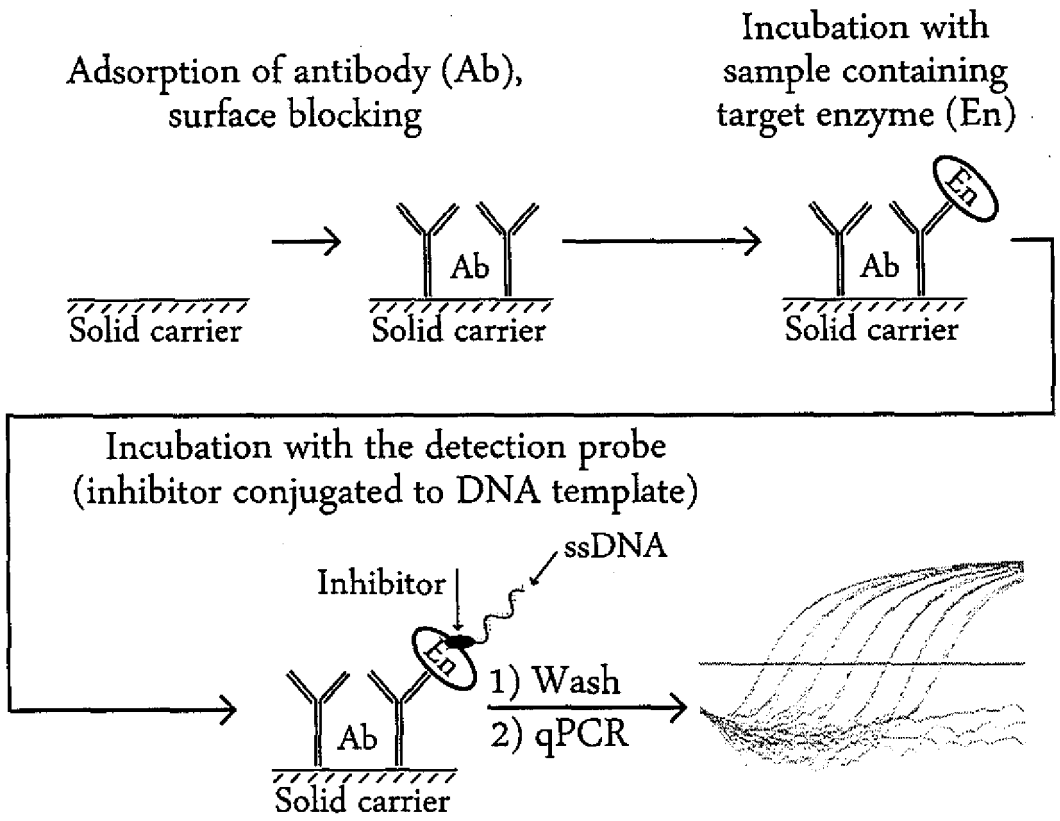
Adsorption of antibody (Ab), surface blocking
Incubation with sample containing target enzyme (En)
Ab
En
Solid carrier
Solid carrier
Solid carrier
Incubation with the detection probe (inhibitor conjugated to DNA template)
ssDNA
Inhibitor
En
Ab
Solid carrier
1) Wash
2) qPCR

Fig. 1

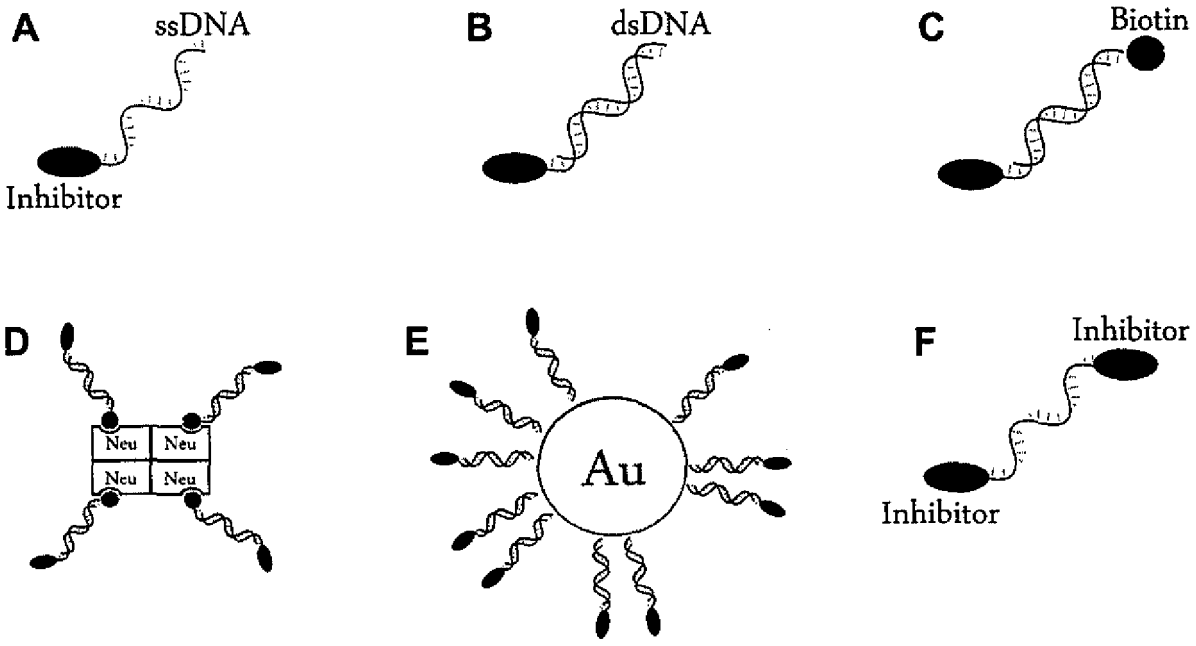
A
ssDNA
Inhibitor
B
dsDNA
C
Biotin
D
Neu
Neu
Neu
Neu
E
Au
F
Inhibitor
Inhibitor

Fig. 2

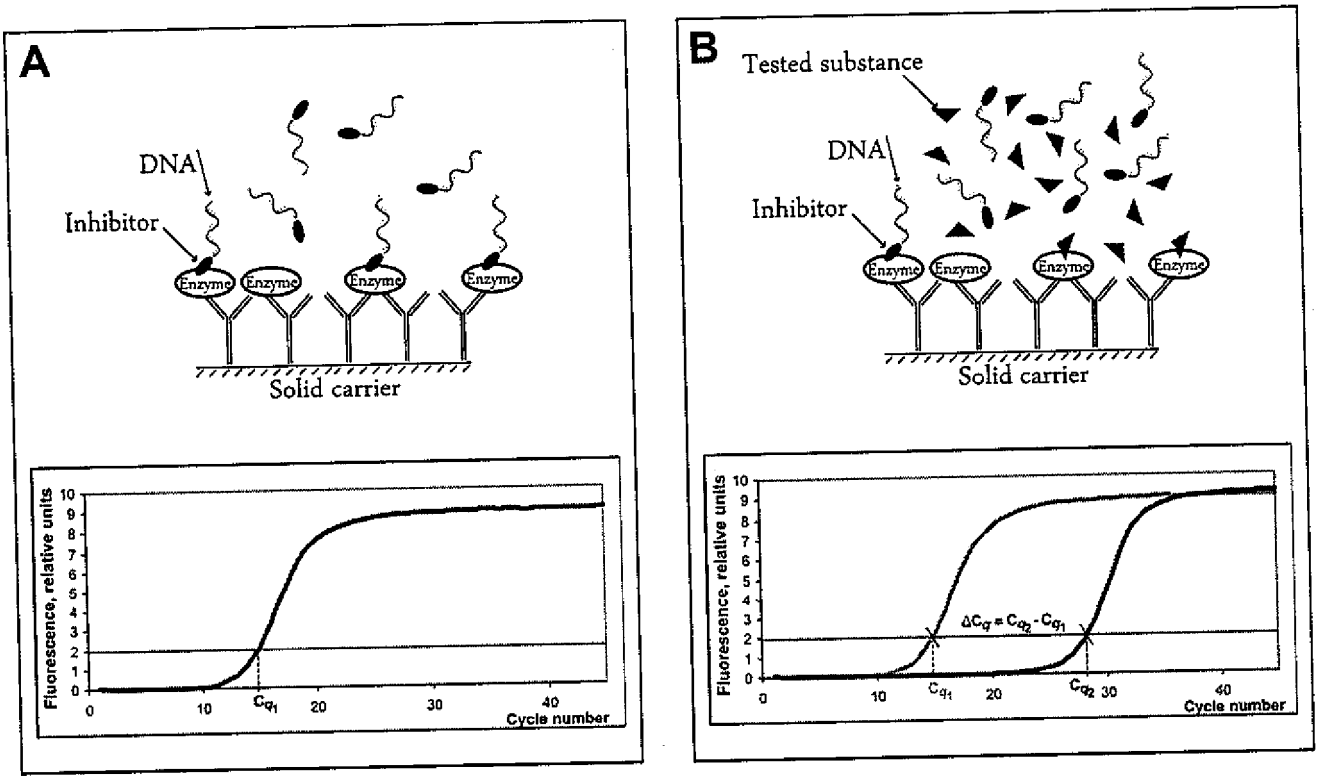
A
DNA
Inhibitor
Enzyme
Solid carrier
Fluorescence, relative units
Cycle number
B
Tested substance
DNA
Inhibitor
Enzyme
Solid carrier
Fluorescence, relative units
Cycle number

Fig. 3

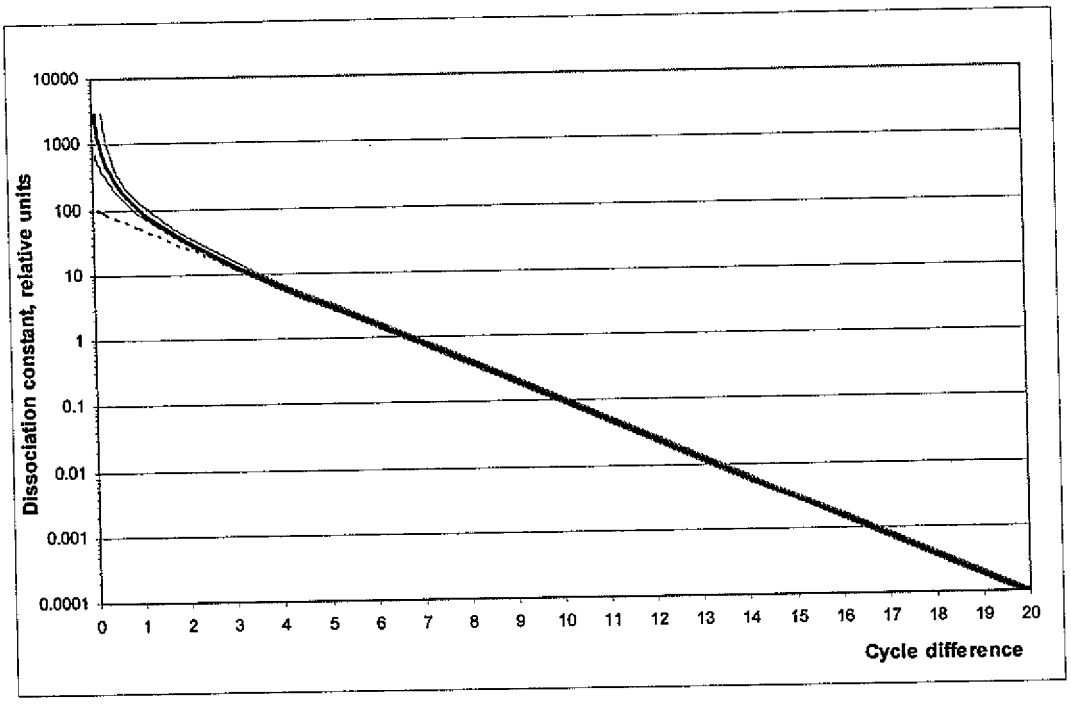
Dissociation constant, relative units
Cycle difference

Fig. 4

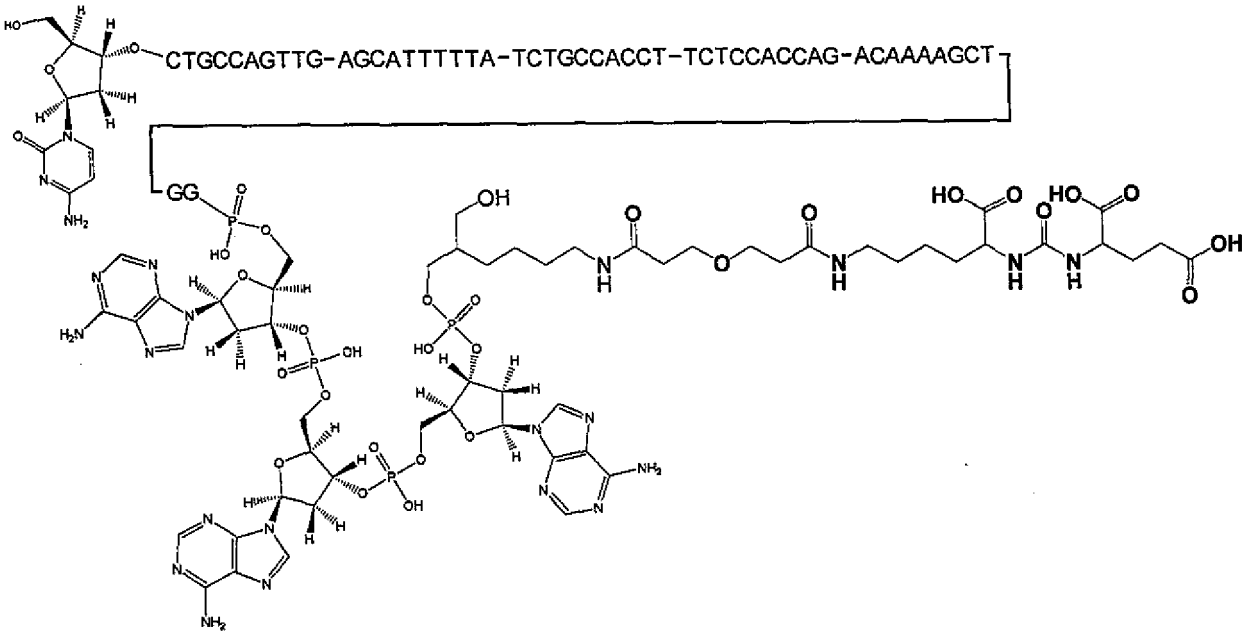
CTGCCAGTTG-AGCATTTTTA-TCTGCCACCT-TCTCCACCAG-ACAAAAGCT
GG
OH
HO
O
N
H
NH2
OH

Fig. 5

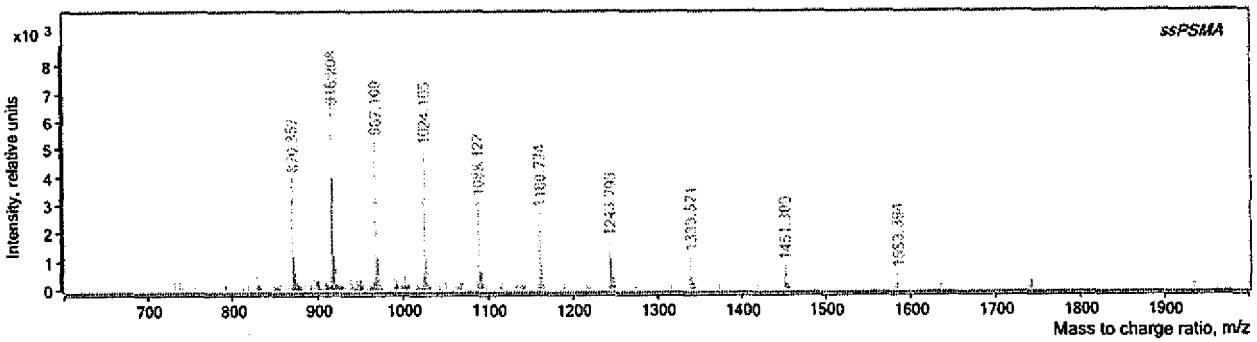
ssPSMA
x10 3
Intensity, relative units
0
1
2
3
4
5
6
7
8
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
Mass to charge ratio, m/z

Fig. 6

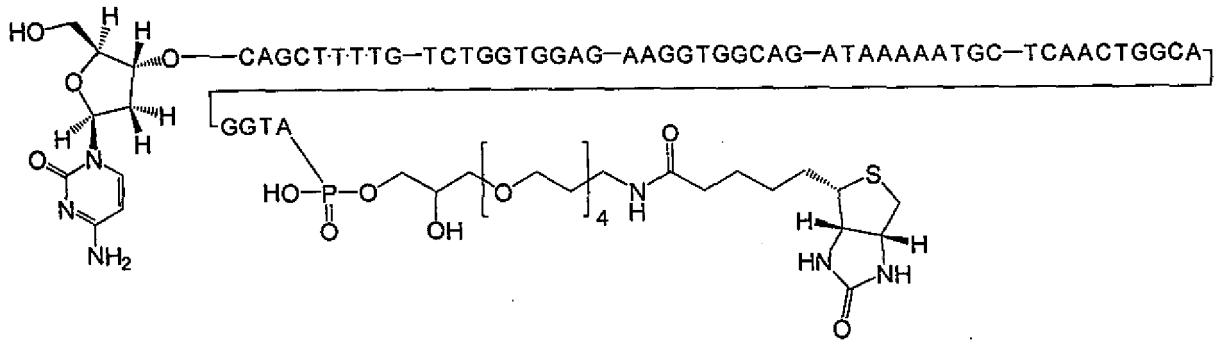
HO
H
H
O
CAGCTTTTG—TCTGGTGGAG—AAGGTGGCAG—ATAAAAATGC—TCAACTGGCA
GGTA
HO—P—O
O
OH
O
4
N
H
O
S
H
H
HN
NH
O
O
H
H
O
N
N
NH2

Fig. 7

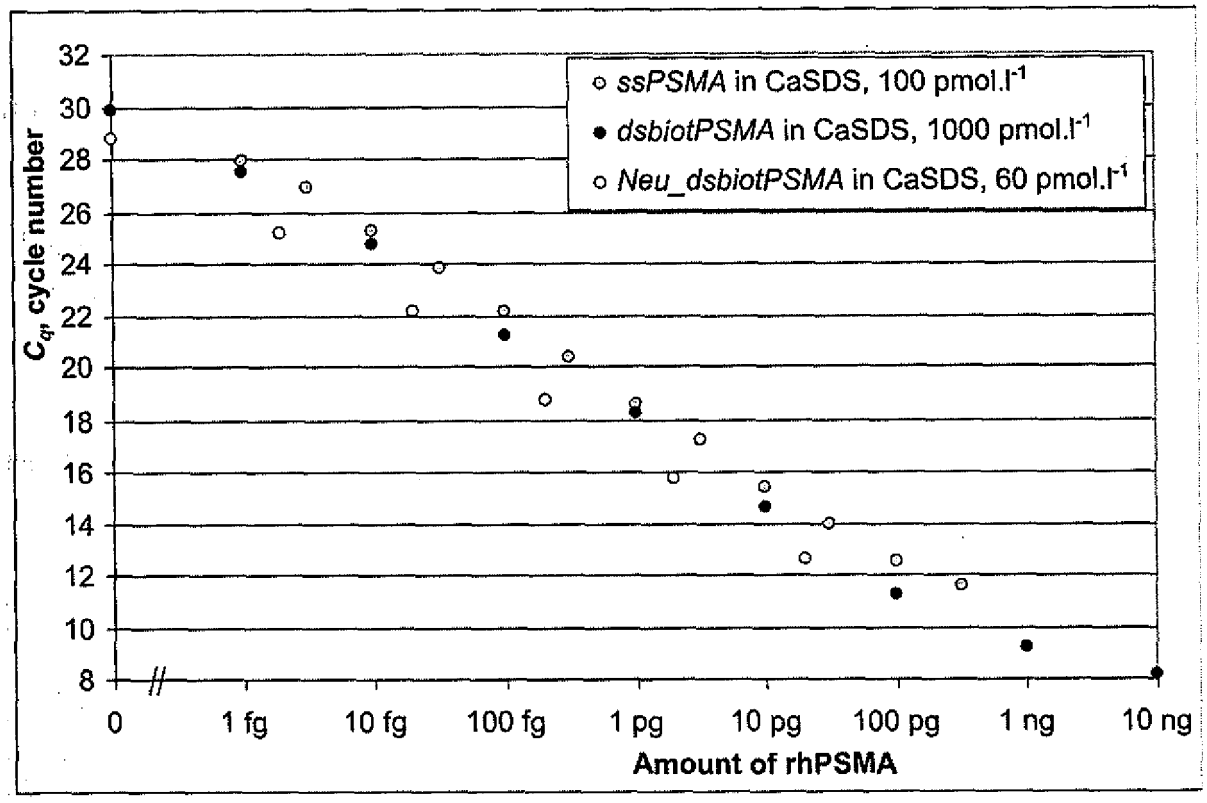
ssPSMA in CaSDS, 100 pmol.l-1
dsbiotPSMA in CaSDS, 1000 pmol.l-1
Neu_dsbiotPSMA in CaSDS, 60 pmol.l-1
Cq, cycle number
32
30
28
26
24
22
20
18
16
14
12
10
8
0
1 fg
10 fg
100 fg
1 pg
10 pg
100 pg
1 ng
10 ng
Amount of rhPSMA

Fig. 8

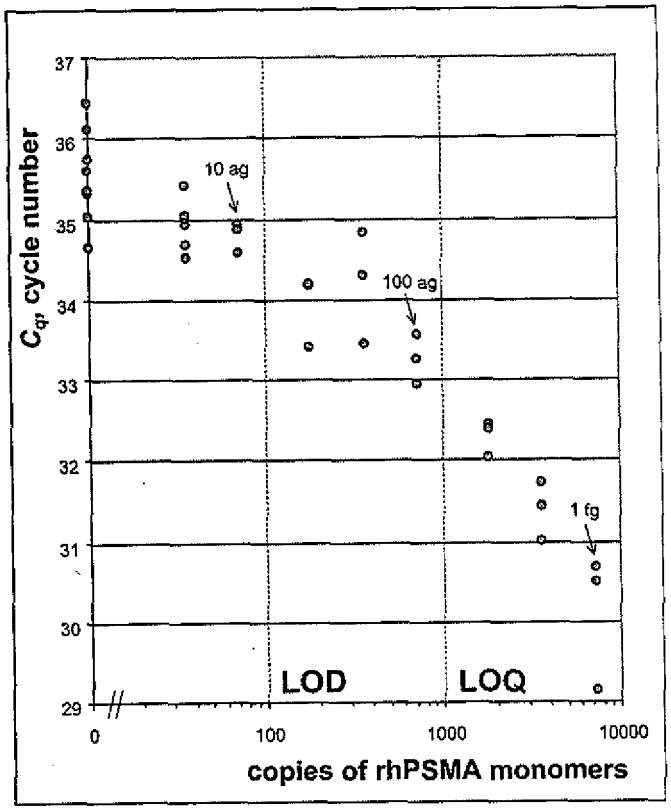
37
36
35
34
33
32
31
30
29
C_{qr} cycle number
10 ag
100 ag
1 fg
LOD
LOQ
0
100
1000
10000
copies of rhPSMA monomers

Fig. 9

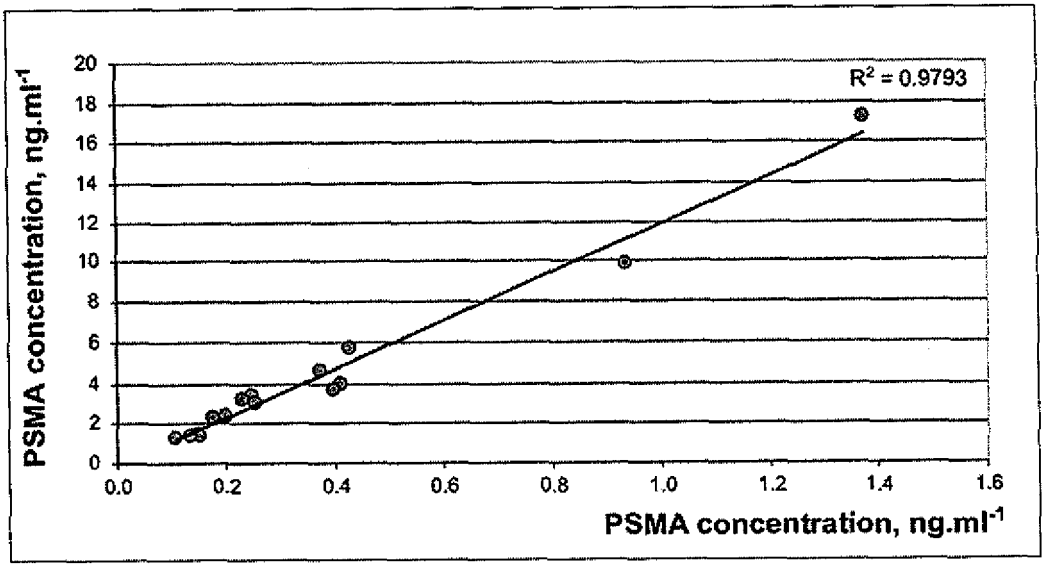
20
18
16
14
12
10
8
6
4
2
0
PSMA concentration, ng.ml^{-1}
R^2 = 0.9793
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
PSMA concentration, ng.ml^{-1}

Fig. 10

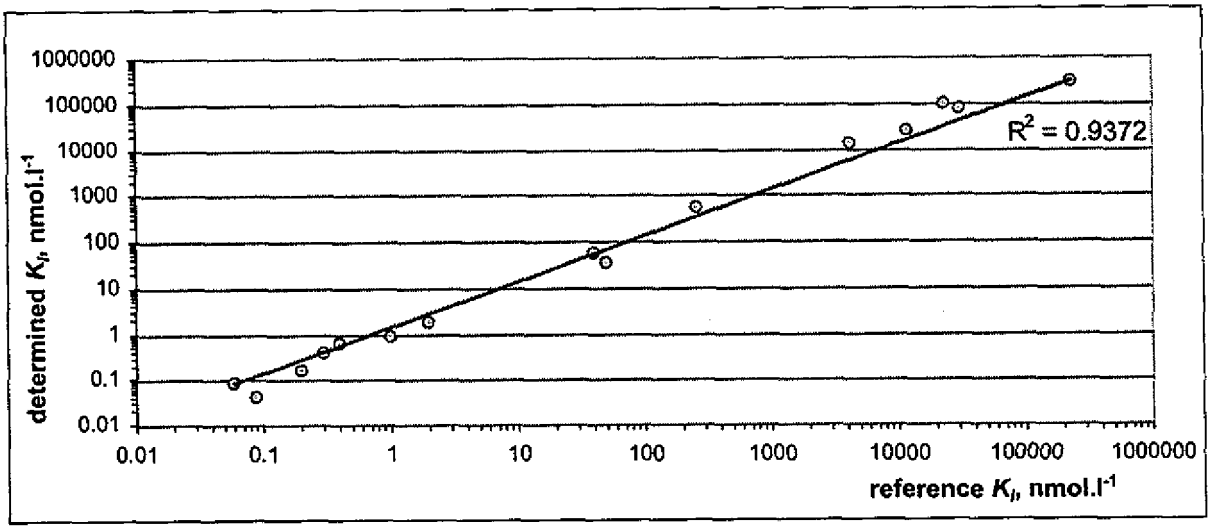
1000000
100000
10000
1000
100
10
1
0.1
0.01
determined K_i, nmol.l^{-1}
R^2 = 0.9372
0.01
0.1
1
10
100
1000
10000
100000
1000000
reference K_i, nmol.l^{-1}

Fig. 11

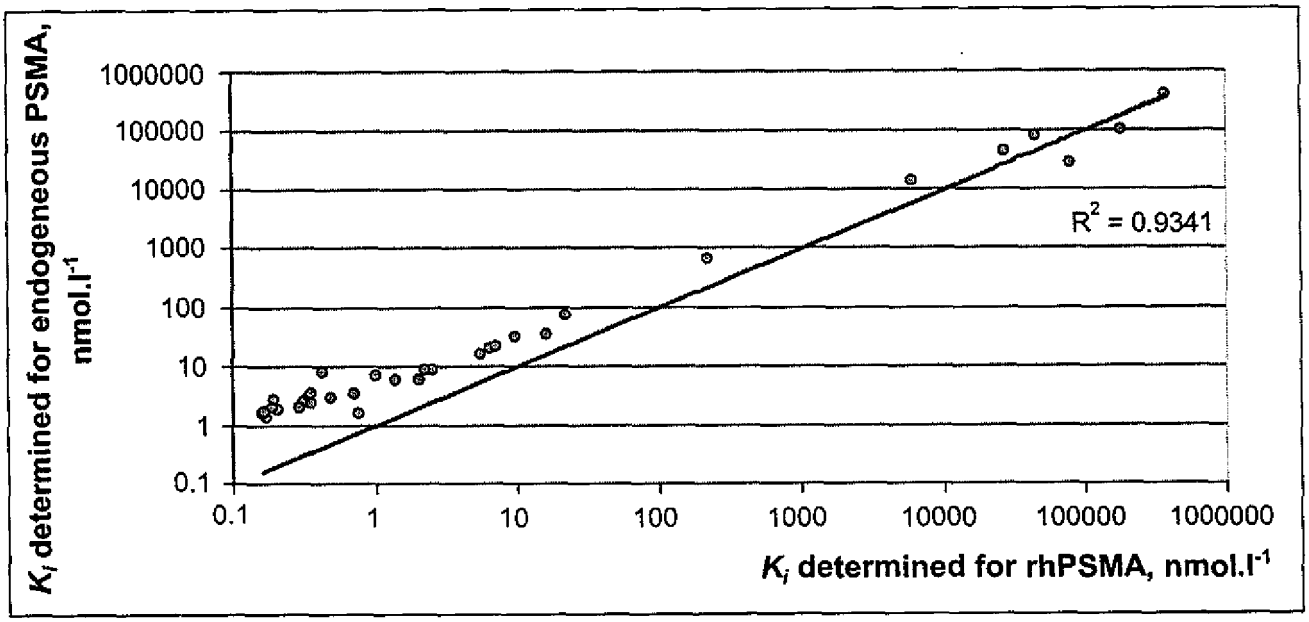
K_i determined for endogeneous PSMA, nmol.l^{-1}
1000000
100000
10000
1000
100
10
1
0.1
R^2 = 0.9341
0.1
1
10
100
1000
10000
100000
1000000
K_i determined for rhPSMA, nmol.l^{-1}

Fig. 12

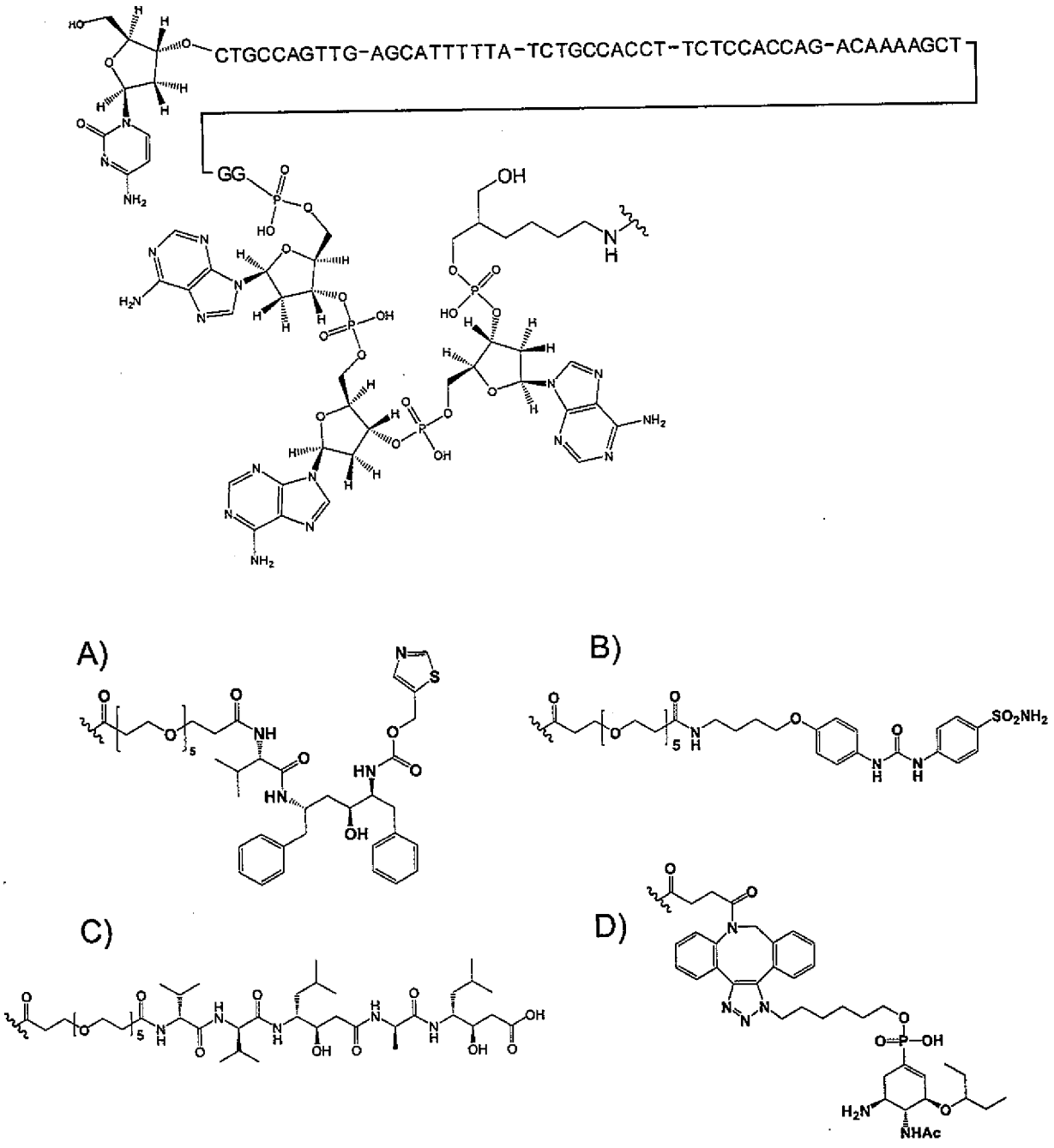
CTGCCAGTTG-AGCATTTTTA-TCTGCCACCT-TCTCCACCAG-ACAAAAGCT
GG
A)
B)
C)
D)
NHAc

Fig. 13

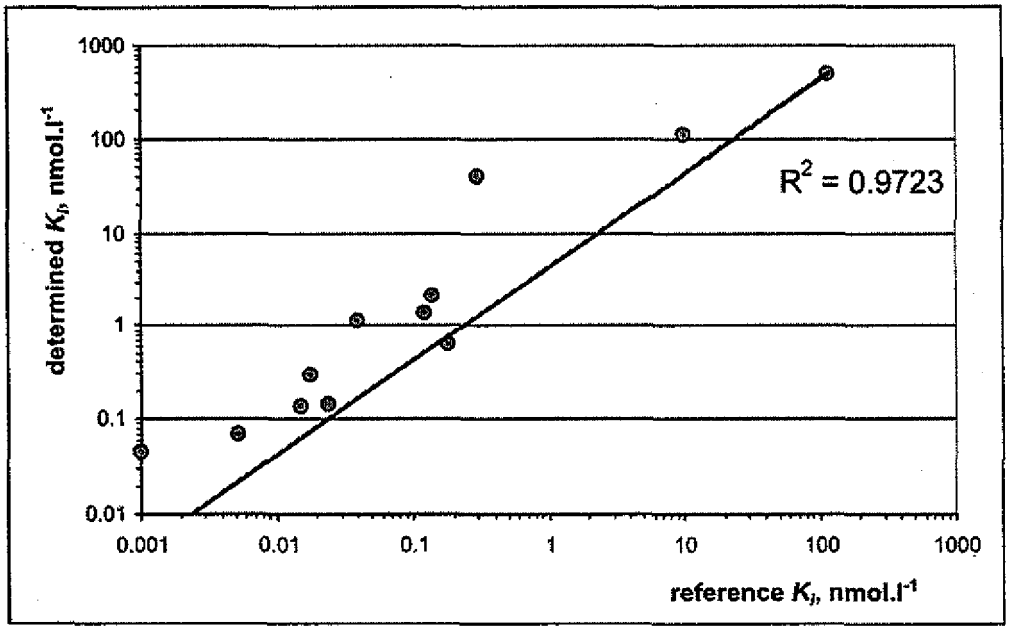
1000
100
10
1
0.1
0.01
determined K_i, nmol.l^{-1}
R^2 = 0.9723
0.001
0.01
0.1
1
10
100
1000
reference K_i, nmol.l^{-1}

Fig. 14

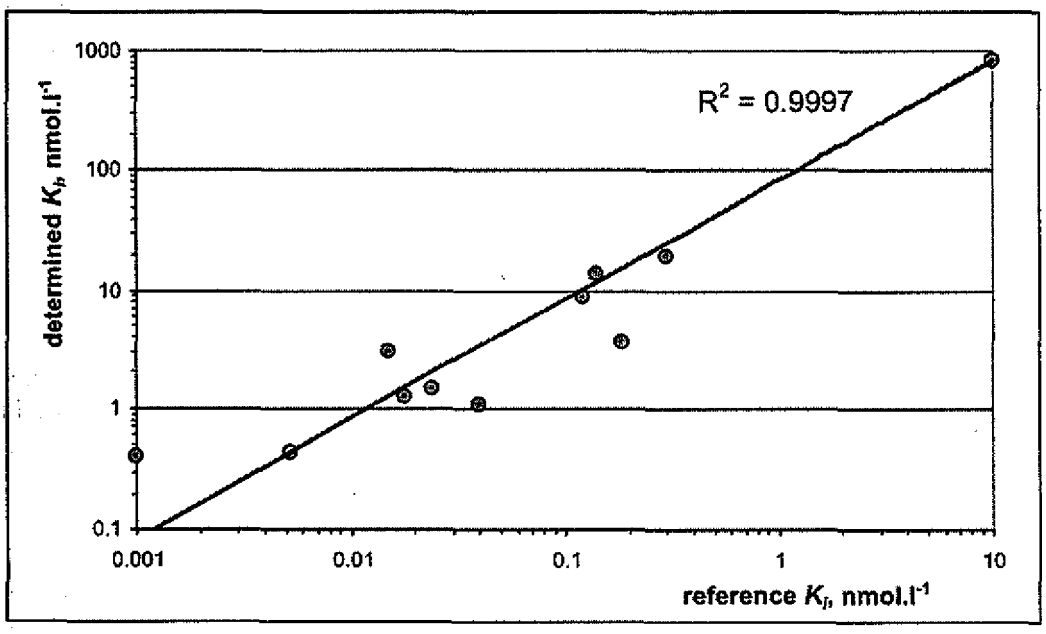
1000
100
10
1
0.1
determined K_i, nmol.l^{-1}
R^2 = 0.9997
0.001
0.01
0.1
1
10
reference K_i, nmol.l^{-1}

Fig. 15

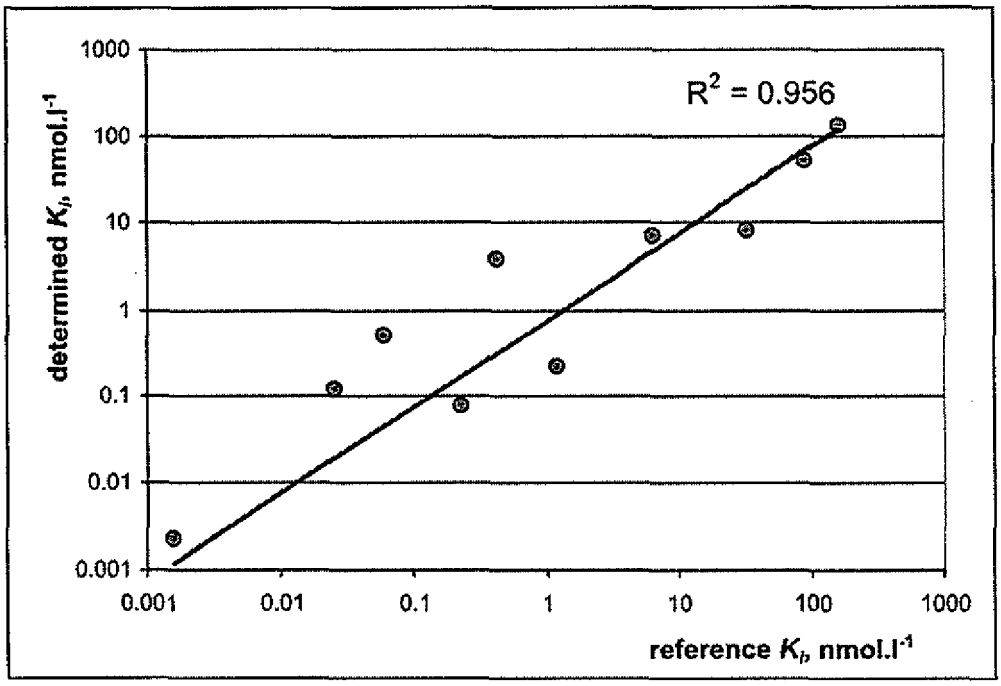
1000
100
10
1
0.1
0.01
0.001
determined K_i, nmol.l^{-1}
R^2 = 0.956
0.001
0.01
0.1
1
10
100
1000
reference K_i, nmol.l^{-1}

Fig. 16

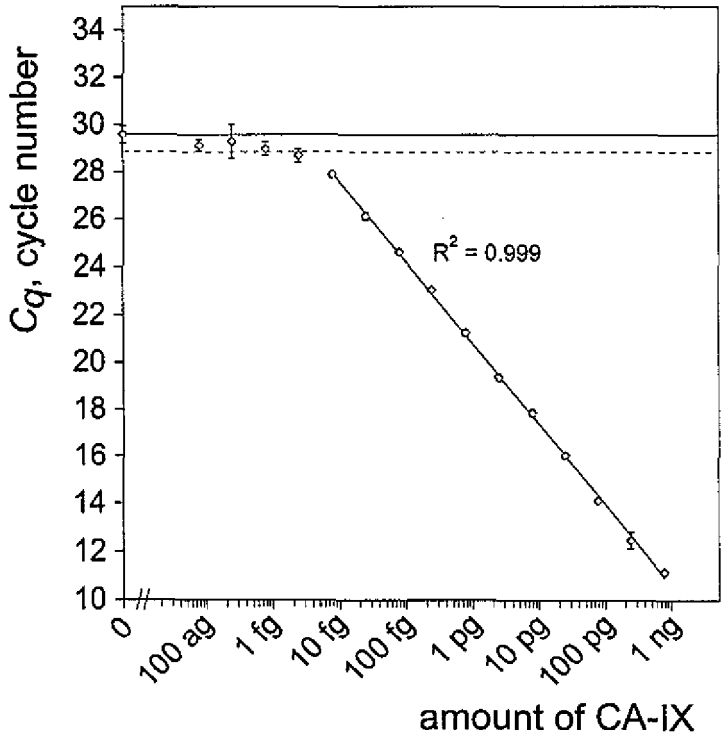
34
32
30
28
26
24
22
20
18
16
14
12
10
C_q, cycle number
R^2 = 0.999
0
100 ag
1 fg
10 fg
100 fg
1 pg
10 pg
100 pg
1 ng
amount of CA-IX

Fig. 17

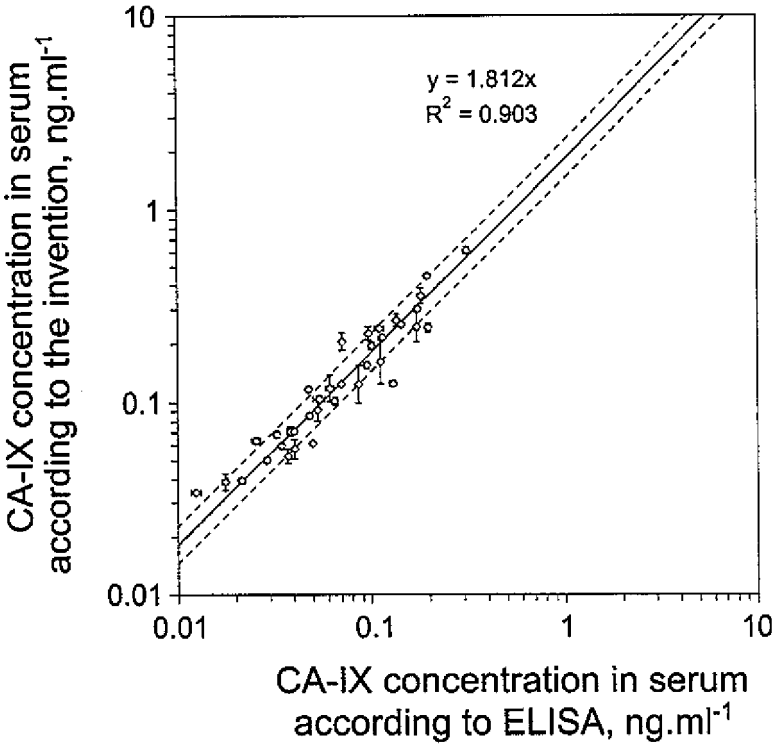
y = 1.812x
R^2 = 0.903
CA-IX concentration in serum according to the invention, ng.ml^{-1}
CA-IX concentration in serum according to ELISA, ng.ml^{-1}
10
1
0.1
0.01
0.01
0.1
1
10

Fig. 18

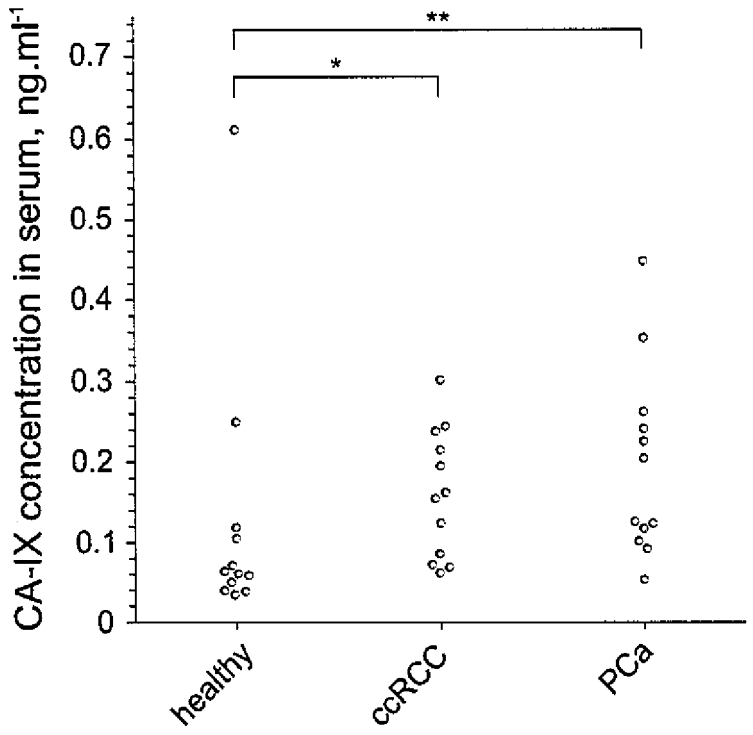
CA-IX concentration in serum, ng.ml^{-1}
**
*
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
healthy
ccRCC
PCa

Fig. 19