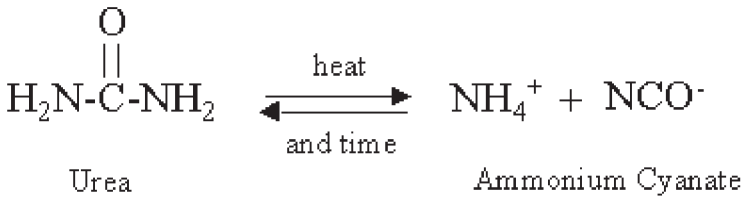
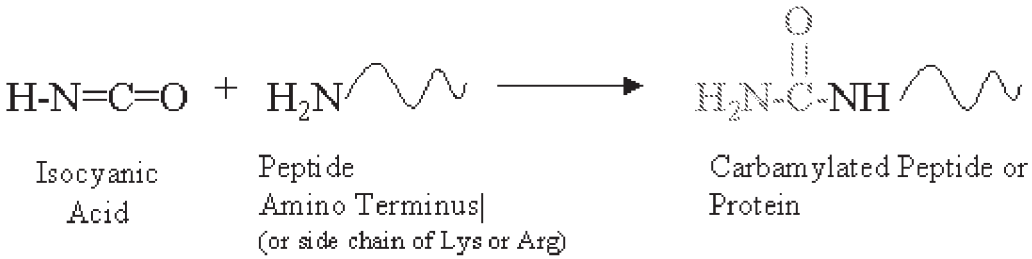
Fig. 1

Decomposition of urea



Carbamoylation of proteins

amino terminus of a peptide used as an example



Amino Acid	Residue Composition	Residue Monoisotopic Mass	Delta Mass
Lysine	$C_6H_{12}N_2O$	128.09496	0
Carbamoyl Lysine	$C_7H_{13}N_3O_2$	171.10078	43.00582
Carbamoylation	* NHCO	43.00582	-
*Note: A proton is lost from the amino group on the protein during carbamoylation and thus the change in composition is NHCO.			

http://ionsource.com/Card/carbam/carbam.htm

Fig. 2

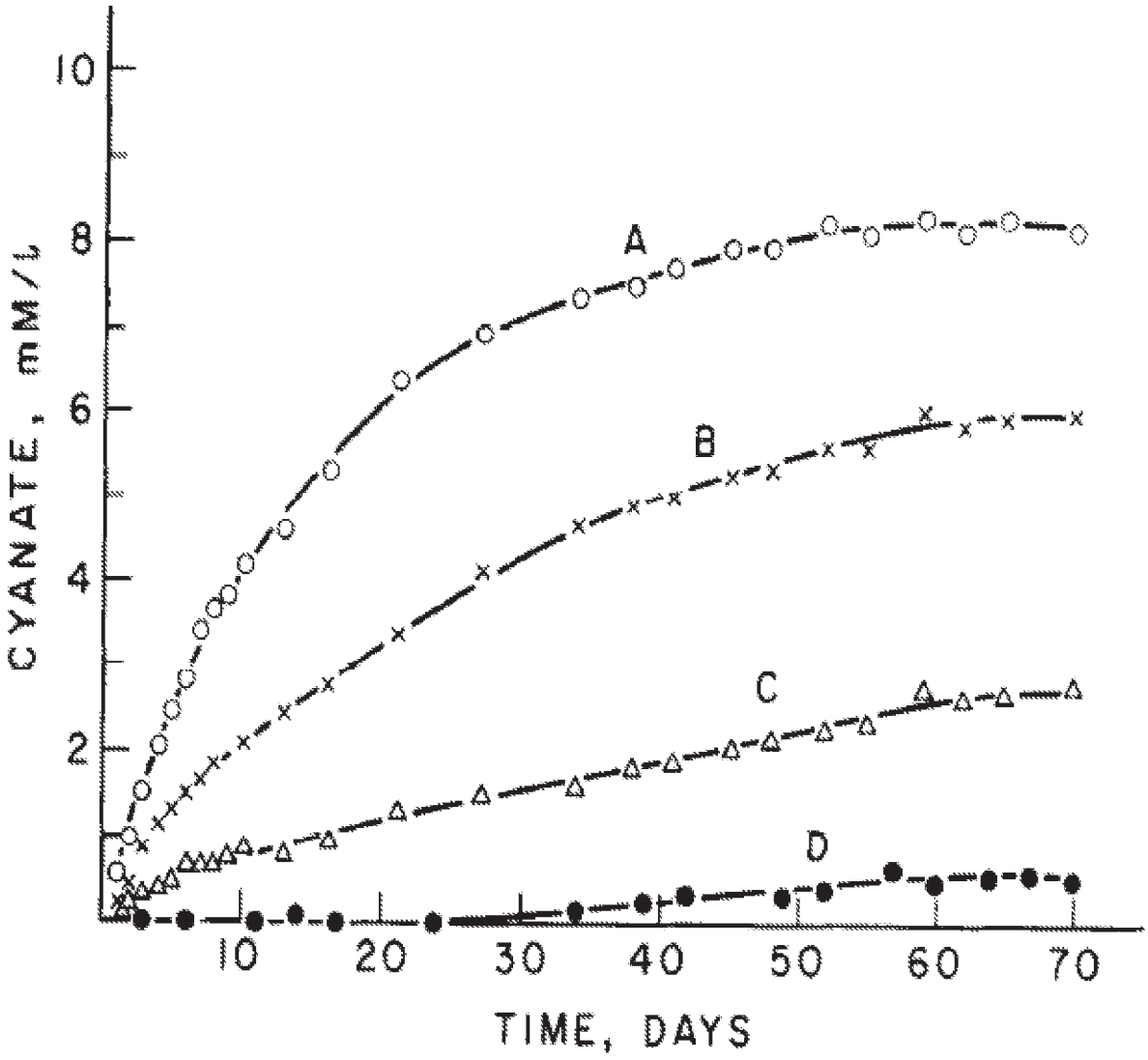


Fig. 3

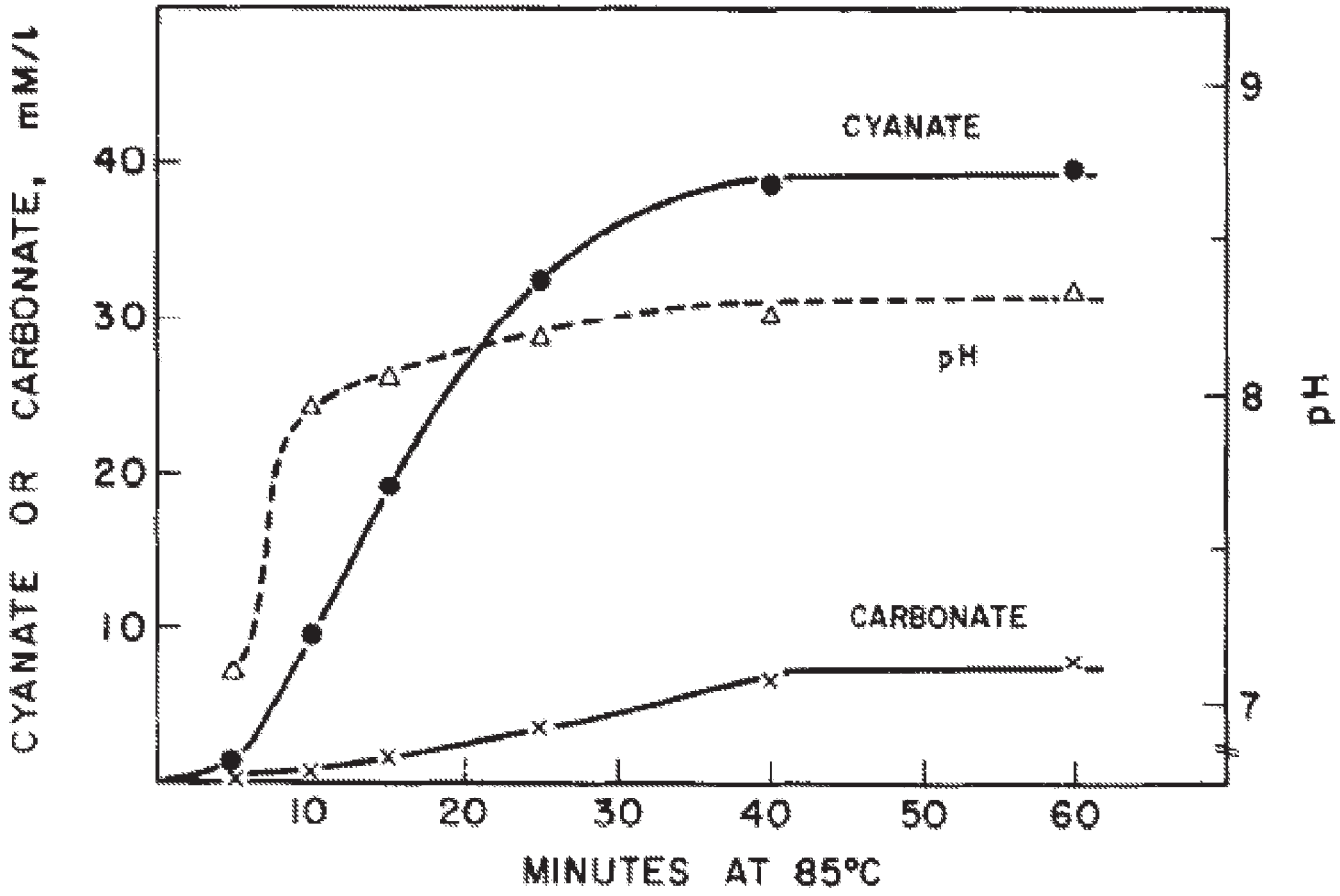

CYANATE OR CARBONATE, mM/L
40
30
20
10
CYANATE
pH
CARBONATE
9
8
7
pH
10
20
30
40
50
60
MINUTES AT 85°C

Fig. 4

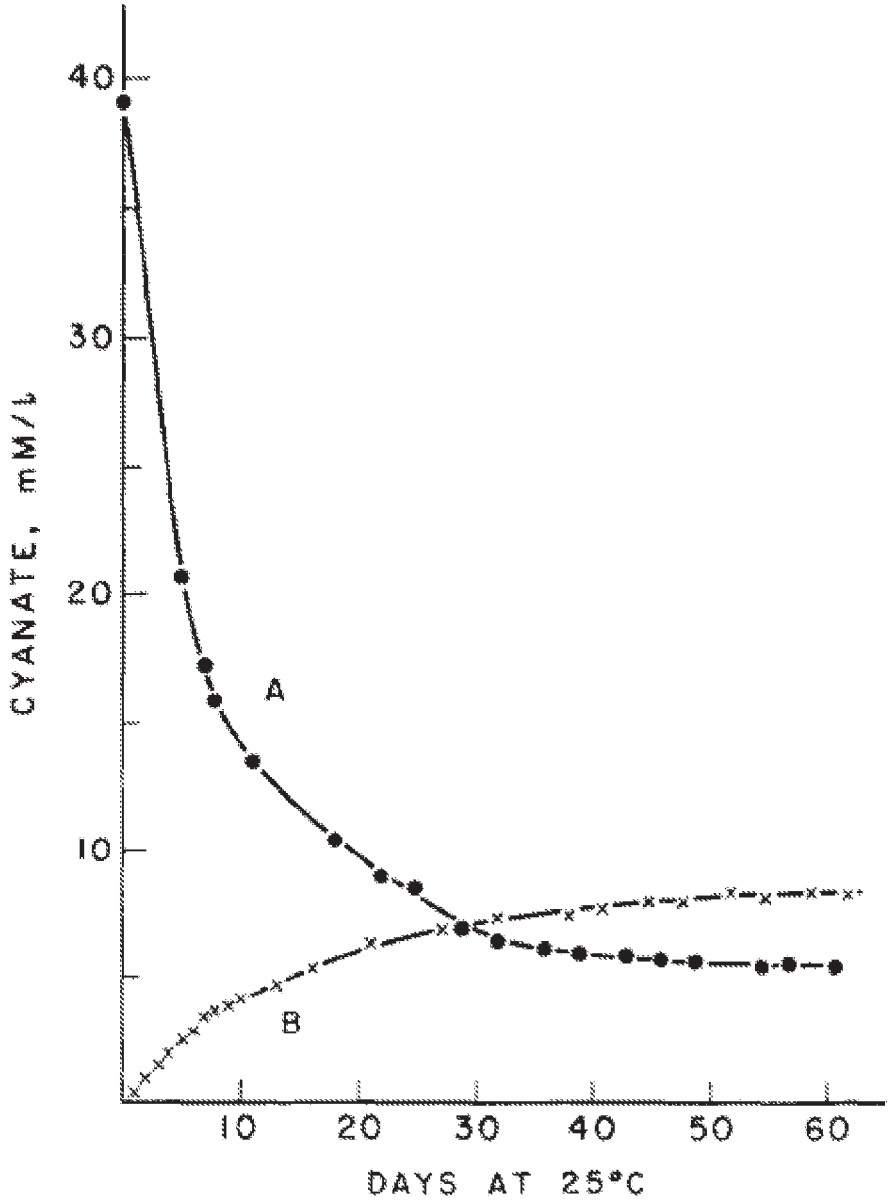
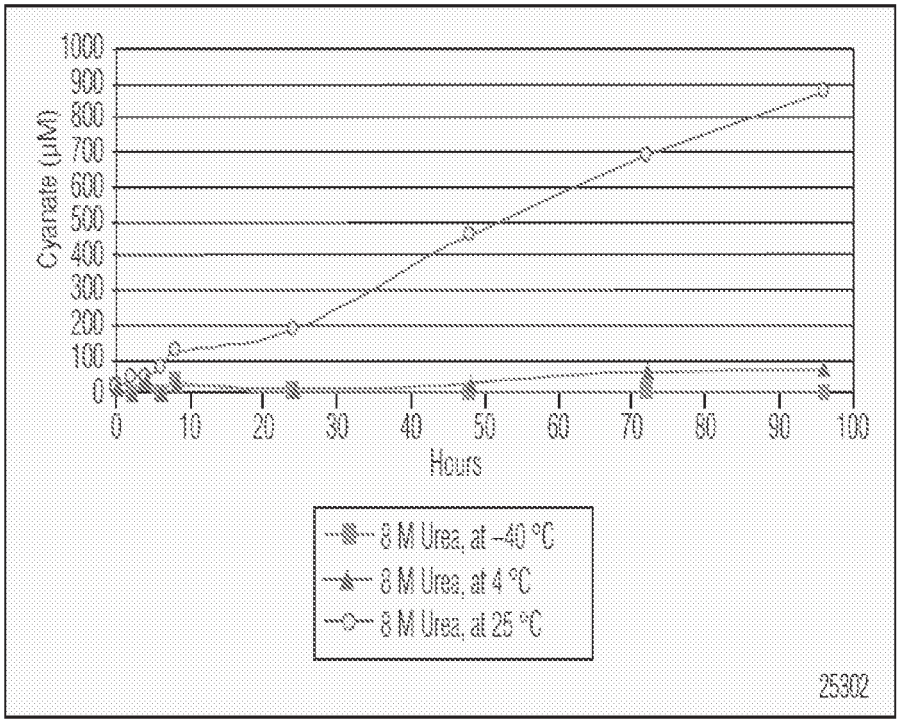


Fig. 5



Christison et al, „*Direct Determination of Cyanate in a Urea Solution and a Urea Containing Protein Buffer Using a Reagent-Free Ion Chromatography System*“, **Dionex (www.dionex.com**)

Fig. 6

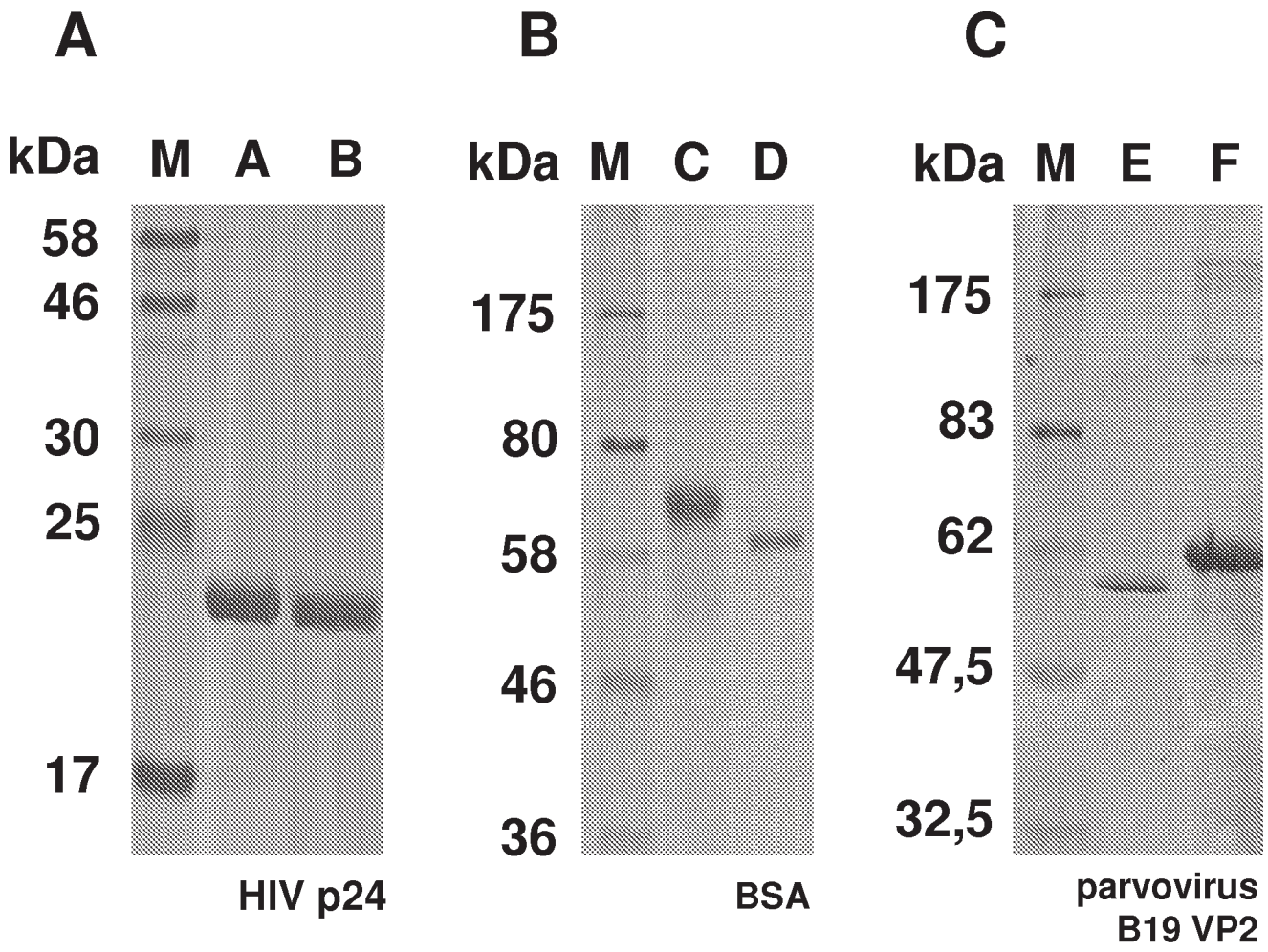

A
kDa
M
A
B
58
46
30
25
17
HIV p24
B
kDa
M
C
D
175
80
58
46
36
BSA
C
kDa
M
E
F
175
83
62
47,5
32,5
parvovirus
B19 VP2

Fig. 7

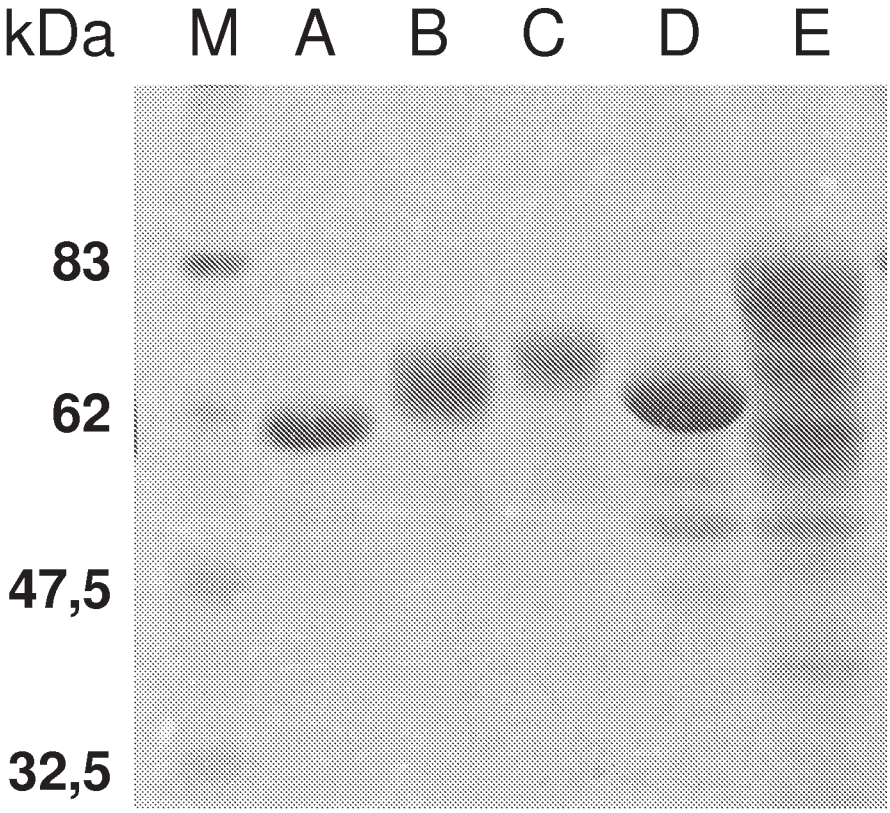


Fig. 8

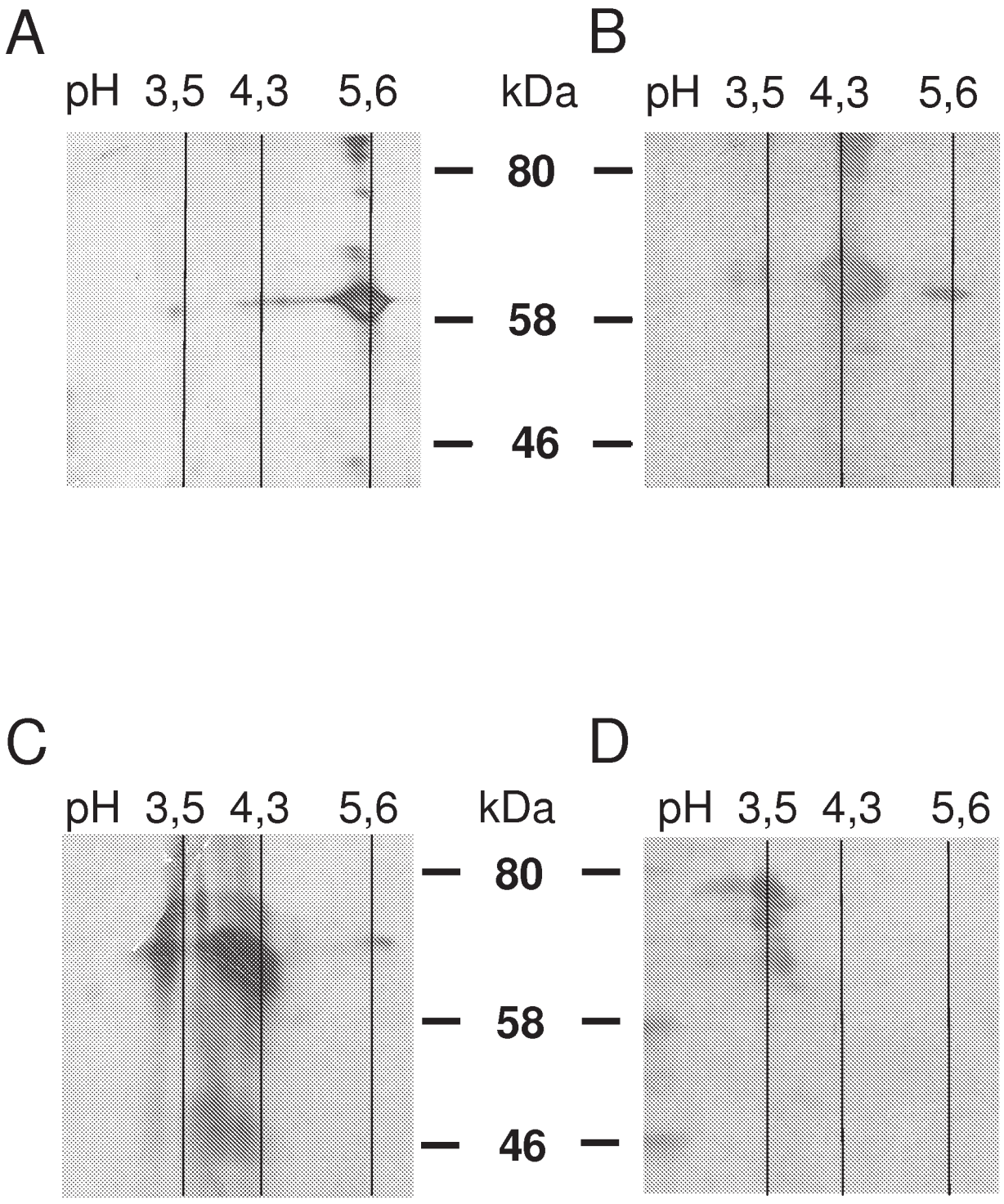

A
pH 3,5 4,3 5,6
kDa
80
58
46
B
pH 3,5 4,3 5,6
C
pH 3,5 4,3 5,6
kDa
80
58
46
D
pH 3,5 4,3 5,6

Fig. 9

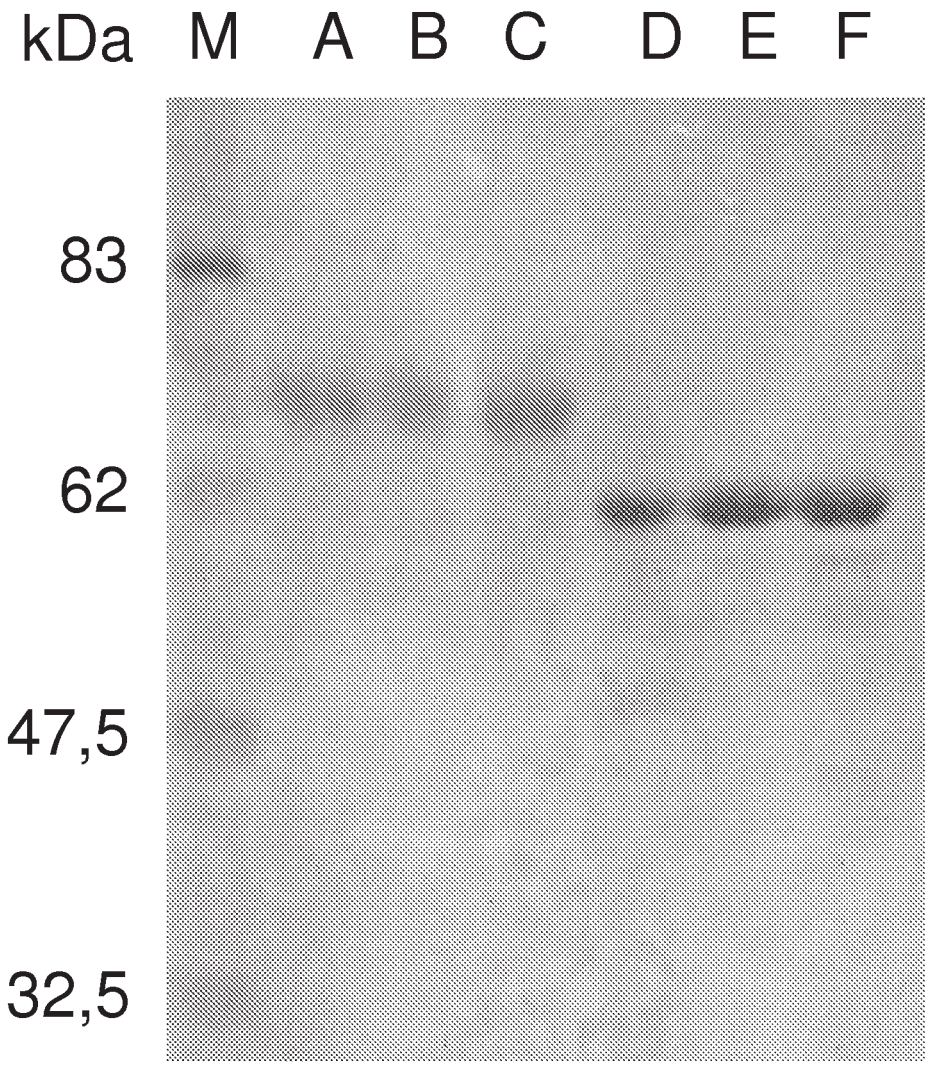


Fig. 10

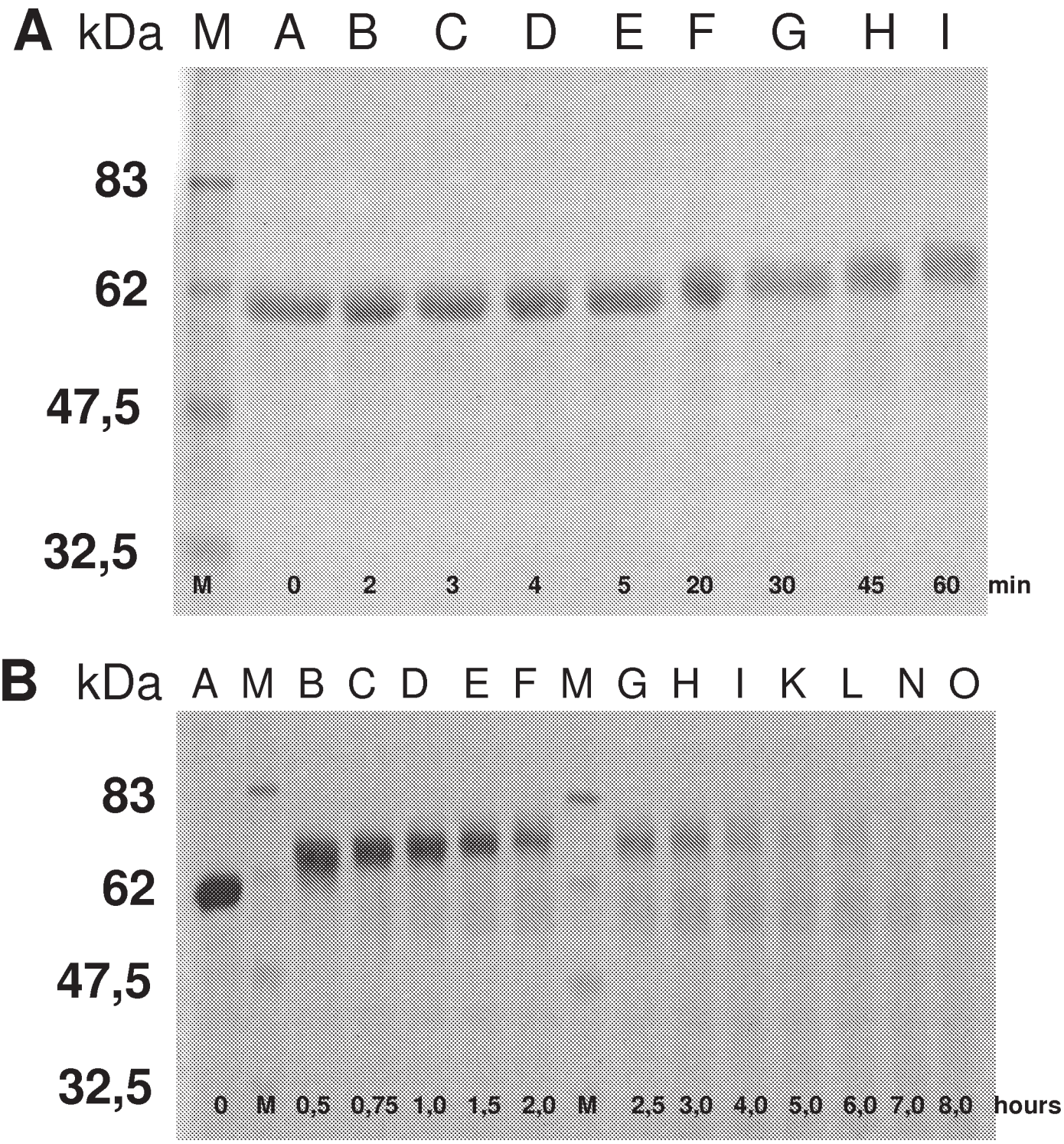

A kDa M A B C D E F G H I
83
62
47,5
32,5
M 0 2 3 4 5 20 30 45 60 min
B kDa A M B C D E F M G H I K L N O
83
62
47,5
32,5
0 M 0,5 0,75 1,0 1,5 2,0 M 2,5 3,0 4,0 5,0 6,0 7,0 8,0 hours

Fig. 11

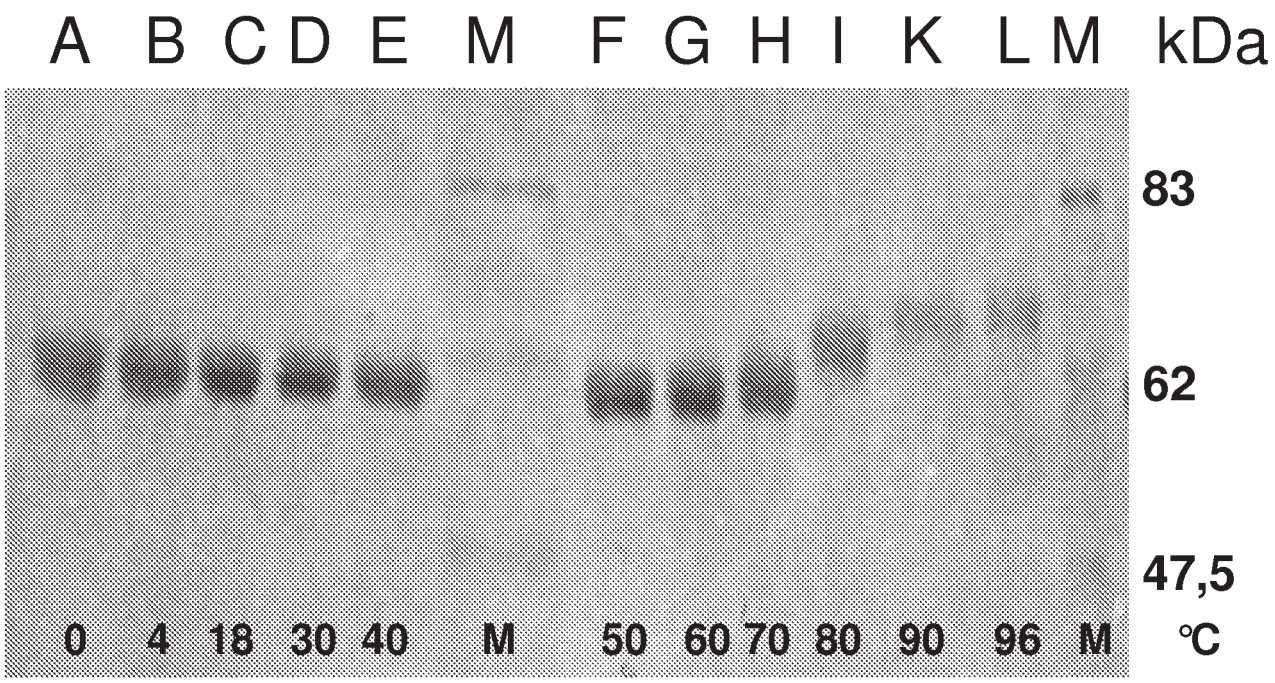
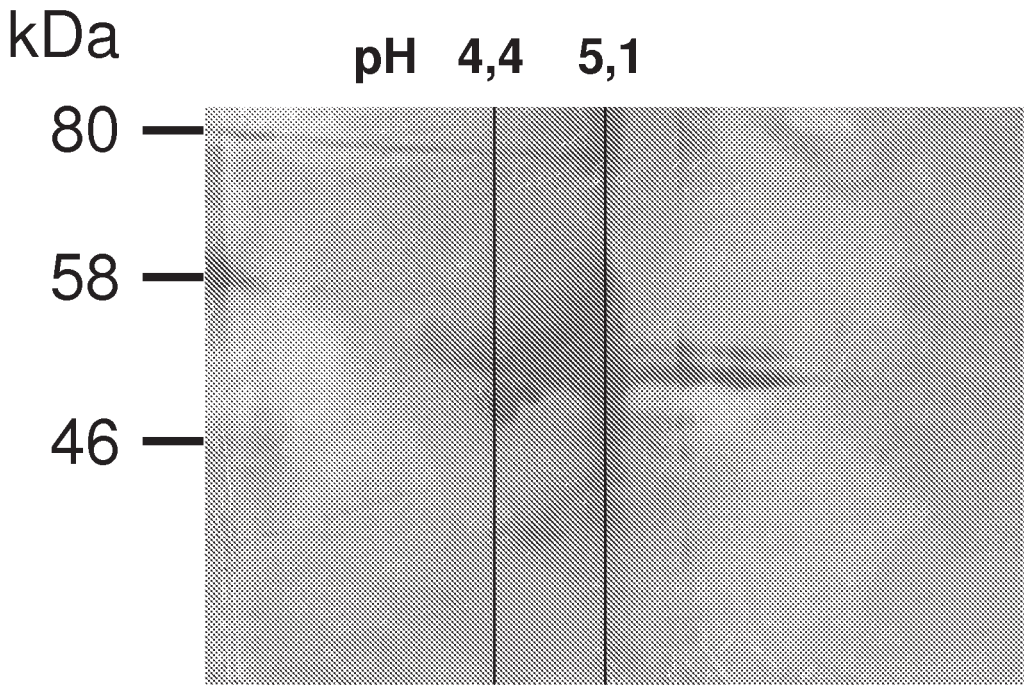
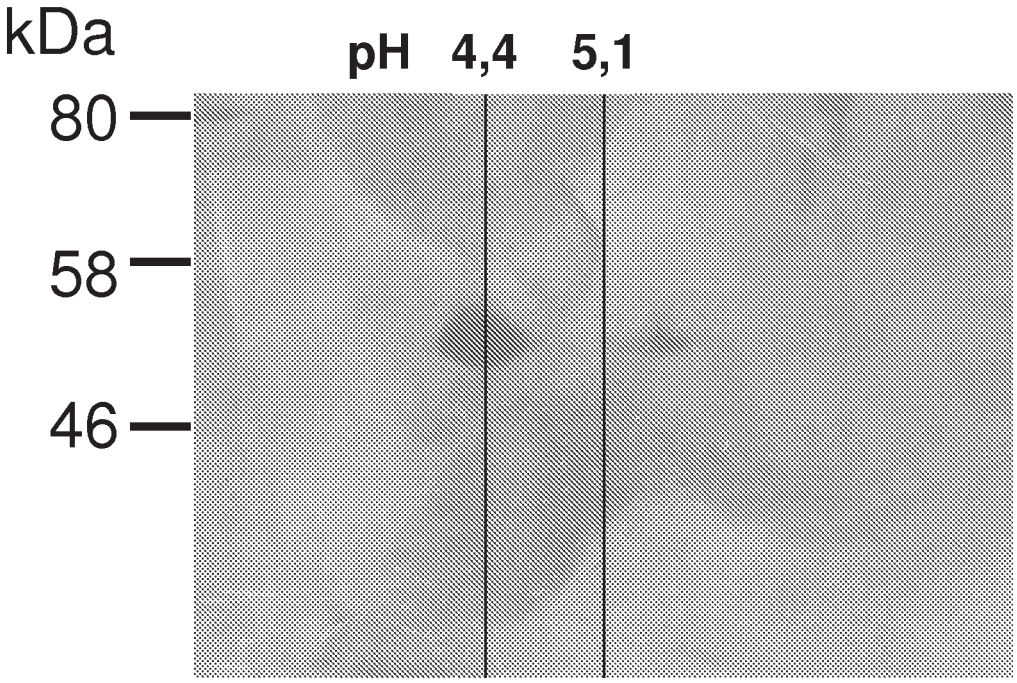


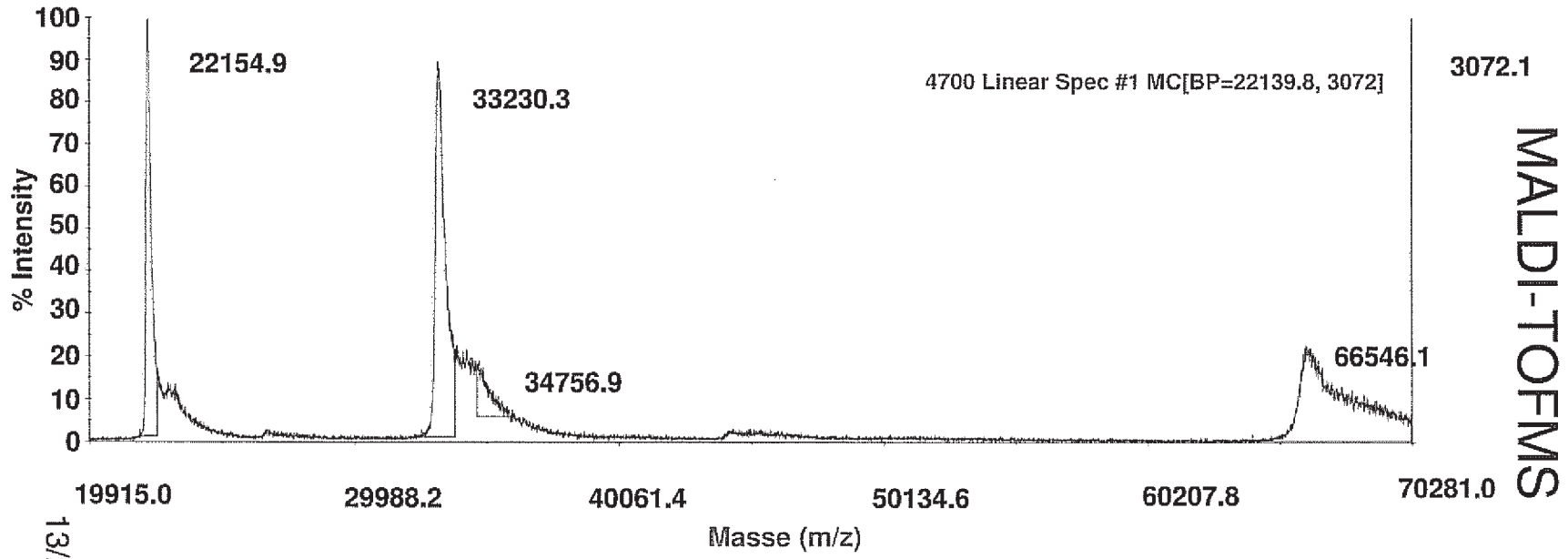
Fig. 12

A



B





MALDI-TOFMS

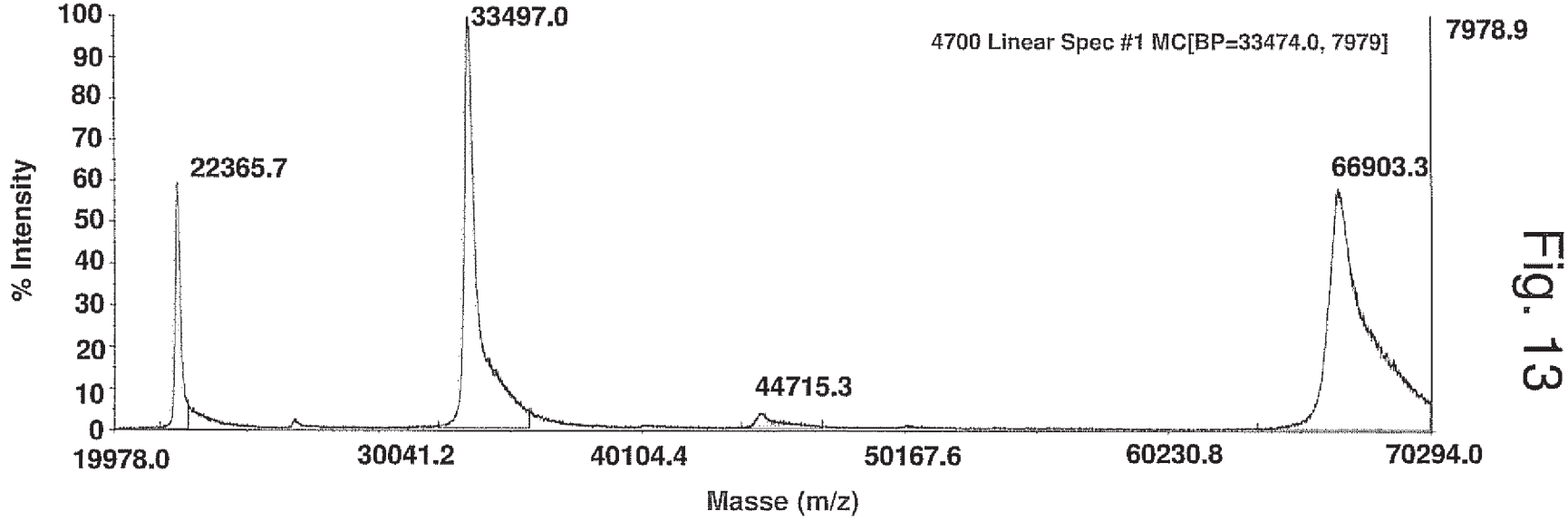


Fig. 13

Fig. 14

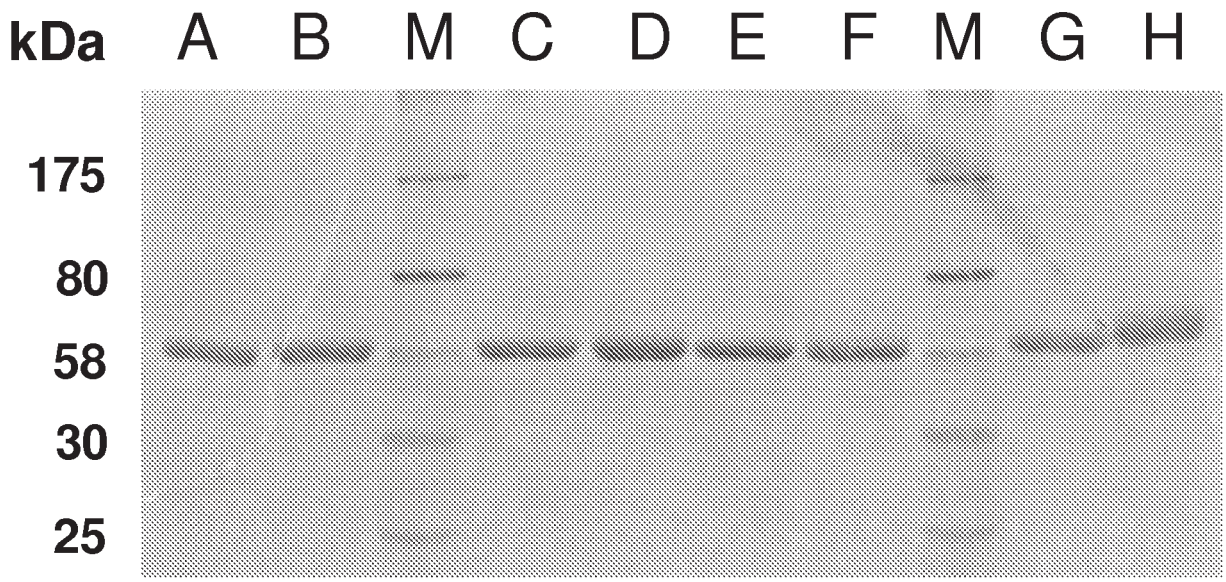


Fig. 15

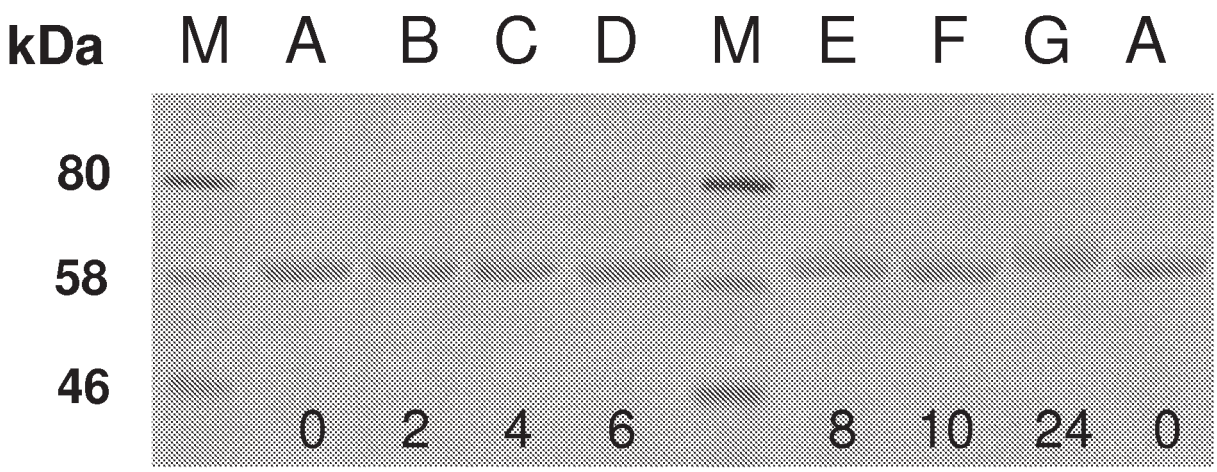


Fig. 16

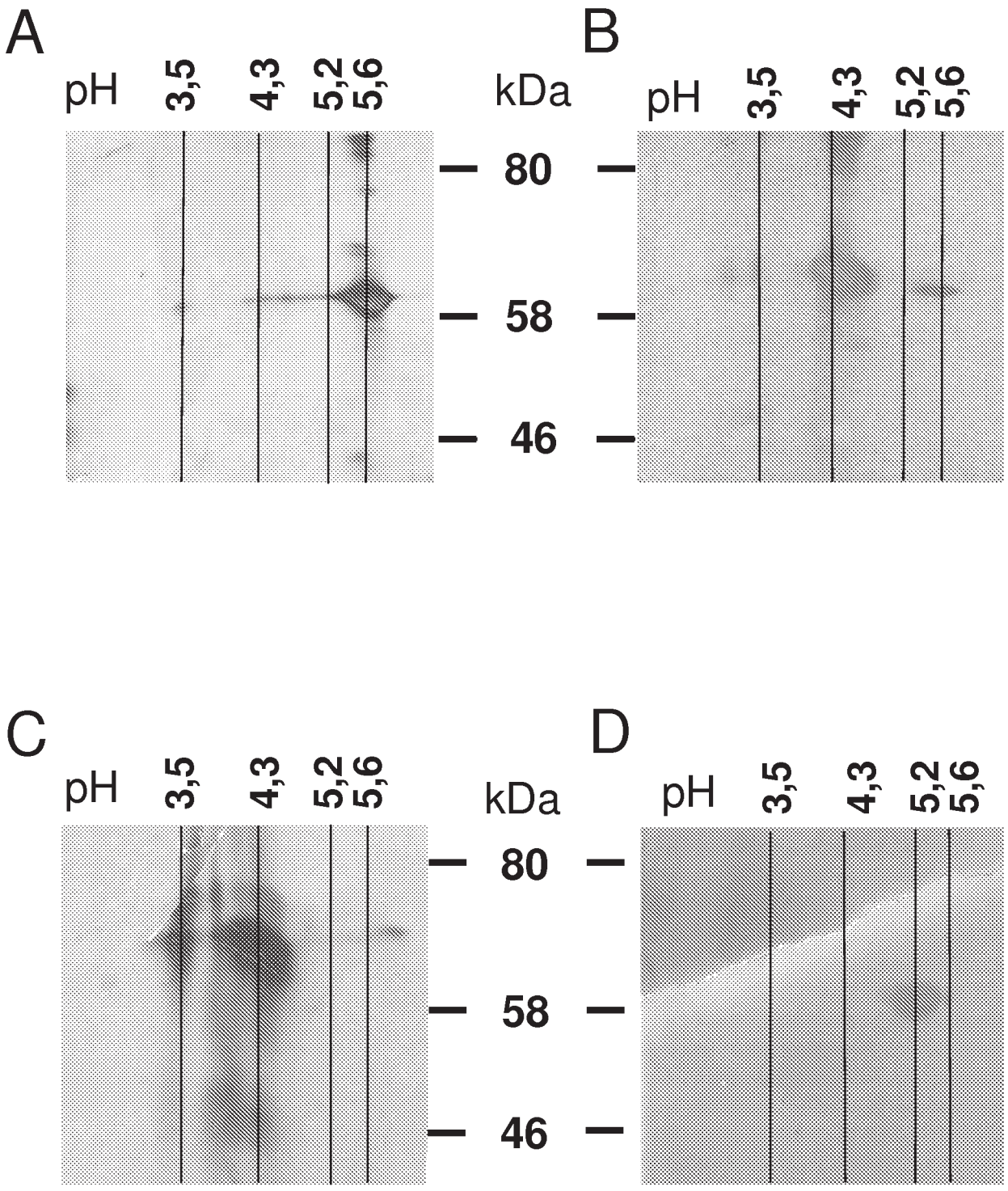

A
pH
3,5
4,3
5,2
5,6
kDa
80
58
46
B
pH
3,5
4,3
5,2
5,6
C
pH
3,5
4,3
5,2
5,6
kDa
80
58
46
D
pH
3,5
4,3
5,2
5,6

Fig. 17

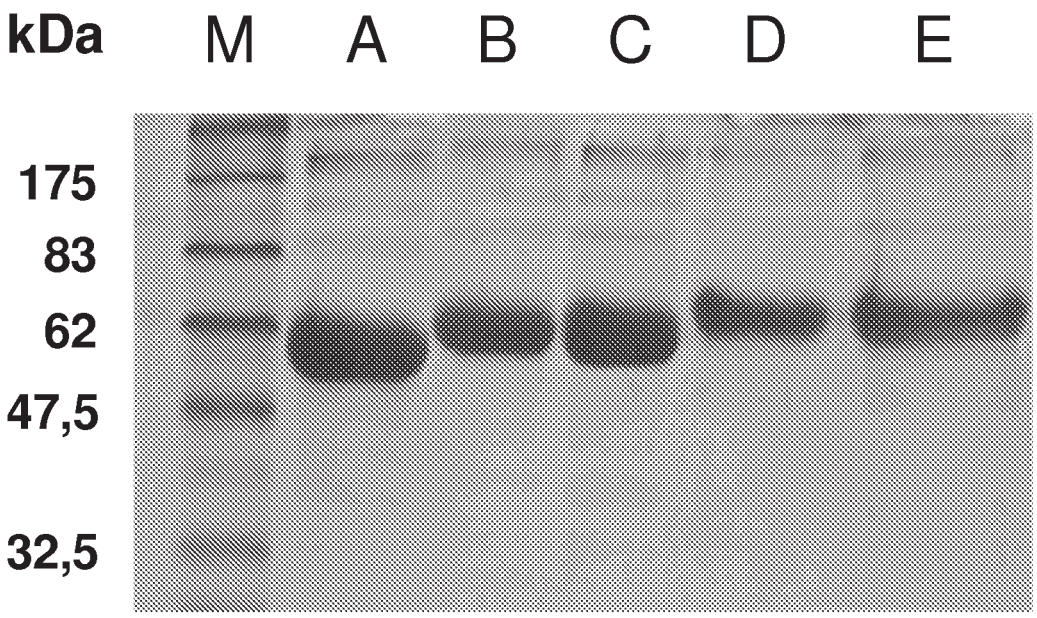


Fig. 18

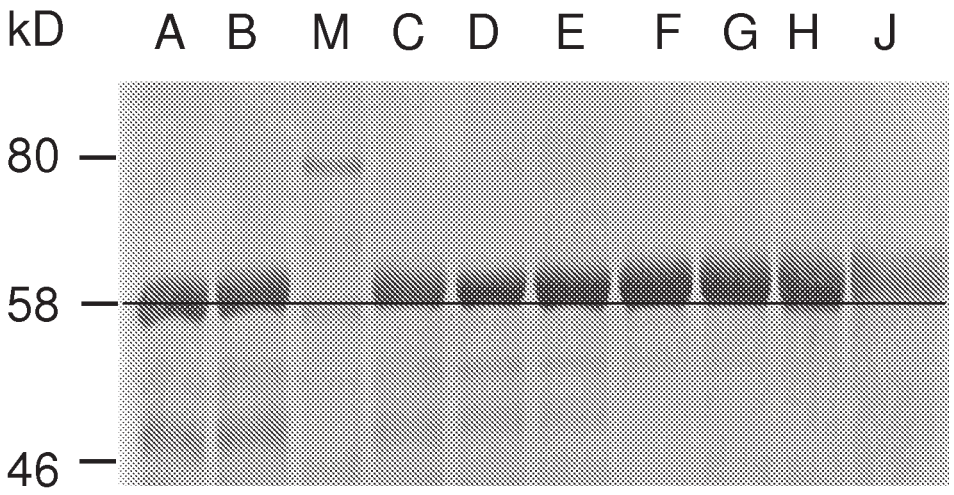


Fig. 19

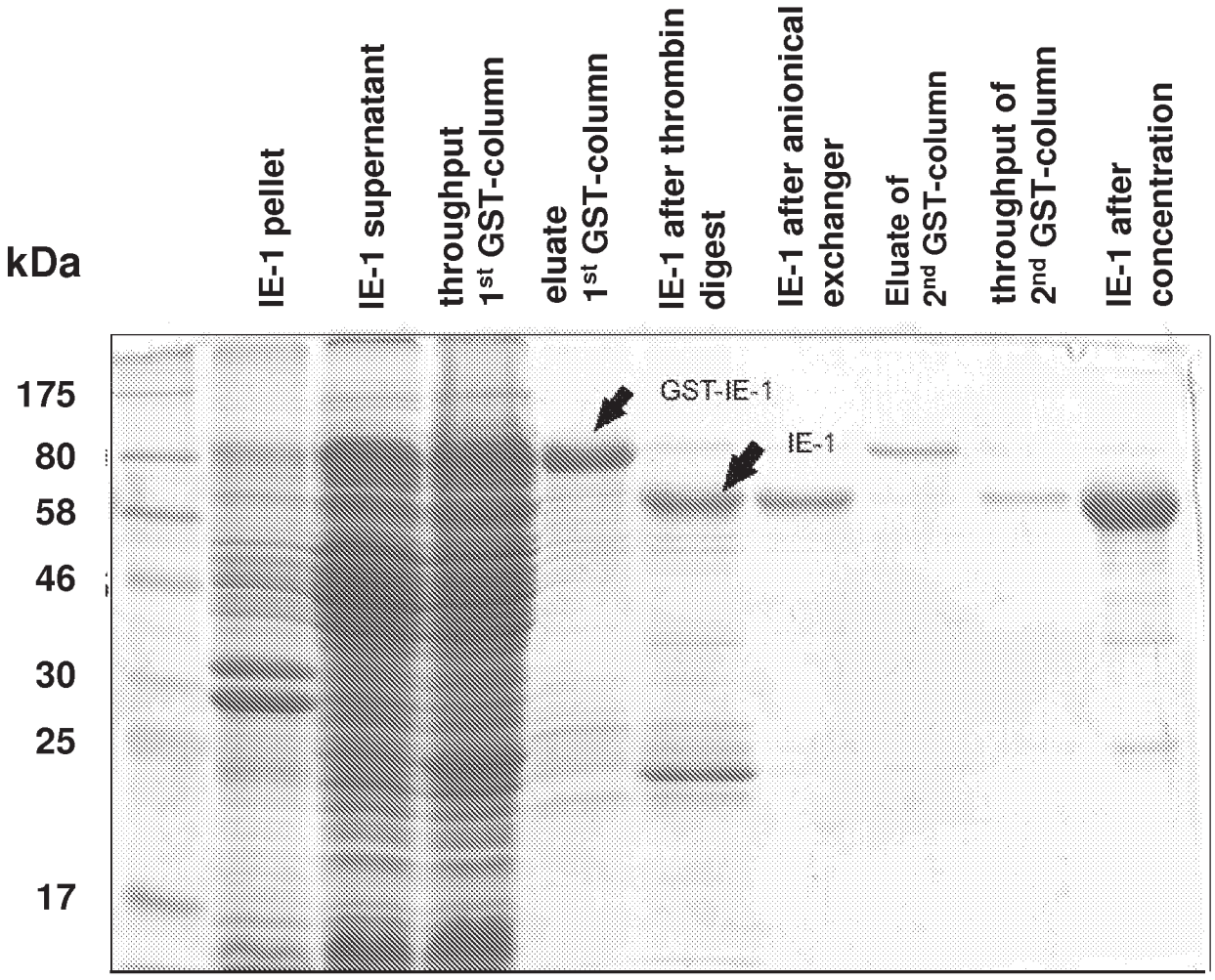
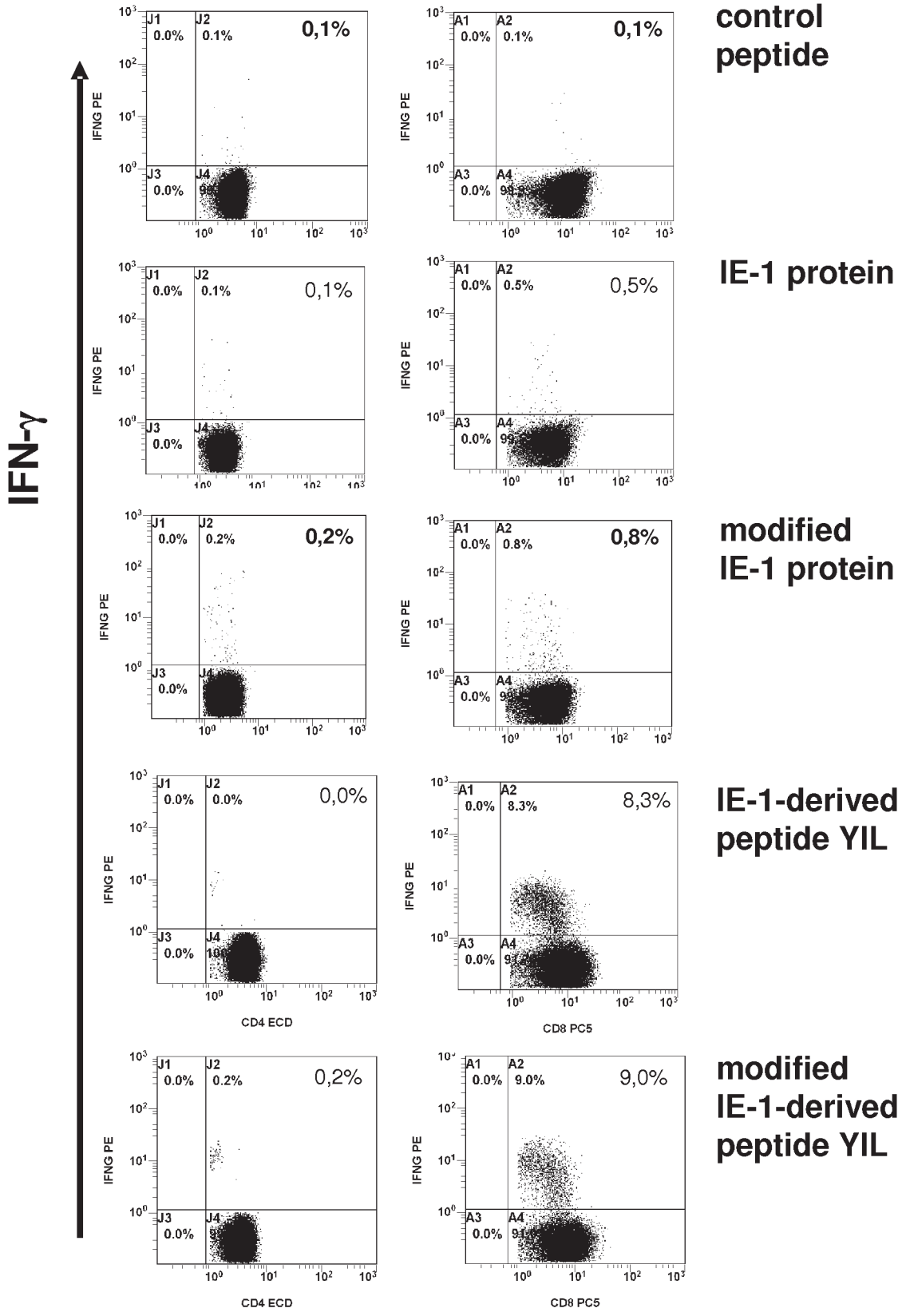

kDa
175
80
58
46
30
25
17
IE-1 pellet
IE-1 supernatant
throughput 1st GST-column
eluate 1st GST-column
IE-1 after thrombin digest
IE-1 after anionical exchanger
Eluate of 2nd GST-column
throughput of 2nd GST-column
IE-1 after concentration
GST-IE-1
IE-1

Fig. 20



control peptide

IE-1 protein

modified IE-1 protein

IE-1-derived peptide YIL

modified IE-1-derived peptide YIL

Fig. 21

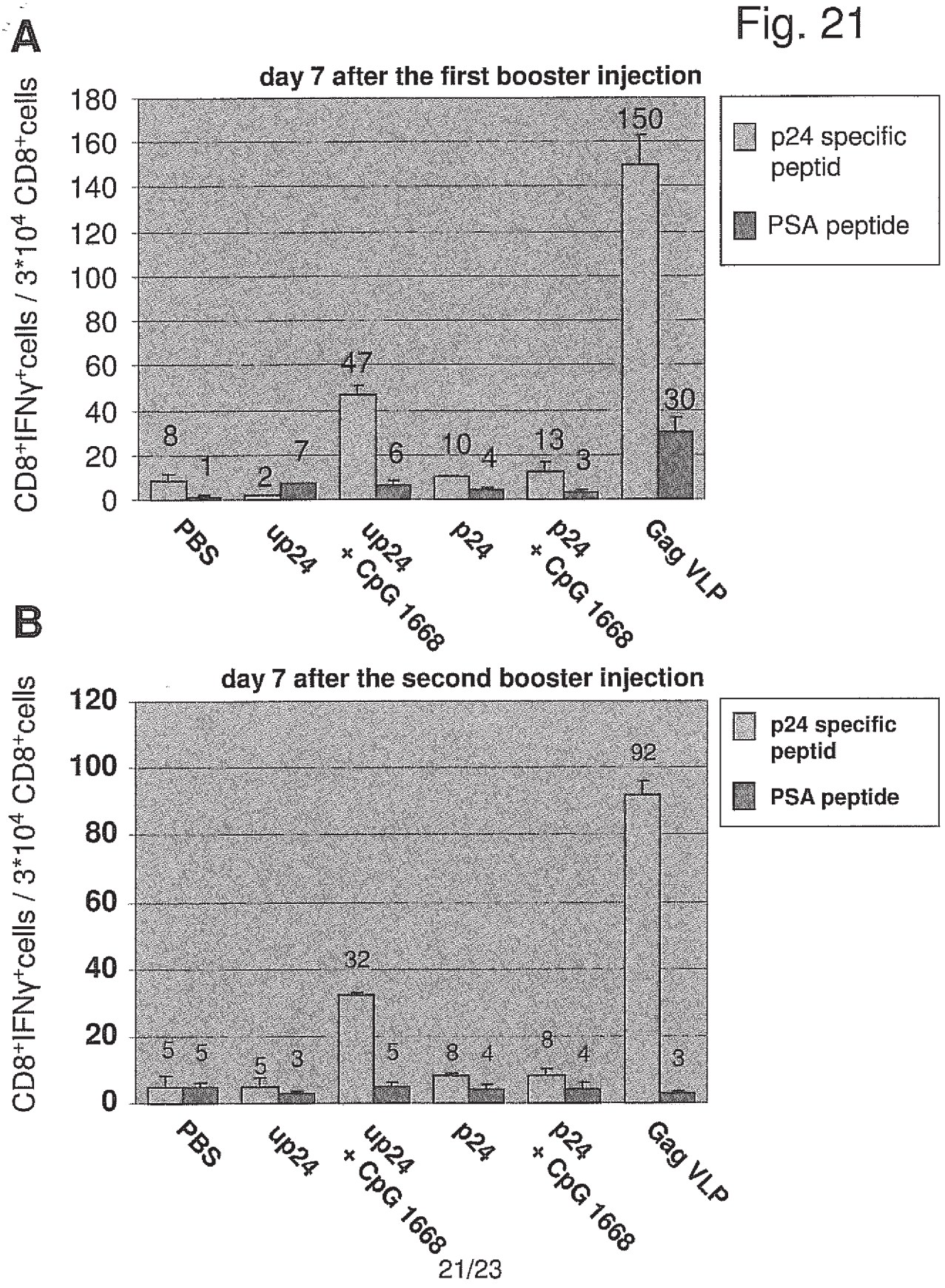

A
day 7 after the first booster injection
CD8+IFNγ+cells / 3*10^4 CD8+cells
180
160
140
120
100
80
60
40
20
0
8
1
2
7
47
6
10
4
13
3
150
30
p24 specific peptid
PSA peptide
PBS
up24
up24 + CpG 1668
p24
p24 + CpG 1668
Gag VLP
B
day 7 after the second booster injection
CD8+IFNγ+cells / 3*10^4 CD8+cells
120
100
80
60
40
20
0
5
5
5
3
32
5
8
4
8
4
92
3
p24 specific peptid
PSA peptide
PBS
up24
up24 + CpG 1668
p24
p24 + CpG 1668
Gag VLP

Fig. 22

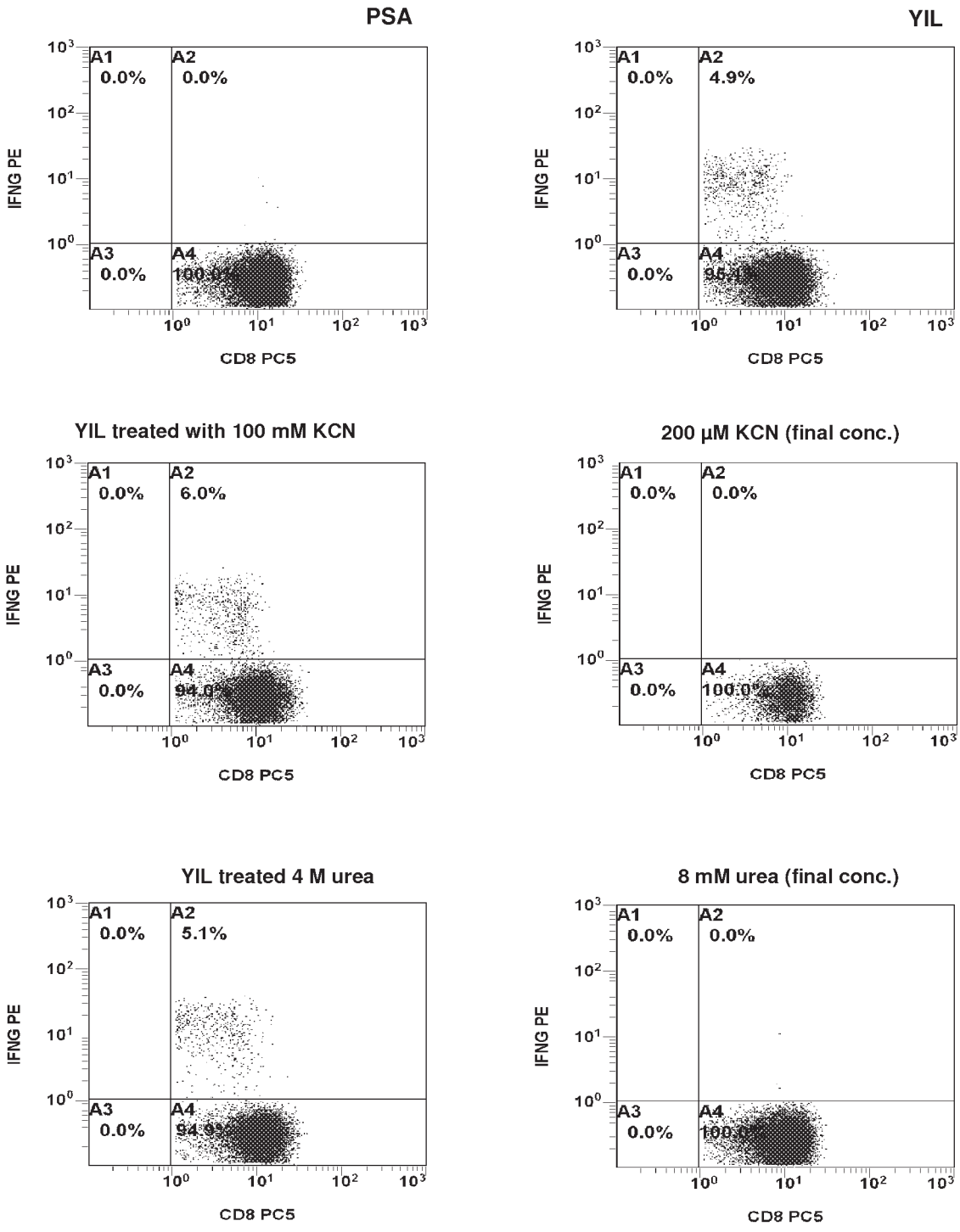
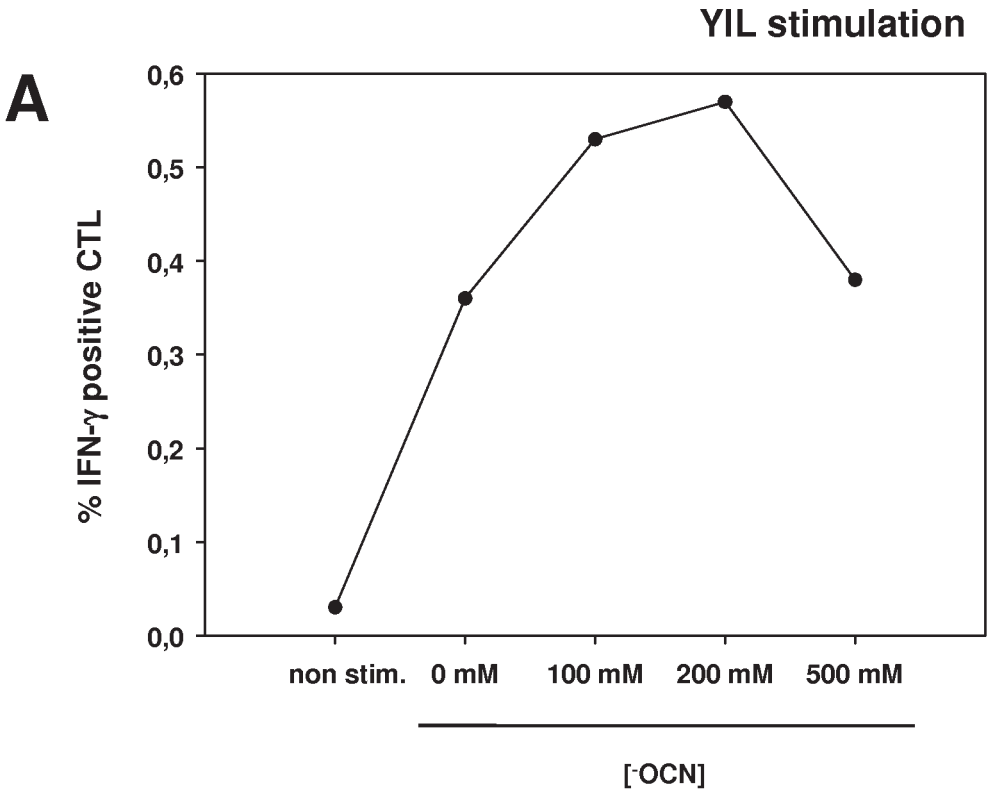
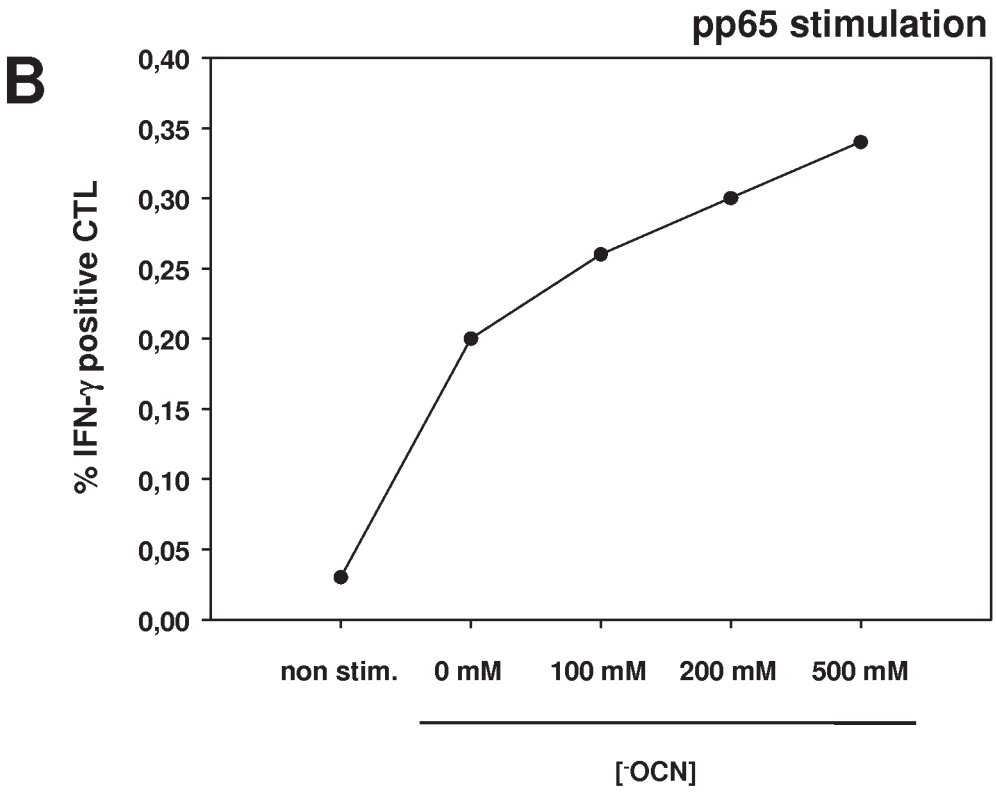

PSA
YIL
YIL treated with 100 mM KCN
200 µM KCN (final conc.)
YIL treated 4 M urea
8 mM urea (final conc.)
IFNG PE
CD8 PC5
A1
A2
A3
A4
0.0%
4.9%
6.0%
5.1%
100.0%
94.0%

Fig. 23


A
YIL stimulation
% IFN-γ positive CTL
0,6
0,5
0,4
0,3
0,2
0,1
0,0
non stim.
0 mM
100 mM
200 mM
500 mM
[-OCN]


B
pp65 stimulation
% IFN-γ positive CTL
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
non stim.
0 mM
100 mM
200 mM
500 mM
[-OCN]