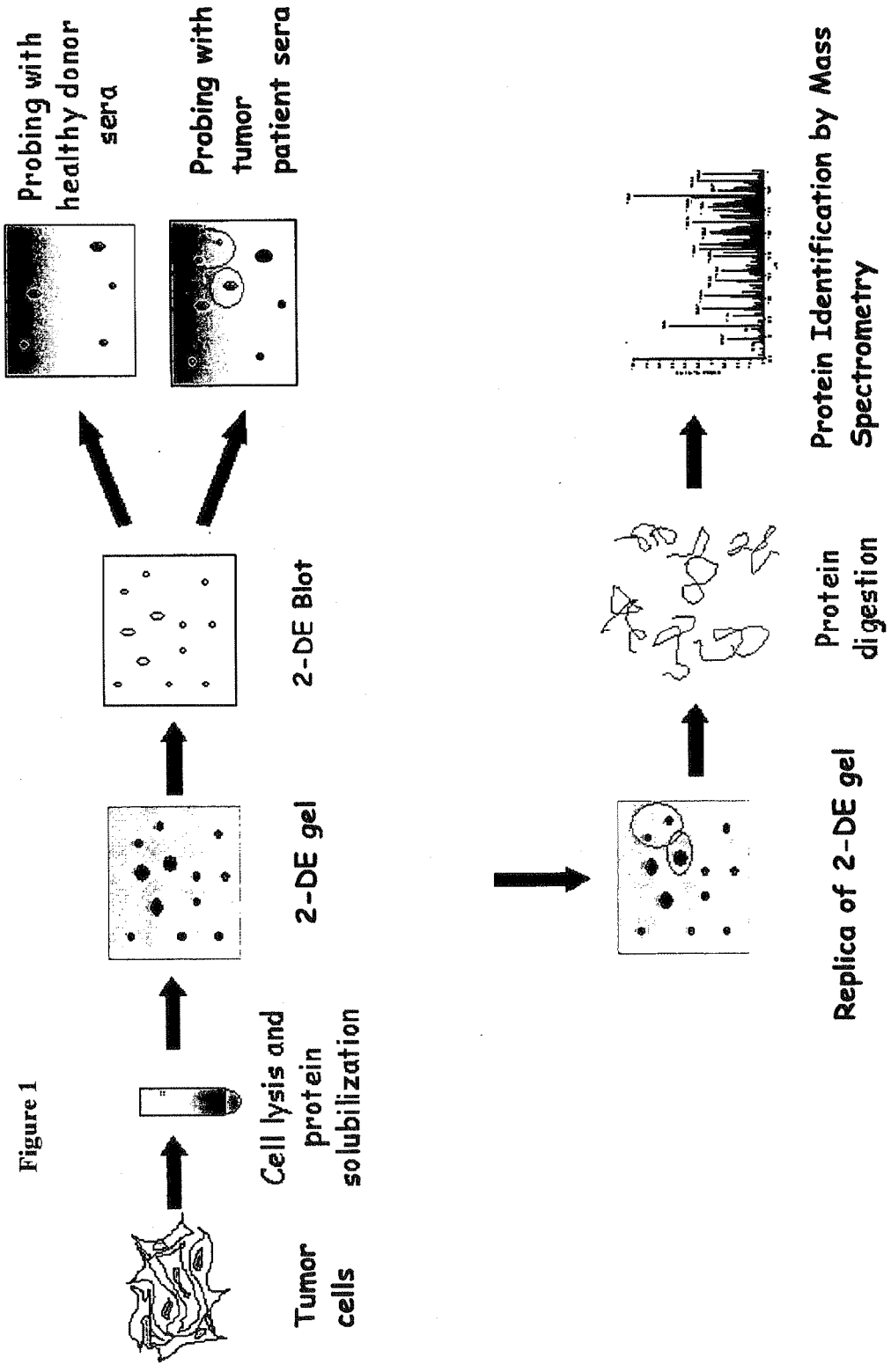
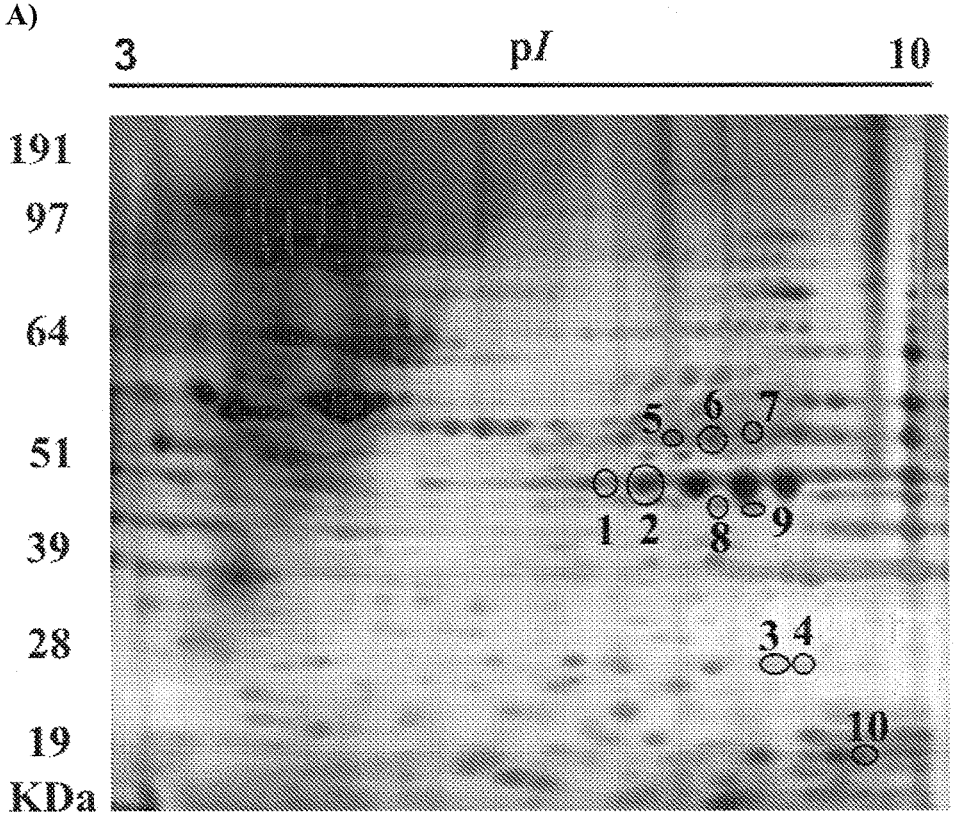

Tumor cells
Cell lysis and protein solubilization
2-DE gel
2-DE Blot
Probing with healthy donor sera
Probing with tumor patient sera
Replica of 2-DE gel
Protein digestion
Protein Identification by Mass Spectrometry

Figure 1

Figure 2

A)


3
pI
10
191
97
64
51
39
28
19
KDa
1
2
3
4
5
6
7
8
9
10

B)

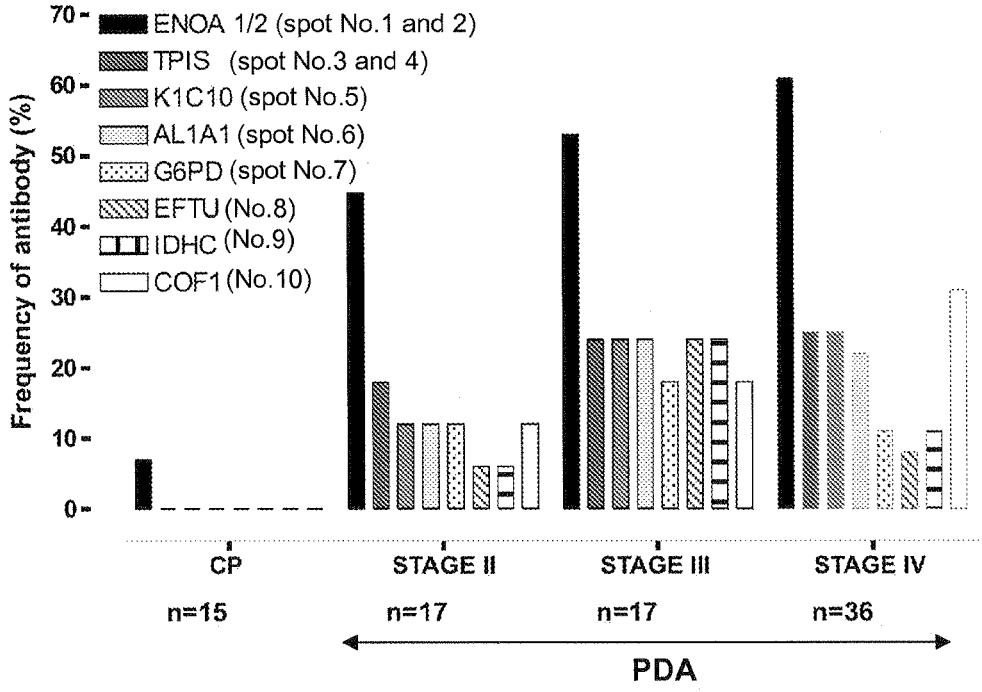
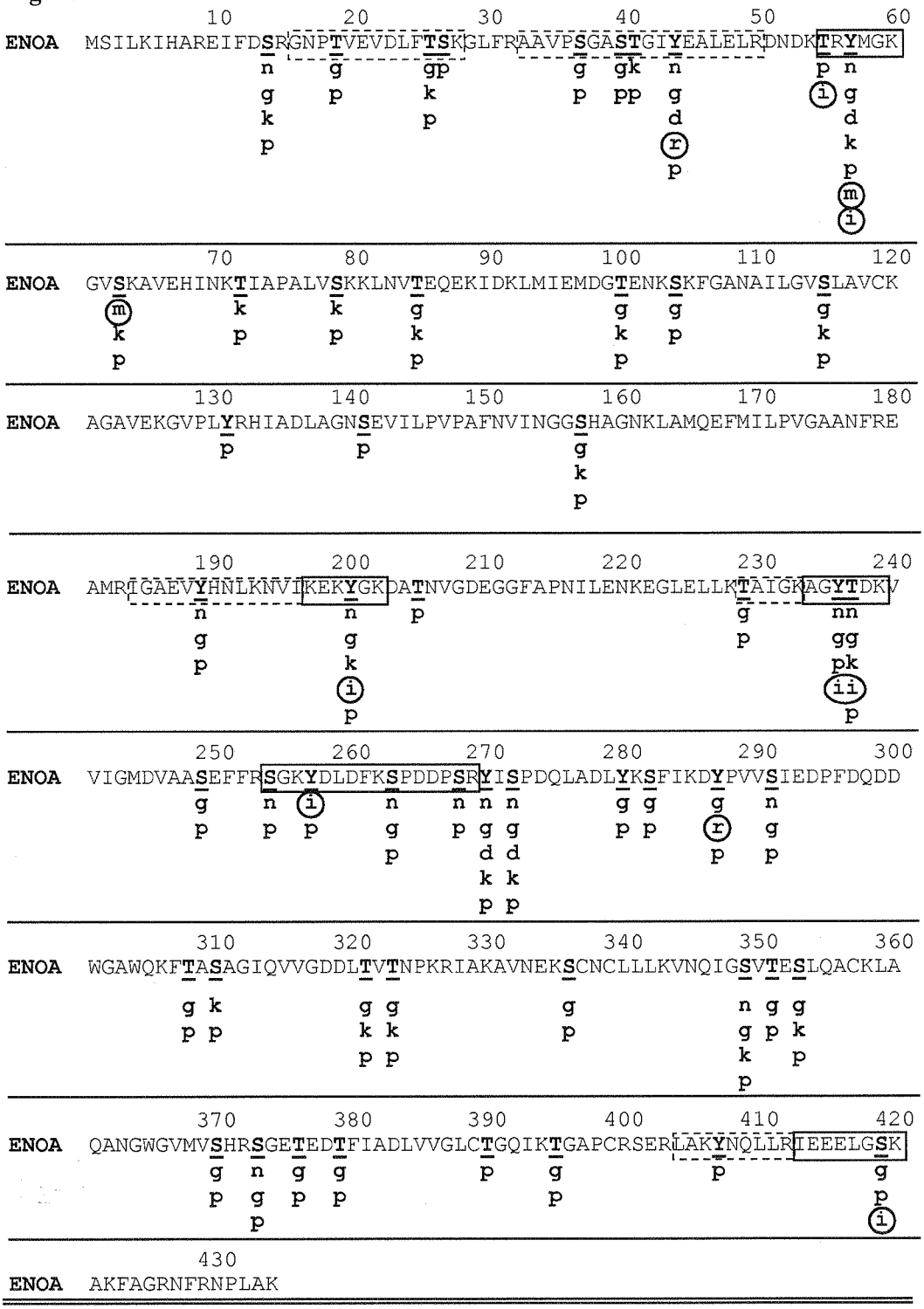

Frequency of antibody (%)
70
60
50
40
30
20
10
0
ENOA 1/2 (spot No.1 and 2)
TPIS (spot No.3 and 4)
K1C10 (spot No.5)
AL1A1 (spot No.6)
G6PD (spot No.7)
EFTU (No.8)
IDHC (No.9)
COF1 (No.10)
CP
STAGE II
STAGE III
STAGE IV
n=15
n=17
n=17
n=36
PDA

Figure 3

```
                10        20        30        40        50        60
ENOA  MSILKIHAREIFDSRGNPTVEVDLFTSKGLFRAAVPSGASTGIYEALELRDNDKTRYMGK
      :: :: ::::.:::::::::::::.:.:::::::::::::::::::::::::.:: ::.:::
ENOG  MSIEKIWAREILDSRGNPTVEVDLYTAKGLFRAAVPSGASTGIYEALELRDGDKQRYLGK

                70        80        90       100       110       120
ENOA  GVSKAVEHINKTIAPALVSKKLNVTEQEKIDKLMIEMDGTENKSKFGANAILGVSLAVCK
      :: :::.:::.:::::::.:. :.:.::::.:.::.:.:::::::::::::::::::::::
ENOG  GVLKAVDHINSTIAPALISSGLSVVEQEKLDNLMLELDGTENKSKFGANAILGVSLAVCK

               130       140       150       160       170       180
ENOA  AGAVEKGVPLYRHIADLAGNSEVILPVPAFNVINGGSHAGNKLAMQEFMILPVGAANFRE
      :::.:..:::::::.:::::. .:::::::::::::::::::::::::::::::: .::.
ENOG  AGAAERELPLYRHIAQLAGNSDLILPVPAFNVINGGSHAGNKLAMQEFMILPVGAESFRD

               190       200       210       220       230       240
ENOA  AMRIGAEVYHNLKNVIKEKYGKDATNVGDEGGFAPNILENKEGLELLKTAIGKAGYTDKV
      :::.:::::::.::.:::.:::::::::::::::::::::::.:.::::.: :: ::::::.:.
ENOG  AMRLGAEVYHTLKGVIKDKYGKDATNVGDEGGFAPNILENSEALELVKEAIDKAGYTEKI

               250       260       270       280       290       300
ENOA  VIGMDVAASEFFRSGKYDLDFKSPDDPSRYISPDQLADLYKSFIKDYPVVSIEDPFDQDD
      :::::::::::.:.:::::::::: :::::::. :::. ::..:..:::::::::::::::
ENOG  VIGMDVAASEFYRDGKYDLDFKSPTDPSRYITGDQLGALYQDFVRDYPVVSIEDPFDQDD

               310       320       330       340       350       360
ENOA  WGAWQKFTASAGIQVVGDDLTVTNPKRIAKAVNEKSCNCLLLKVNQIGSVTESLQACKLA
      :.::.::::..:::.:::::::::::::: .::.::.:::::::::::::::..::::::
ENOG  WAAWSKFTANVGIQIVGDDLTVTNPKRIERAVEEKACNCLLLKVNQIGSVTEAIQACKLA

               370       380       390       400       410       420
ENOA  QANGWGVMVSHRSGETEDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQLLRIEEELGSK
      : :::::::::::::::::::::::::::::::::::::::::::::::::.:::::::..
ENOG  QENGWGVMVSHRSGETEDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQLMRIEEELGDE

               430
ENOA  AKFAGRNFRNPLAK
      :.::::.:::::
ENOG  ARFAGHNFRNPSVL
```

Figure 4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ENOA AGAVEKGVPLYRHIADLAGNSEVILPVPAFNVINGGSHAGNKLAMQEFMILPVGAANFRE
ENOA AMRIGAEVYHNLKNVIKEKYGKDATNVGDEGGFAPNILENKEGLELLKTAIGKAGYTDKV
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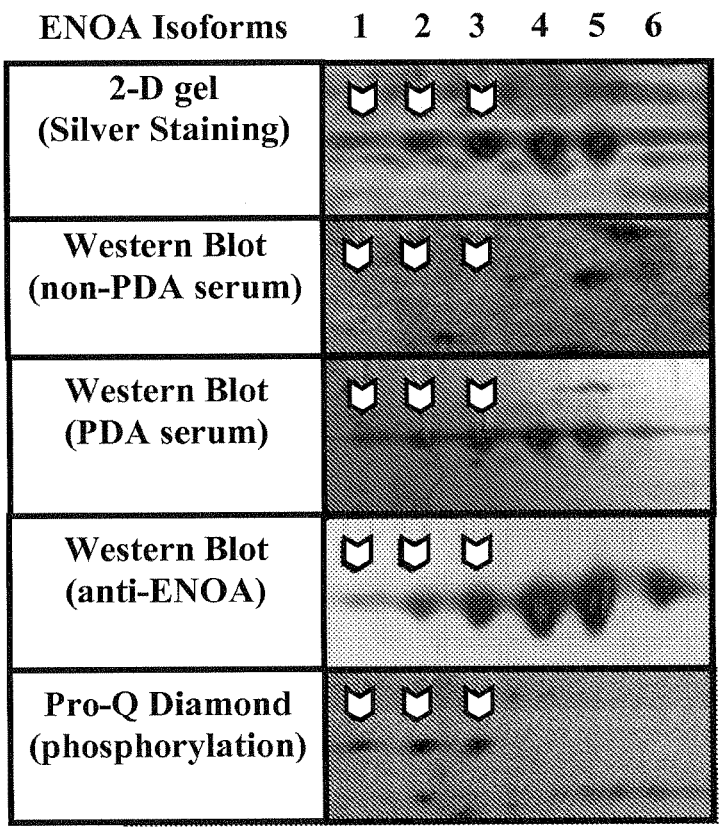
ENOA QANGWGVMVSHRSGETEDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQLLRIEEELGSK
ENOA AKFAGRNFRNPLAK
n NetPhos
g GPS
d dbPTM
k NetPhosk
p PSPP
m Identified in Molina H et al.,2007
r Identified in Rush J et al., 2005
i Identified in the ENOA Isoform 3 isolated from CF-PAC-1 cell extracts by 2-DE

Figure 5

A)


ENOA Isoforms 1 2 3 4 5 6
2-D gel (Silver Staining)
Western Blot (non-PDA serum)
Western Blot (PDA serum)
Western Blot (anti-ENOA)
Pro-Q Diamond (phosphorylation)

B)

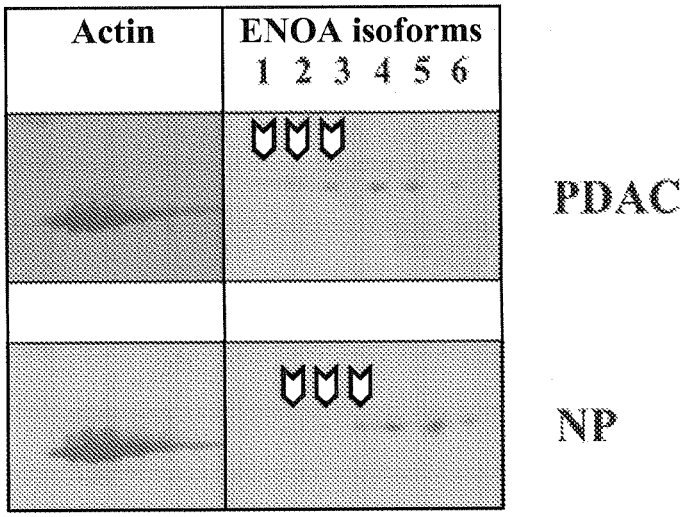
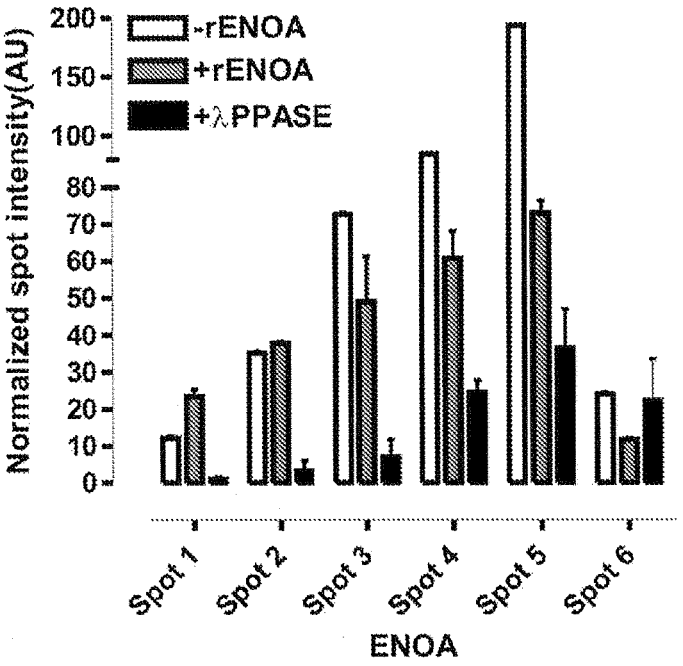

Actin
ENOA isoforms
1 2 3 4 5 6
PDAC
NP

Figure 6


Normalized spot intensity(AU)
200
150
100
80
70
60
50
40
30
20
10
0
-rENOA
+rENOA
+λPPASE
Spot 1
Spot 2
Spot 3
Spot 4
Spot 5
Spot 6
ENOA

SEQUENCE LISTING

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Bioline Diagnostici S.R.L.

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35 40 45

Leu Arg Asp Asn Asp Lys Thr Arg Tyr Met Gly Lys Gly Val Ser Lys
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85 90 95

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Pro Leu Tyr Arg His Ile Ala Asp Leu Ala Gly Asn Ser Glu Val Ile
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Leu Pro Val Pro Ala Phe Asn Val Ile Asn Gly Gly Ser His Ala Gly
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Asn Lys Leu Ala Met Gln Glu Phe Met Ile Leu Pro Val Gly Ala Ala
165 170 175

Asn Phe Arg Glu Ala Met Arg Ile Gly Ala Glu Val Tyr His Asn Leu
180 185 190

Lys Asn Val Ile Lys Glu Lys Tyr Gly Lys Asp Ala Thr Asn Val Gly
195 200 205

Asp Glu Gly Gly Phe Ala Pro Asn Ile Leu Glu Asn Lys Glu Gly Leu
210 215 220

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

Val Ile Gly Met Asp Val Ala Ala Ser Glu Phe Phe Arg Ser Gly Lys
245 250 255

Tyr Asp Leu Asp Phe Lys Ser Pro Asp Asp Pro Ser Arg Tyr Ile Ser
260 265 270

Pro Asp Gln Leu Ala Asp Leu Tyr Lys Ser Phe Ile Lys Asp Tyr Pro
275 280 285

Val Val Ser Ile Glu Asp Pro Phe Asp Gln Asp Asp Trp Gly Ala Trp
290 295 300

Gln Lys Phe Thr Ala Ser Ala Gly Ile Gln Val Val Gly Asp Asp Leu
305 310 315 320

Thr Val Thr Asn Pro Lys Arg Ile Ala Lys Ala Val Asn Glu Lys Ser
325 330 335

Cys Asn Cys Leu Leu Leu Lys Val Asn Gln Ile Gly Ser Val Thr Glu
340 345 350

Ser Leu Gln Ala Cys Lys Leu Ala Gln Ala Asn Gly Trp Gly Val Met
355 360 365

Val Ser His Arg Ser Gly Glu Thr Glu Asp Thr Phe Ile Ala Asp Leu
370 375 380

Val Val Gly Leu Cys Thr Gly Gln Ile Lys Thr Gly Ala Pro Cys Arg
385 390 395 400

Ser Glu Arg Leu Ala Lys Tyr Asn Gln Leu Leu Arg Ile Glu Glu Glu
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Leu Gly Ser Lys Ala Lys Phe Ala Gly Arg Asn Phe Arg Asn Pro Leu
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Ala Lys

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<212> PRT
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            20                  25                  30

Ala Ala Val Pro Ser Gly Ala Ser Thr Gly Ile Tyr Glu Ala Leu Glu
        35                  40                  45

Leu Arg Asp Gly Asp Lys Gln Arg Tyr Leu Gly Lys Gly Val Leu Lys
    50                  55                  60

Ala Val Asp His Ile Asn Ser Thr Ile Ala Pro Ala Leu Ile Ser Ser
65                  70                  75                  80

Gly Leu Ser Val Val Glu Gln Glu Lys Leu Asp Asn Leu Met Leu Glu
                85                  90                  95

Leu Asp Gly Thr Glu Asn Lys Ser Lys Phe Gly Ala Asn Ala Ile Leu
            100                 105                 110

Gly Val Ser Leu Ala Val Cys Lys Ala Gly Ala Ala Glu Arg Glu Leu
        115                 120                 125

Pro Leu Tyr Arg His Ile Ala Gln Leu Ala Gly Asn Ser Asp Leu Ile
    130                 135                 140

Leu Pro Val Pro Ala Phe Asn Val Ile Asn Gly Gly Ser His Ala Gly
145                 150                 155                 160

Asn Lys Leu Ala Met Gln Glu Phe Met Ile Leu Pro Val Gly Ala Glu
                165                 170                 175

Ser Phe Arg Asp Ala Met Arg Leu Gly Ala Glu Val Tyr His Thr Leu
            180                 185                 190

Lys Gly Val Ile Lys Asp Lys Tyr Gly Lys Asp Ala Thr Asn Val Gly
        195                 200                 205

Asp Glu Gly Gly Phe Ala Pro Asn Ile Leu Glu Asn Ser Glu Ala Leu
    210                 215                 220

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

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

Tyr Asp Leu Asp Phe Lys Ser Pro Thr Asp Pro Ser Arg Tyr Ile Thr
260 265 270

Gly Asp Gln Leu Gly Ala Leu Tyr Gln Asp Phe Val Arg Asp Tyr Pro
275 280 285

Val Val Ser Ile Glu Asp Pro Phe Asp Gln Asp Asp Trp Ala Ala Trp
290 295 300

Ser Lys Phe Thr Ala Asn Val Gly Ile Gln Ile Val Gly Asp Asp Leu
305 310 315 320

Thr Val Thr Asn Pro Lys Arg Ile Glu Arg Ala Val Glu Glu Lys Ala
325 330 335

Cys Asn Cys Leu Leu Leu Lys Val Asn Gln Ile Gly Ser Val Thr Glu
340 345 350

Ala Ile Gln Ala Cys Lys Leu Ala Gln Glu Asn Gly Trp Gly Val Met
355 360 365

Val Ser His Arg Ser Gly Glu Thr Glu Asp Thr Phe Ile Ala Asp Leu
370 375 380

Val Val Gly Leu Cys Thr Gly Gln Ile Lys Thr Gly Ala Pro Cys Arg
385 390 395 400

Ser Glu Arg Leu Ala Lys Tyr Asn Gln Leu Met Arg Ile Glu Glu Glu
405 410 415

Leu Gly Asp Glu Ala Arg Phe Ala Gly His Asn Phe Arg Asn Pro Ser
420 425 430

Val Leu