctatgatggc 720
atcgtagcg ctggcgtggg taacggcaac ctgtataaat ccgtatttga cacccttgca 780
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atccaacaaa tcttcaatca gtac 984

<210> 5

<211> 980

<212> DNA

<213> Escherichia coli

<400> 5

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cgcaactaaa agacattgcg aacgttaaag gcgagcaggt agtgaatata ggtcccagg 180
acatgaacga taatgtctgg ctgacactgg cgaaaaaat taacaccgac tgcgataaaa 240
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<210> 6

<211> 975

<212> DNA

<213> *Erwinia carotovora*

<400> 6

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gaaaatatga ccagcgatgt tctgctgaag ctgagcaagc gcgtgaacga gctgctggca 240
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ccggcaacgg caatcagtgc tgacggcccc atgaacctgt acggtgcagt aaaagtggca 420
gcggataaaa actctcgcgg ccgcggcgtg ctggctcgtg ttaacgaccg tattggttct 480
gctcgtttca tcagcaaaac caacgcttct acgctggata cctttaaagc gccagaagaa 540
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cacaccacgc gttccgtatt tgacgtgacc aacgtcgata aactgccagc cgttgacatt 660
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tatttccacg cgtat

975

<210> 7

<211> 30

<212> DNA

<213> Sequência Artificial

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<223> Iniciador frag 1/2 direto

<400> 7

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30

<210> 8

<211> 26

<212> DNA

<213> Sequência Artificial

<220>

<223> Iniciador frag 1/2 reverso

<400> 8

catgccatgg gtaatcacia accatc

26

<210> 9

<211> 28

<212> DNA

<213> Sequência Artificial

<220>

<223> Iniciador frag 3 direto

<400> 9

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28

<210> 10

<211> 27

<212> DNA

<213> Sequência Artificial

<220>

<223> Iniciador frag 3 reverso

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27

<210> 11

<211> 27

<212> DNA

<213> Sequência Artificial

<220>

<223> Iniciador Ecar-LANS direto

<400> 11

catatgaacc tgccgaacat tgtgatt

27

<210> 12

<211> 30

<212> DNA

<213> Sequência Artificial

<220>

<223> Iniciador Ecar-LANS reverso

<400> 12

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30