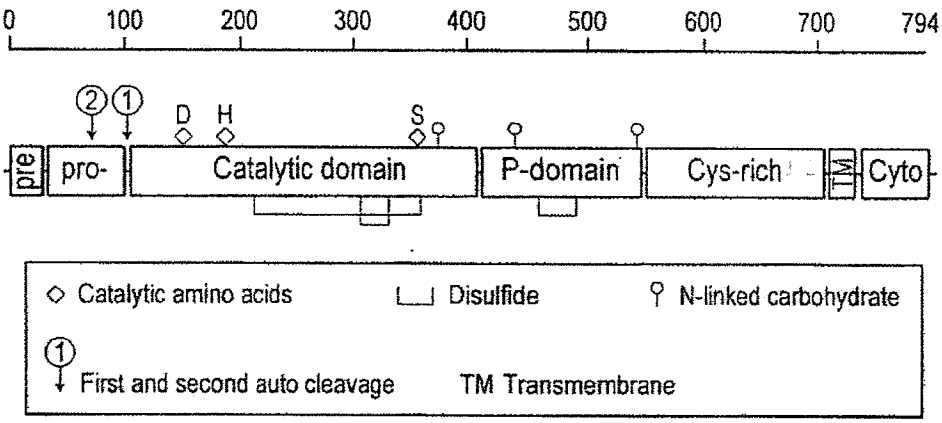

0
100
200
300
400
500
600
700
794
2
1
D
H
S
pre
pro-
Catalytic domain
P-domain
Cys-rich
TM
Cyto
◇ Catalytic amino acids
Disulfide
N-linked carbohydrate
1
First and second auto cleavage
TM Transmembrane

FIG. 1

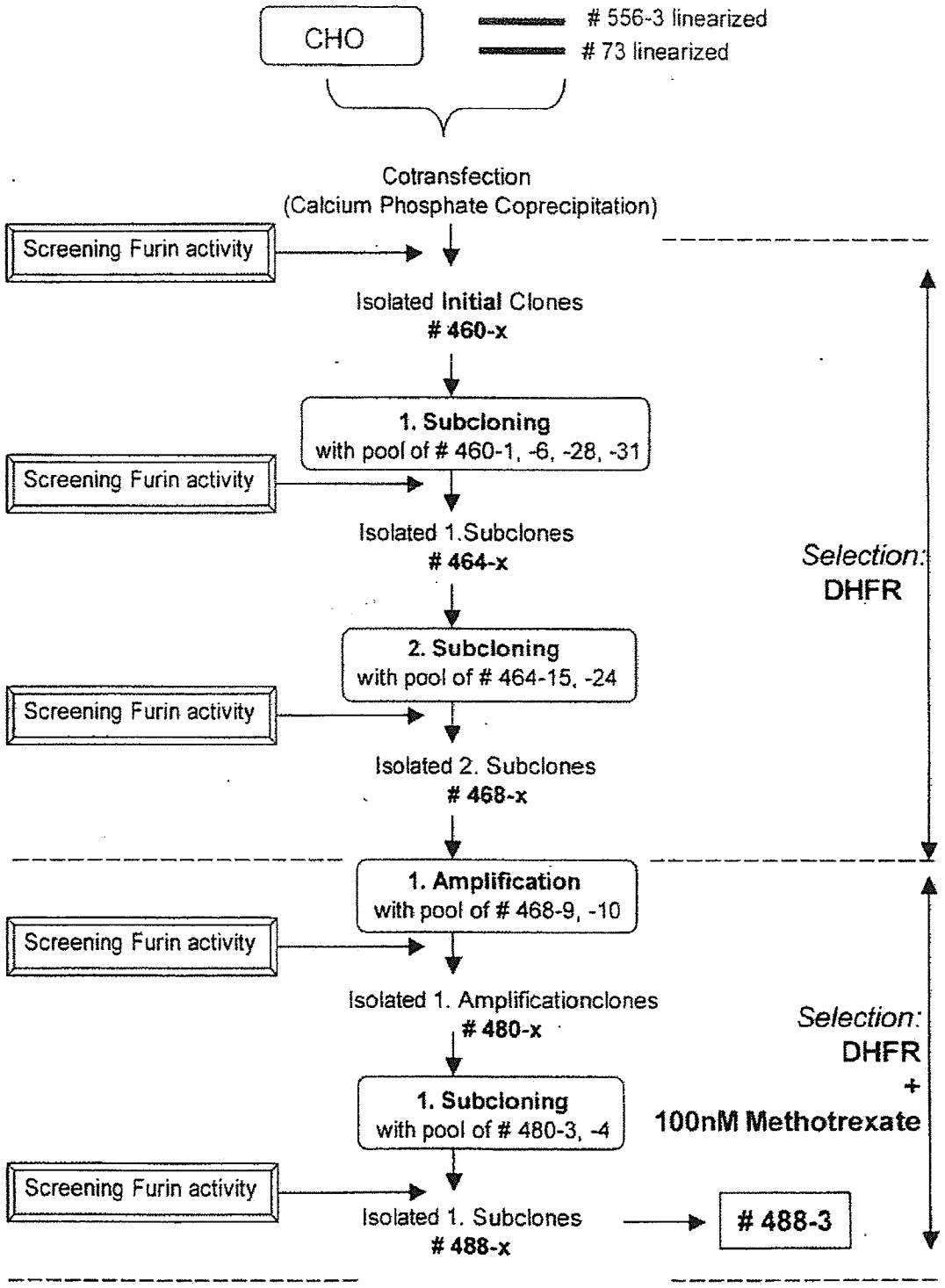

CHO
556-3 linearized
73 linearized
Cotransfection
(Calcium Phosphate Coprecipitation)
Screening Furin activity
Isolated Initial Clones
460-x
1. Subcloning
with pool of # 460-1, -6, -28, -31
Screening Furin activity
Isolated 1.Subclones
464-x
2. Subcloning
with pool of # 464-15, -24
Screening Furin activity
Isolated 2. Subclones
468-x
Selection:
DHFR
1. Amplification
with pool of # 468-9, -10
Screening Furin activity
Isolated 1. Amplificationclones
480-x
1. Subcloning
with pool of # 480-3, -4
Screening Furin activity
Isolated 1. Subclones
488-x
488-3
Selection:
DHFR
+
100nM Methotrexate

FIG. 2

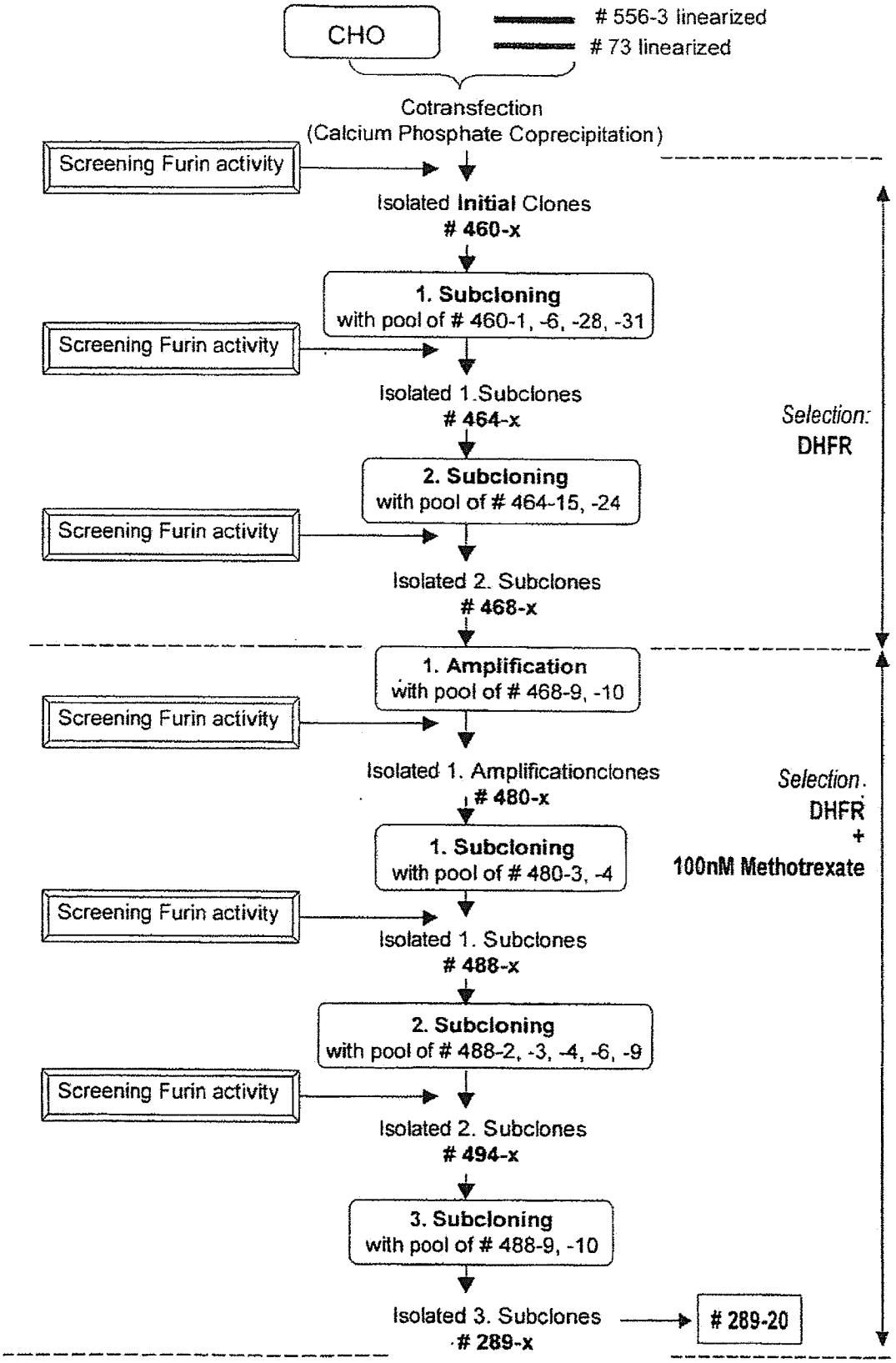

CHO
556-3 linearized
73 linearized
Cotransfection
(Calcium Phosphate Coprecipitation)
Screening Furin activity
Isolated Initial Clones
460-x
1. Subcloning
with pool of # 460-1, -6, -28, -31
Screening Furin activity
Isolated 1.Subclones
464-x
2. Subcloning
with pool of # 464-15, -24
Screening Furin activity
Isolated 2. Subclones
468-x
Selection:
DHFR
1. Amplification
with pool of # 468-9, -10
Screening Furin activity
Isolated 1. Amplificationclones
480-x
1. Subcloning
with pool of # 480-3, -4
Screening Furin activity
Isolated 1. Subclones
488-x
2. Subcloning
with pool of # 488-2, -3, -4, -6, -9
Screening Furin activity
Isolated 2. Subclones
494-x
3. Subcloning
with pool of # 488-9, -10
Isolated 3. Subclones
289-x
289-20
Selection:
DHFR
+
100nM Methotrexate

FIG. 3

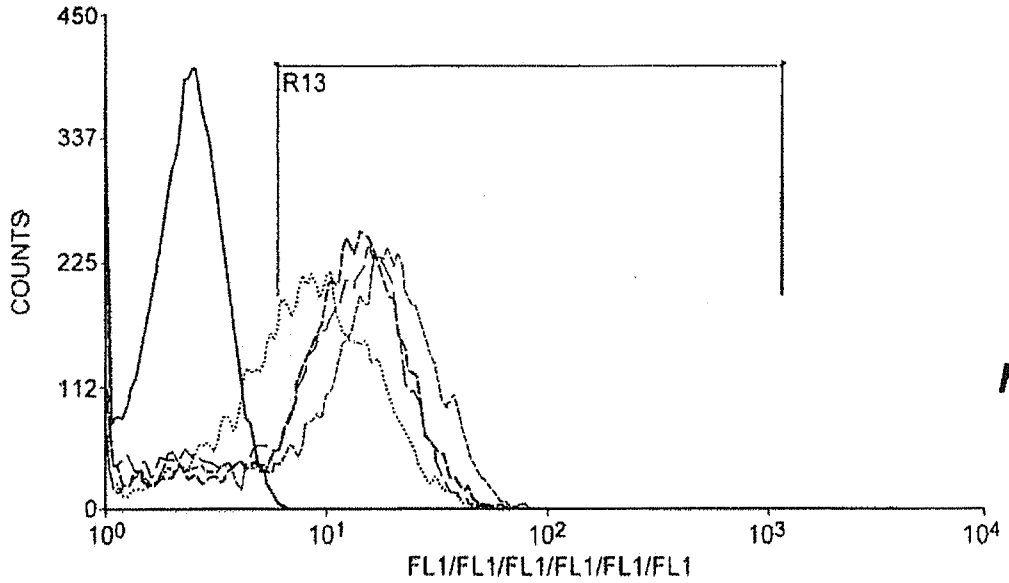


FIG. 4A

Comments to FACS results

Cell population	Sample code	% Furin positive	Mean	Median
Control ———	CHO DUKX DXB11neg	0.25	2.52	2.37
PMCB#01 — — — -	RE06-076-07	74.06	12.55	11.55
Irrelevant sample	RE06-077-07	81.02	17.59	15.96
PMCB#04 --------	RE06-079-07	80.74	13.71	12.41
Irrelevant sample ------	SK06-63-28	67.89	10.22	8.35

FIG. 4B

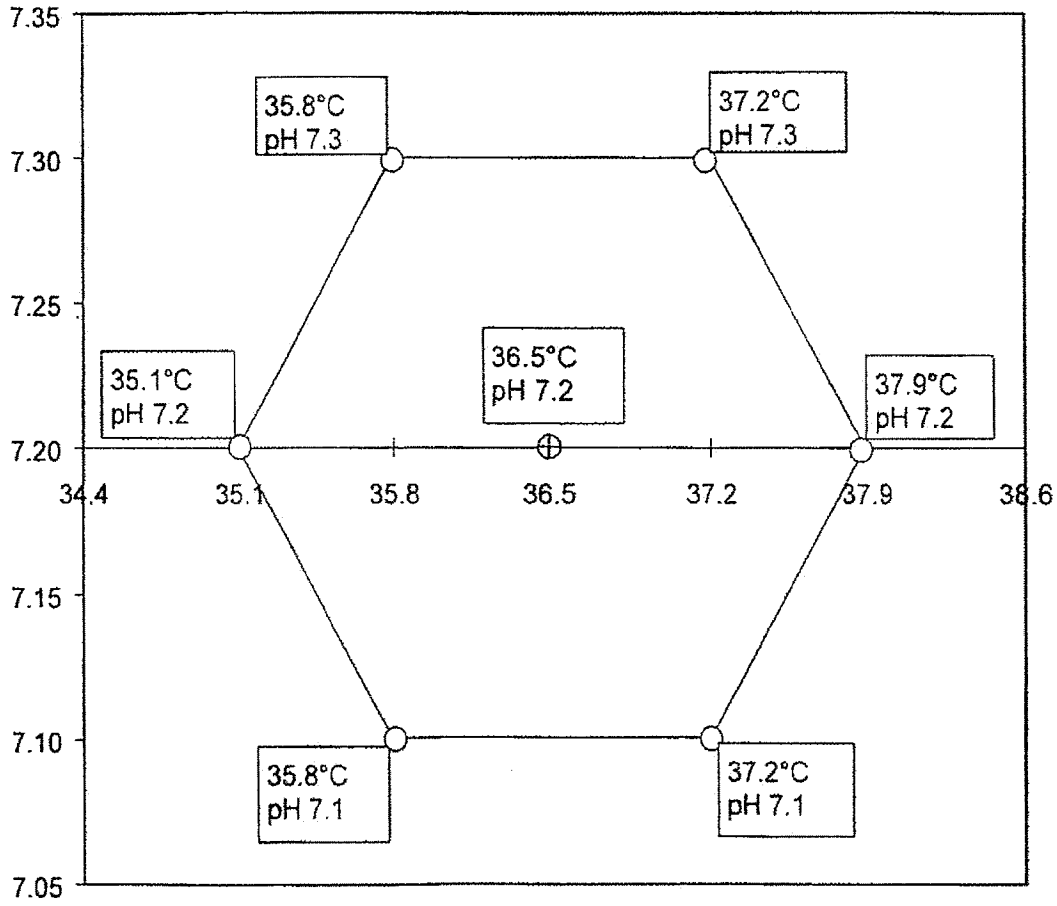

7.35
7.30
7.25
7.20
7.15
7.10
7.05
34.4
35.1
35.8
36.5
37.2
37.9
38.6
35.8°C
pH 7.3
37.2°C
pH 7.3
35.1°C
pH 7.2
36.5°C
pH 7.2
37.9°C
pH 7.2
35.8°C
pH 7.1
37.2°C
pH 7.1

FIG. 5

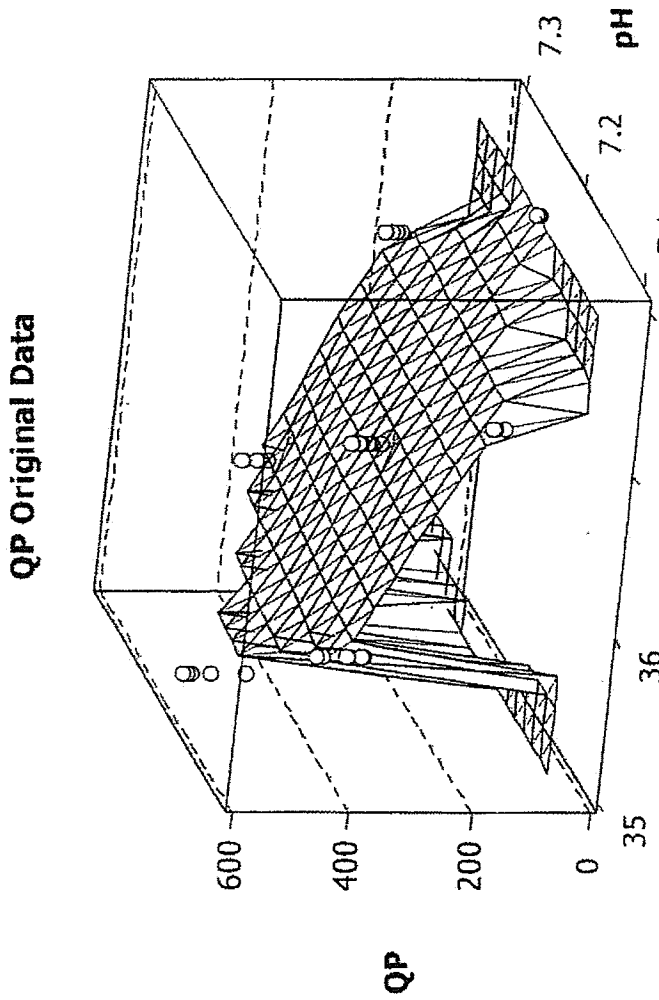


FIG. 6

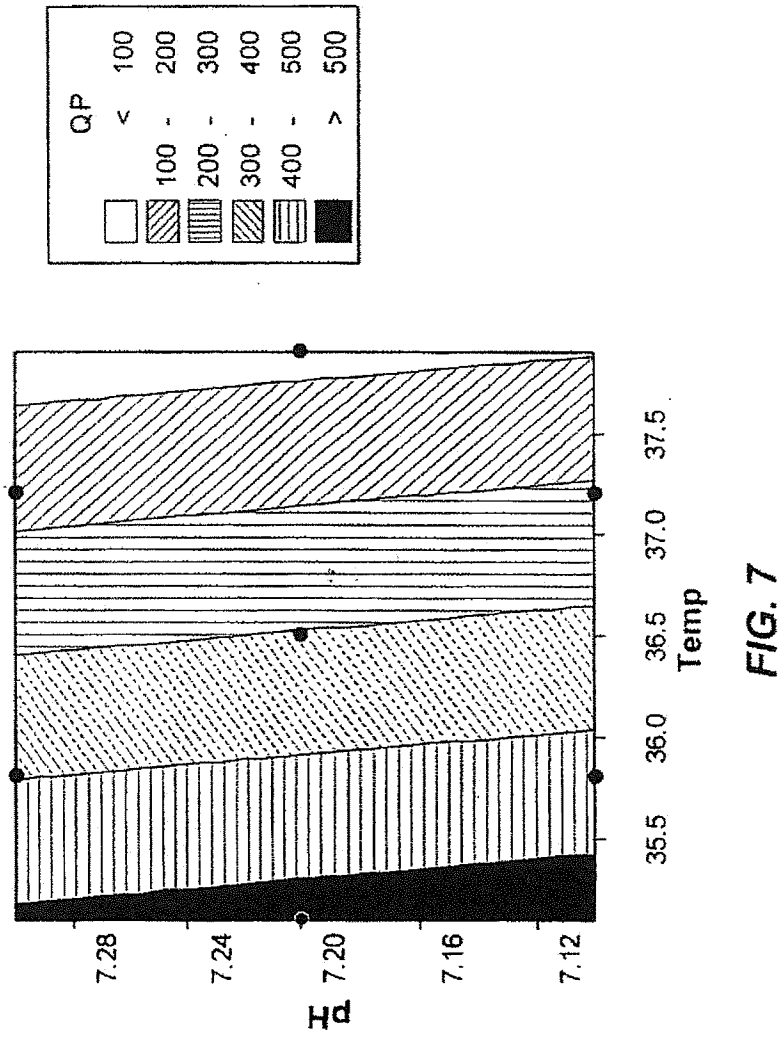

QP
< 100
100 - 200
200 - 300
300 - 400
400 - 500
> 500
pH
7.28
7.24
7.20
7.16
7.12
Temp
35.5
36.0
36.5
37.0
37.5

FIG. 7

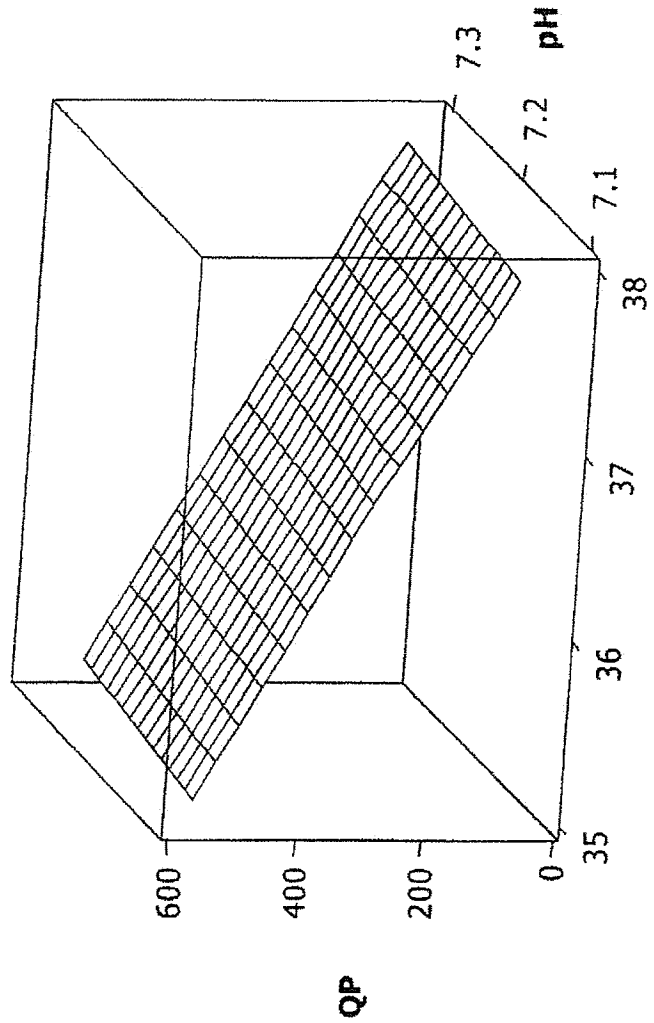


FIG. 8

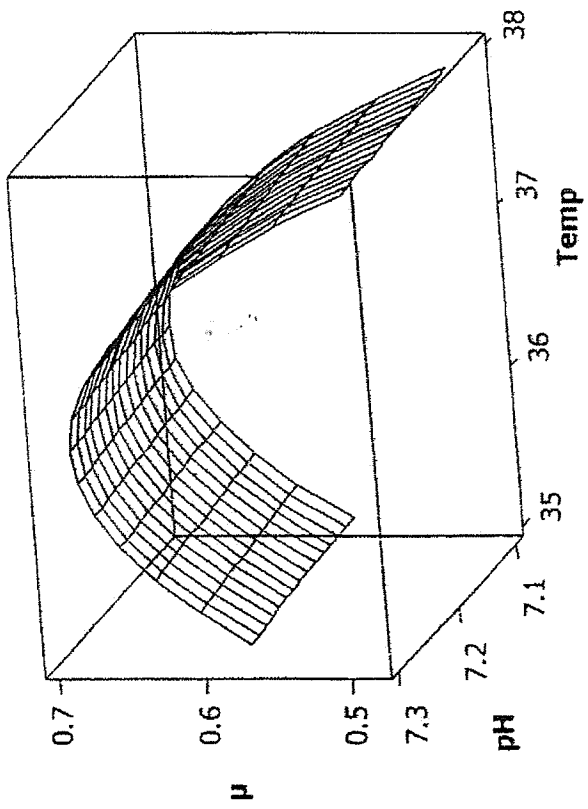


FIG. 9

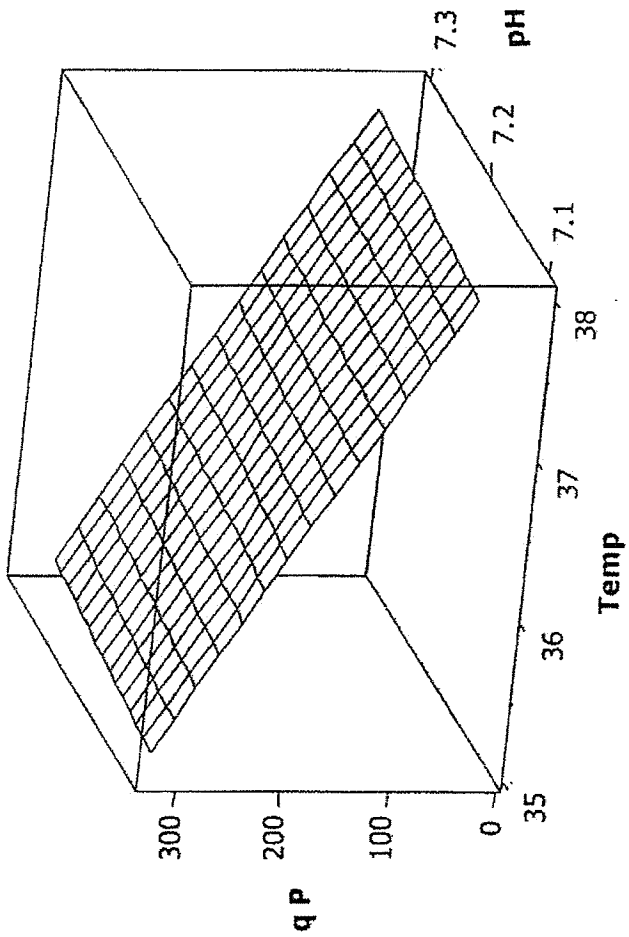


FIG. 10

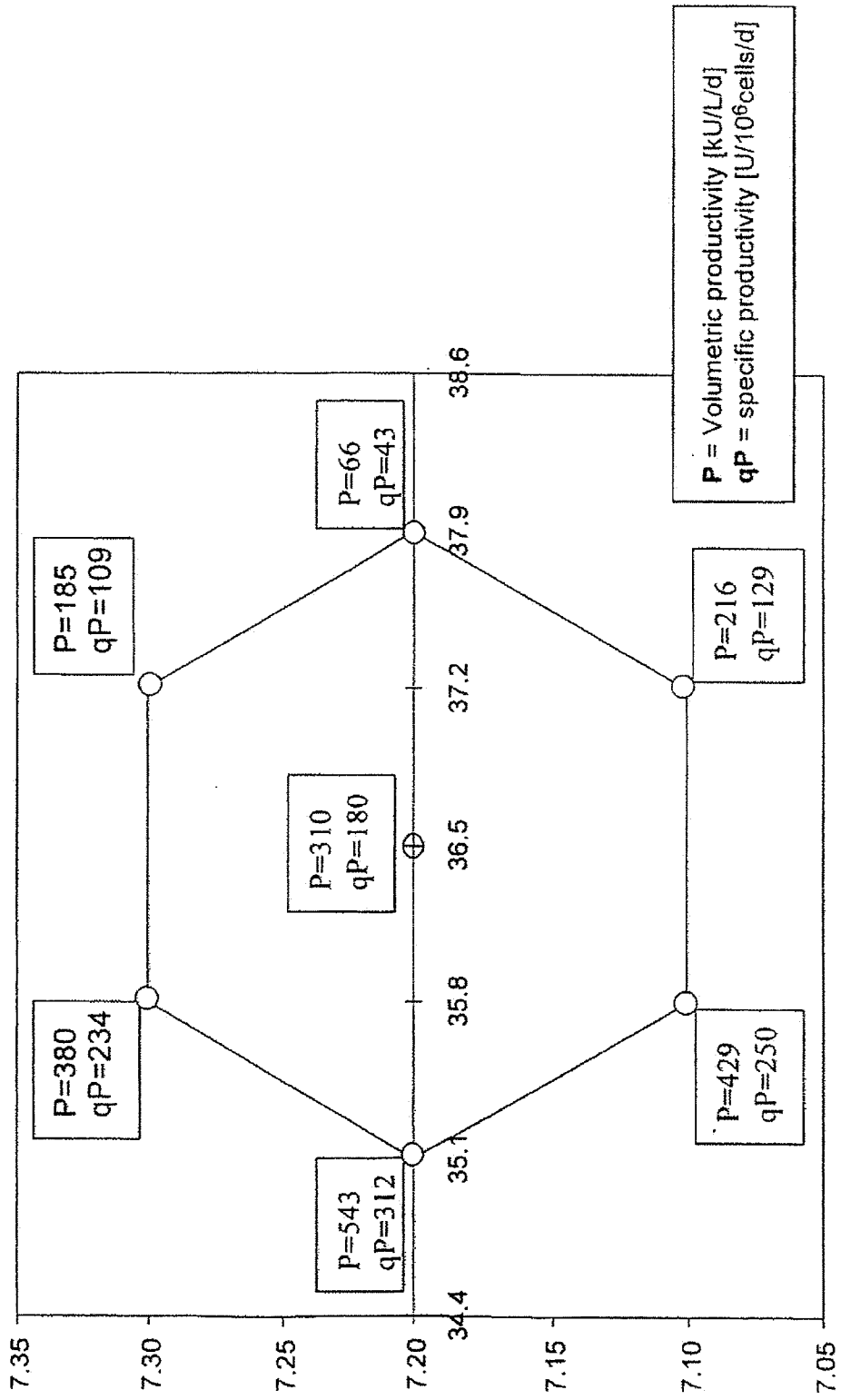
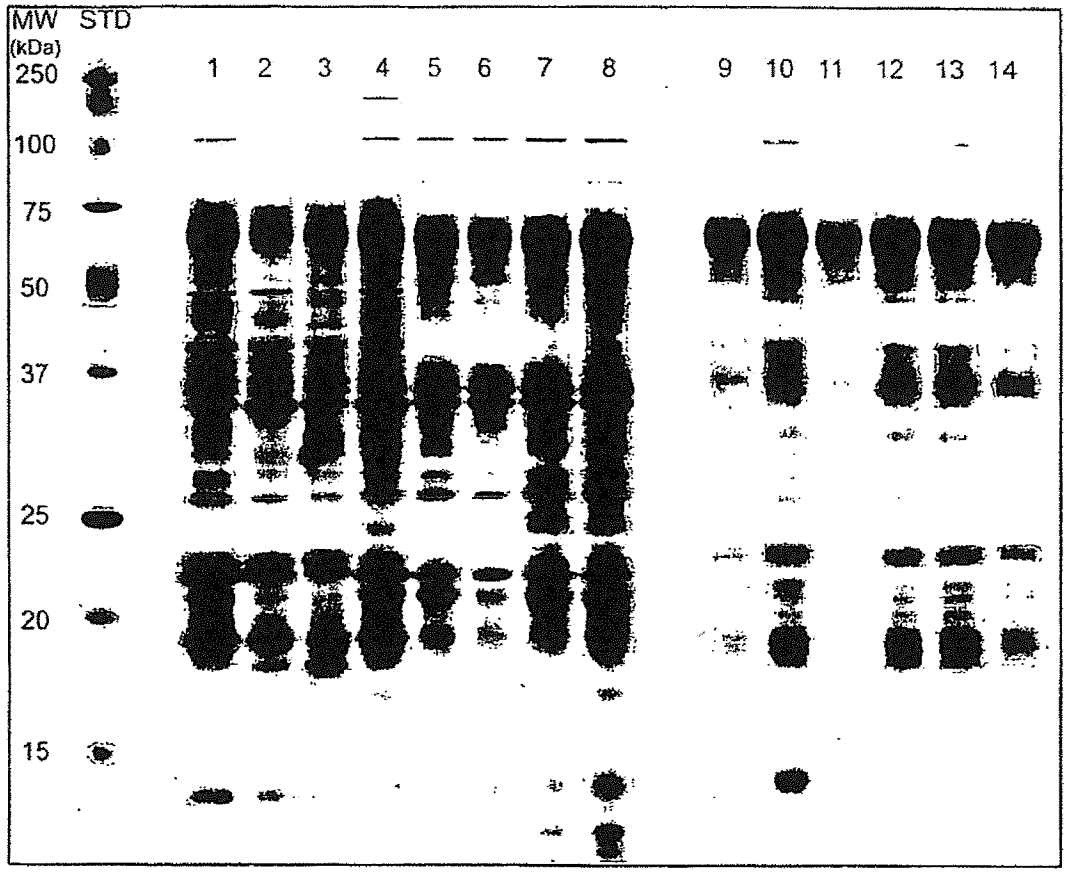

P = Volumetric productivity [kU/L/d]
qP = specific productivity [U/10^6cells/d]
P=185
qP=109
P=66
qP=43
P=380
qP=234
P=310
qP=180
P=216
qP=129
P=543
qP=312
P=429
qP=250
7.35
7.30
7.25
7.20
7.15
7.10
7.05
38.6
37.9
37.2
36.5
35.8
35.1
34.4

FIG. 11

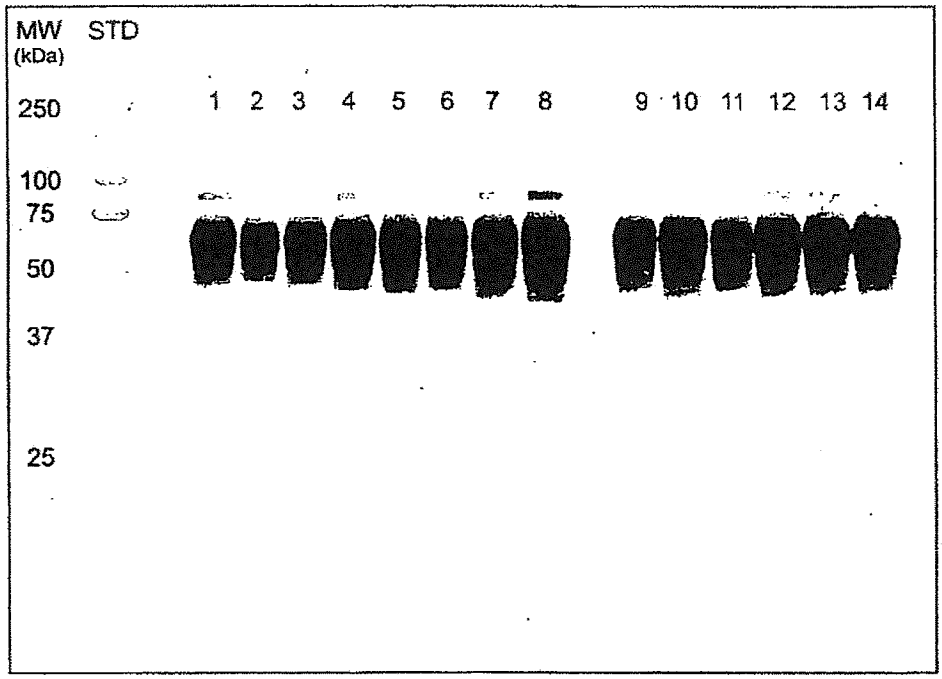


The samples were analyzed by SDS-PAGE on 8% gels. The separated polypeptides were visualized by silver staining

ORFU06002:
Lane 1: MMC 01
Lane 2: MMC 02
Lane 3: MMC 03
Lane 4: MMC 04
Lane 5: MMC 05
Lane 6: MMC 06
Lane 7: MMC 07
Lane 8: MMC 08

ORFU07002:
Lane 9: MMC 01
Lane 10: MMC 02
Lane 11: MMC 03
Lane 12: MMC 04
Lane 13: MMC 05
Lane 14: MMC 06

FIG. 12



The samples were analyzed by SDS-PAGE on 8% gels. The separated polypeptides were visualized by Western blot, using a monoclonal Goat-anti Furin antibody (Alexis Mon-148) as first antibody.

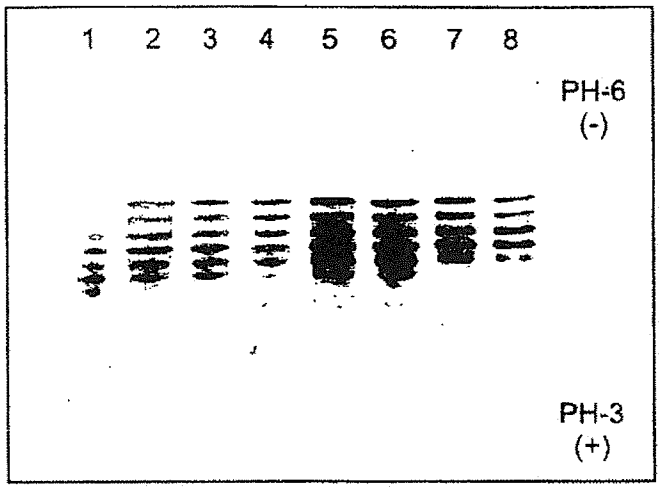
ORFU06002:

Lane 1: MMC 01
Lane 2: MMC 02
Lane 3: MMC 03
Lane 4: MMC 04
Lane 5: MMC 05
Lane 6: MMC 06
Lane 7: MMC 07
Lane 8: MMC 08

ORFU07002:

Lane 9: MMC 01
Lane 10: MMC 02
Lane 11: MMC 03
Lane 12: MMC 04
Lane 13: MMC 05
Lane 14: MMC 06

FIG. 13



The samples were analyzed on vertical IEF using a pH gradient of PH 7.0 – pH 3.0. The separated polypeptides were visualized by Western blotting using a monoclonal Goat-anti Furin antibody (Alexis Mon-148) as first antibody.

ORFU06002:

Lane 1: MMC 01

Lane 2: MMC 02

Lane 3: MMC 03

Lane 4: MMC 04

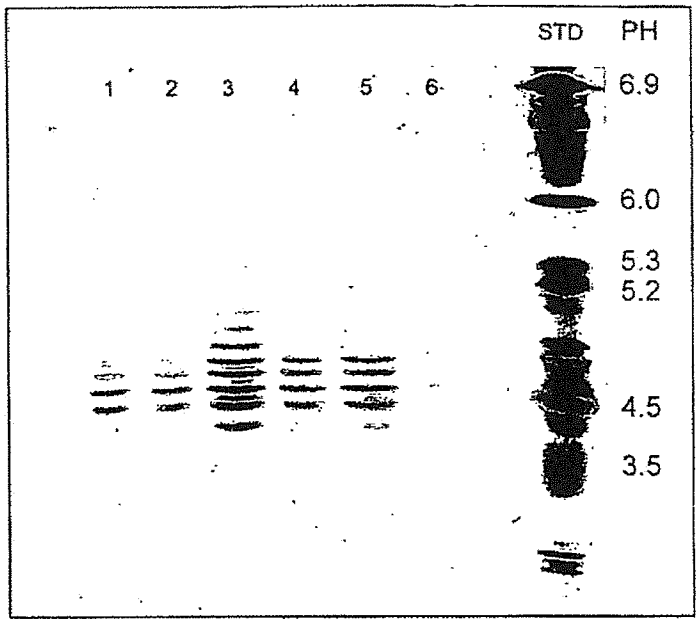
Lane 5: MMC 05

Lane 6: MMC 06

Lane 7: MMC 07

Lane 8: MMC 08

FIG. 14



The samples were analyzed by vertical IEF pH 7.0 – 3.0. The separated polypeptides were visualized by Coomassie staining.

ORFU07002:

Lane 1: MMC 01

Lane 2: MMC 02

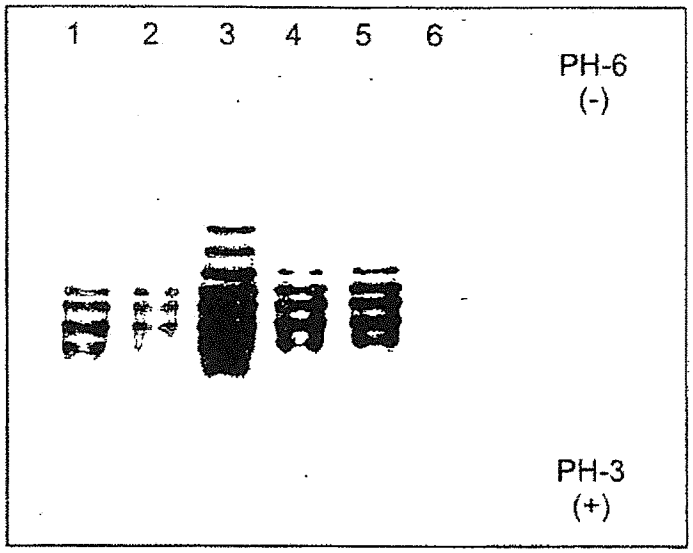
Lane 3: MMC 03

Lane 4: MMC 04

Lane 5: MMC 05

Lane 6: MMC 06

FIG. 15



The samples were analyzed by vertical IEF pH 7.0 – 3.0. The separated polypeptides were visualized by Western blotting, using a monoclonal Goat-anti Furin antibody (Alexis Mon-148) as first antibody.

ORFU07002:

Lane 1: MMC 01

Lane 2: MMC 02

Lane 3: MMC 03

Lane 4: MMC 04

Lane 5: MMC 05

Lane 6: MMC 06

FIG. 16

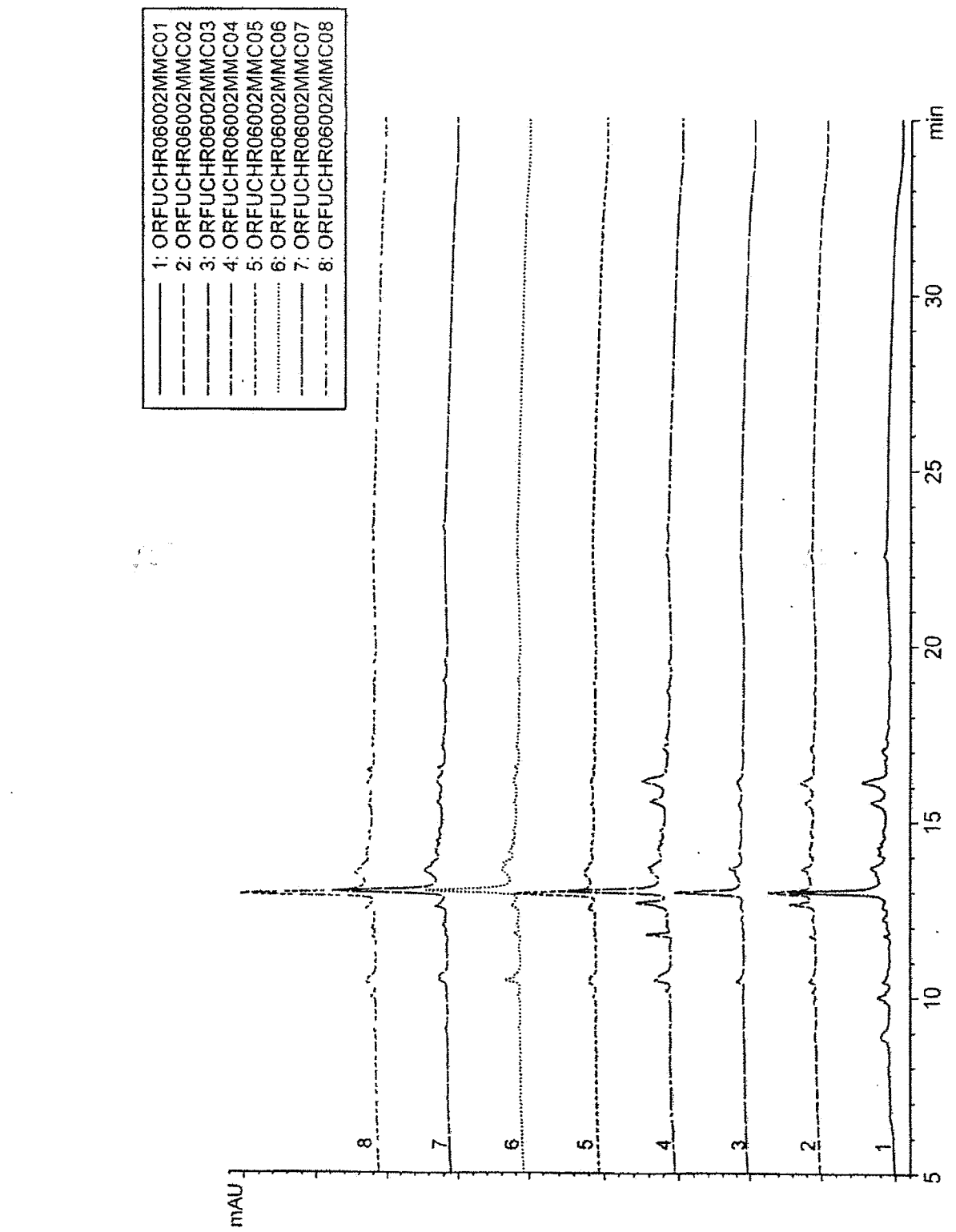

1: ORFUCHR06002MMC01
2: ORFUCHR06002MMC02
3: ORFUCHR06002MMC03
4: ORFUCHR06002MMC04
5: ORFUCHR06002MMC05
6: ORFUCHR06002MMC06
7: ORFUCHR06002MMC07
8: ORFUCHR06002MMC08
mAU
min
5
10
15
20
25
30
1
2
3
4
5
6
7
8

FIG. 17

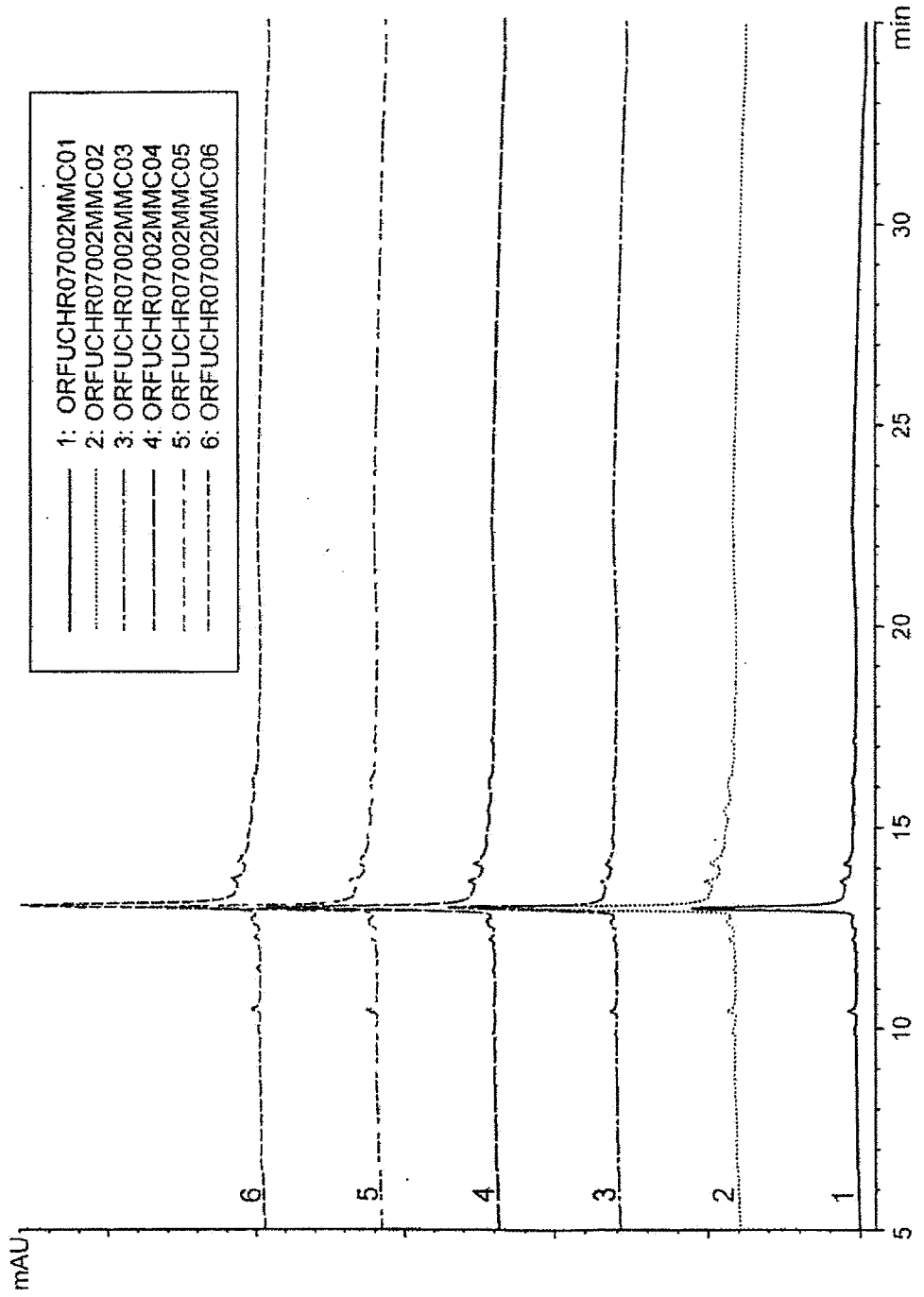

1: ORFUCHR07002MMC01
2: ORFUCHR07002MMC02
3: ORFUCHR07002MMC03
4: ORFUCHR07002MMC04
5: ORFUCHR07002MMC05
6: ORFUCHR07002MMC06
mAU
min
5
10
15
20
25
30
1
2
3
4
5
6

FIG. 18