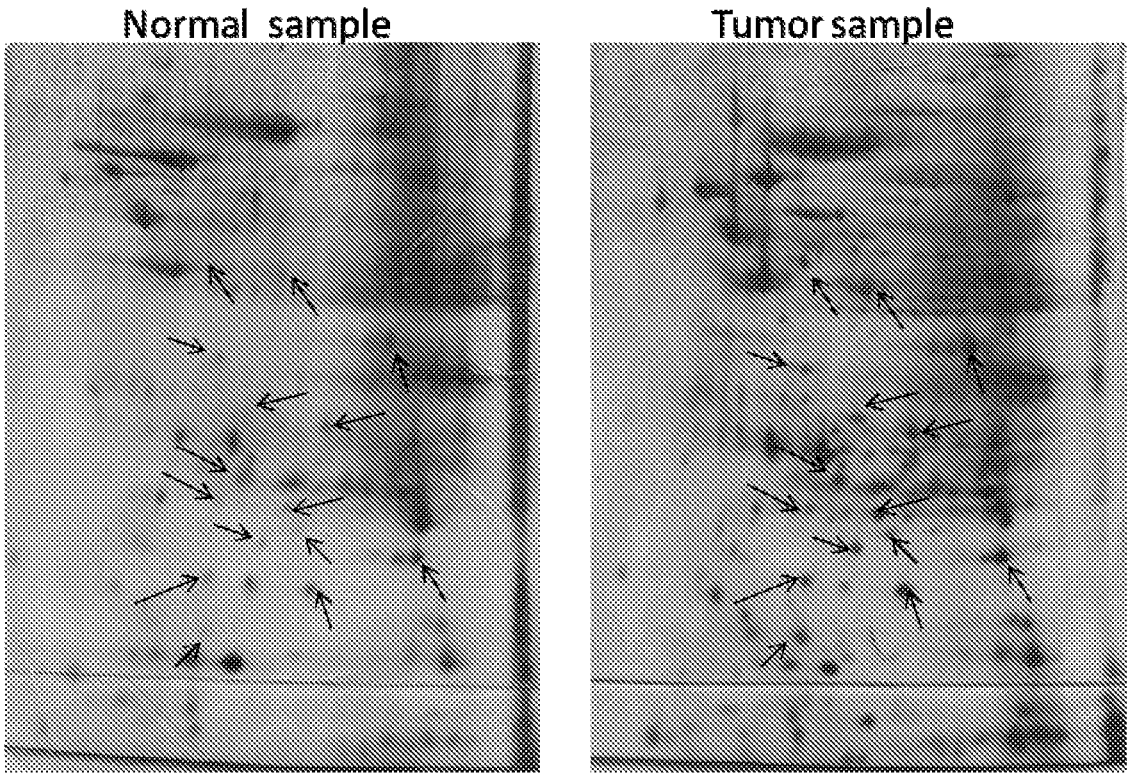


FIG. 1

Spot	Protein	MW (kD)
1	Bmi1	37
2	VCC1	13.6
3	SUMO-4	11
4	RhoA	22
5	TXN	12
6	ET-1	24
7	UBE2C	20
8	HDGF2	74
9	FGF21	20
10	LECT2	16
11	SOD1	16
12	STMN4	22
13	Midkine	19
14	IL-17A	17.5
15	IL-26	20

FIG. 2

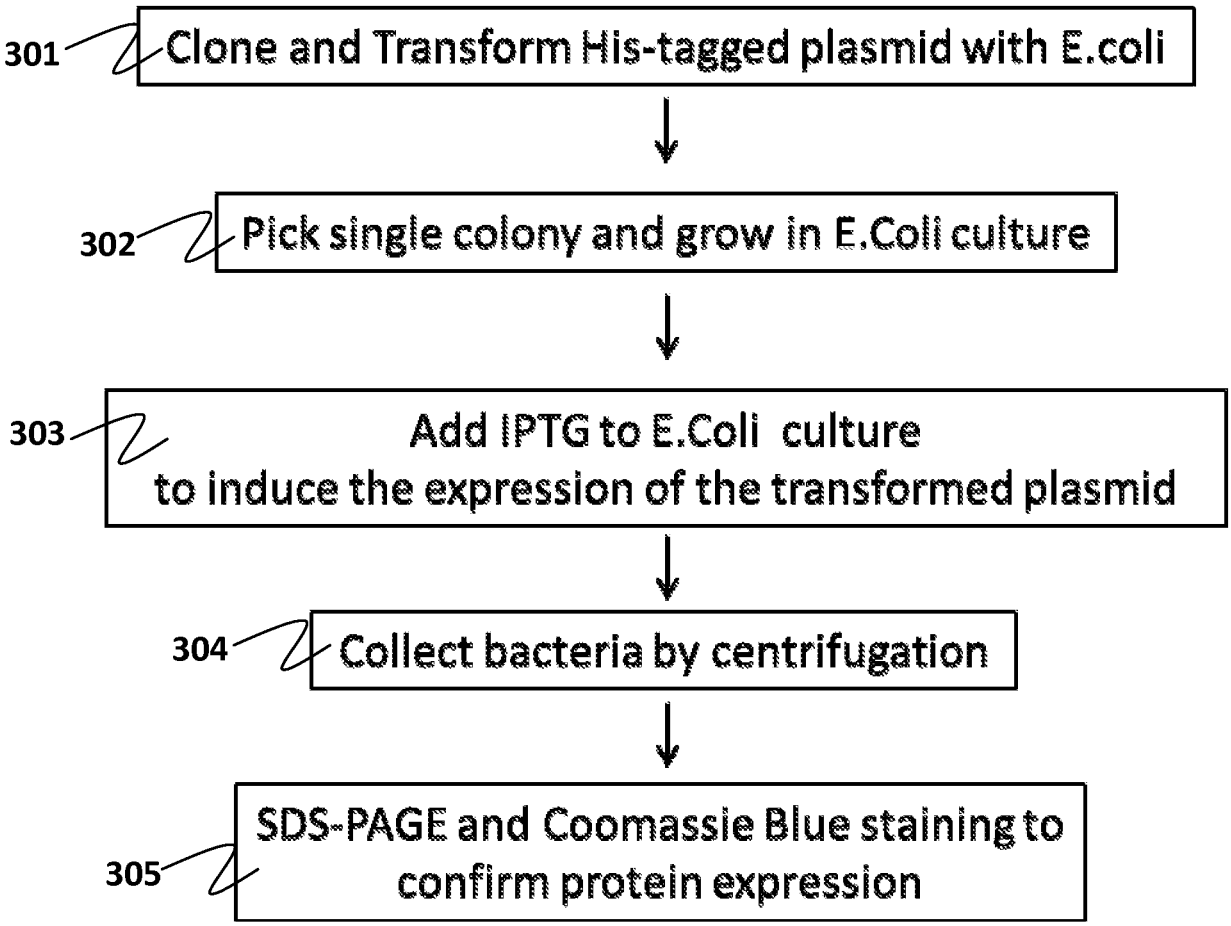

301
Clone and Transform His-tagged plasmid with E.coli
302
Pick single colony and grow in E.Coli culture
303
Add IPTG to E.Coli culture
to induce the expression of the transformed plasmid
304
Collect bacteria by centrifugation
305
SDS-PAGE and Coomassie Blue staining to
confirm protein expression

FIG. 3

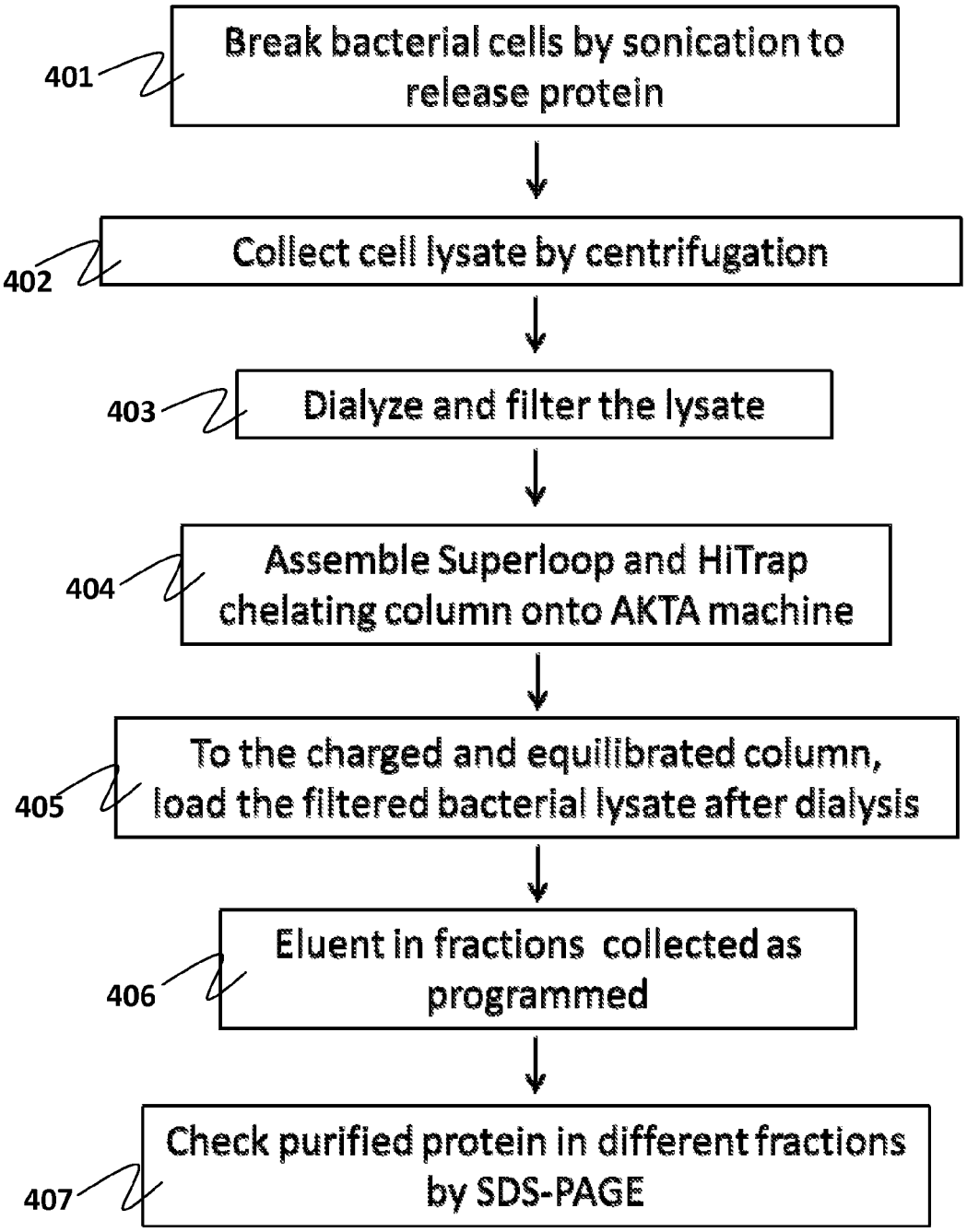

401
Break bacterial cells by sonication to release protein
402
Collect cell lysate by centrifugation
403
Dialyze and filter the lysate
404
Assemble Superloop and HiTrap chelating column onto AKTA machine
405
To the charged and equilibrated column, load the filtered bacterial lysate after dialysis
406
Eluent in fractions collected as programmed
407
Check purified protein in different fractions by SDS-PAGE

FIG. 4

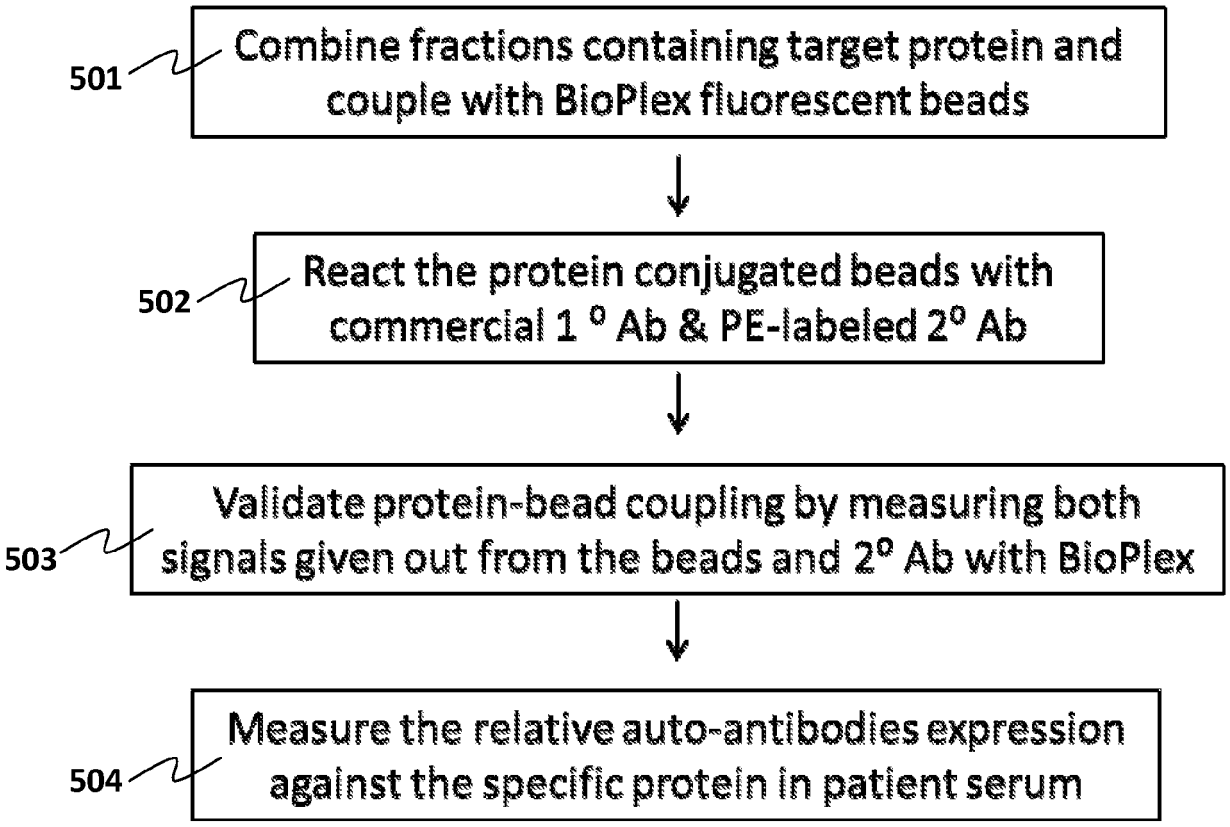

501
Combine fractions containing target protein and couple with BioPlex fluorescent beads
502
React the protein conjugated beads with commercial 1 º Ab & PE-labeled 2º Ab
503
Validate protein-bead coupling by measuring both signals given out from the beads and 2º Ab with BioPlex
504
Measure the relative auto-antibodies expression against the specific protein in patient serum

FIG. 5

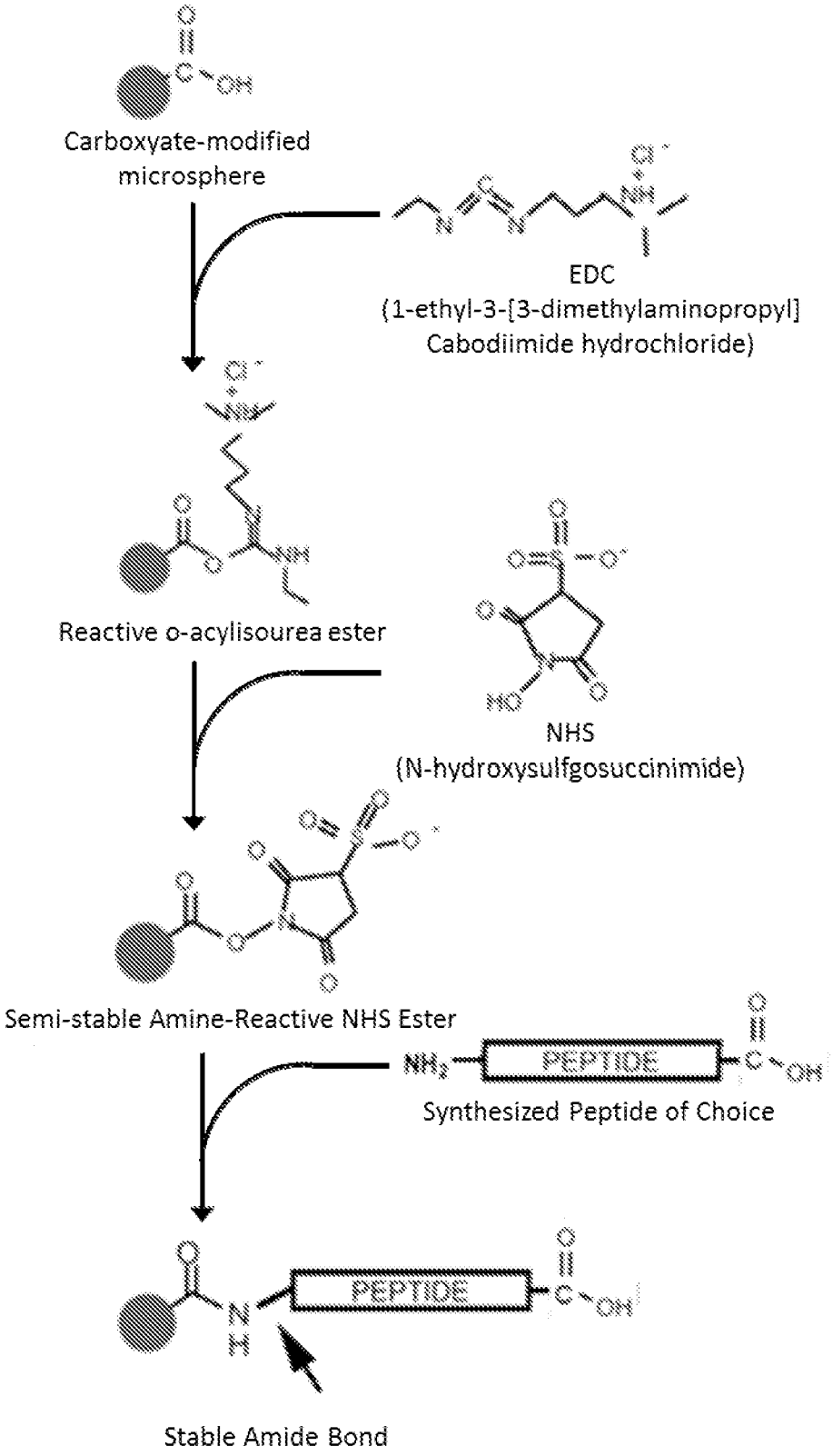

Carboxyate-modified microsphere
EDC
(1-ethyl-3-[3-dimethylaminopropyl] Cabodiimide hydrochloride)
Reactive o-acylisourea ester
NHS
(N-hydroxysulfgosuccinimide)
Semi-stable Amine-Reactive NHS Ester
PEPTIDE
Synthesized Peptide of Choice
PEPTIDE
Stable Amide Bond

FIG. 6

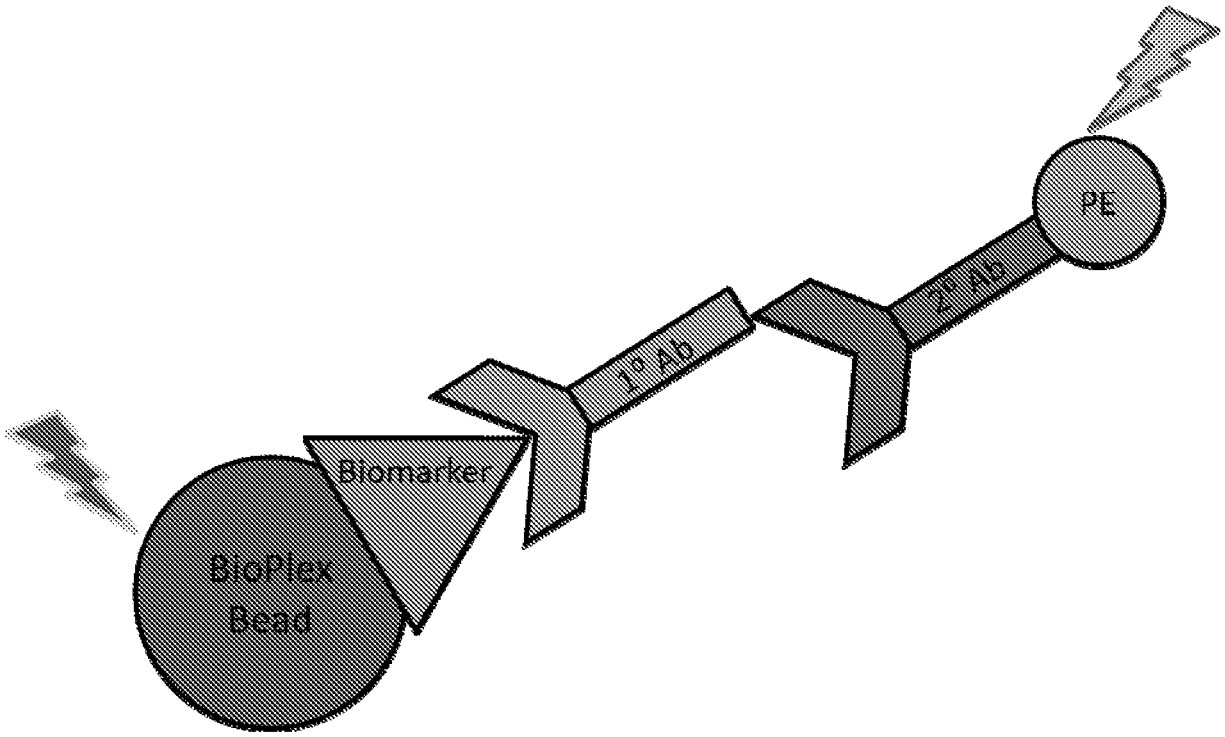


FIG. 7

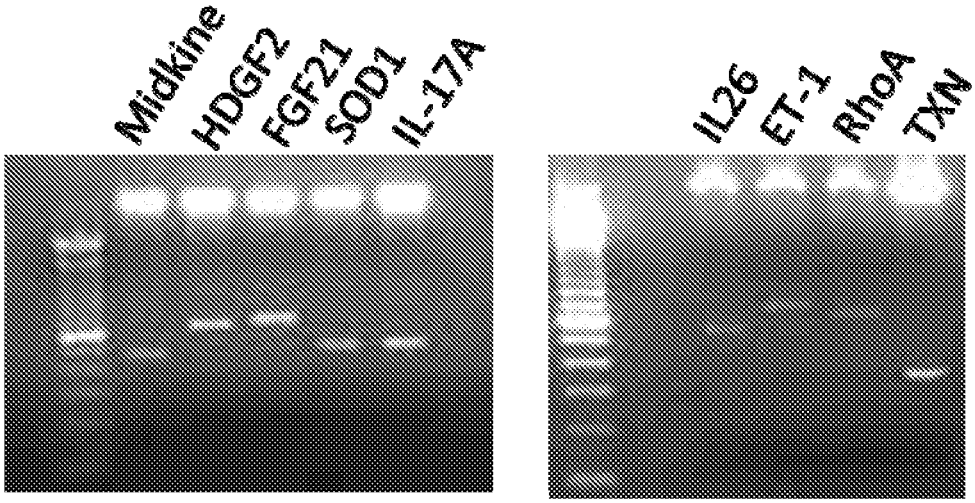


FIG. 8

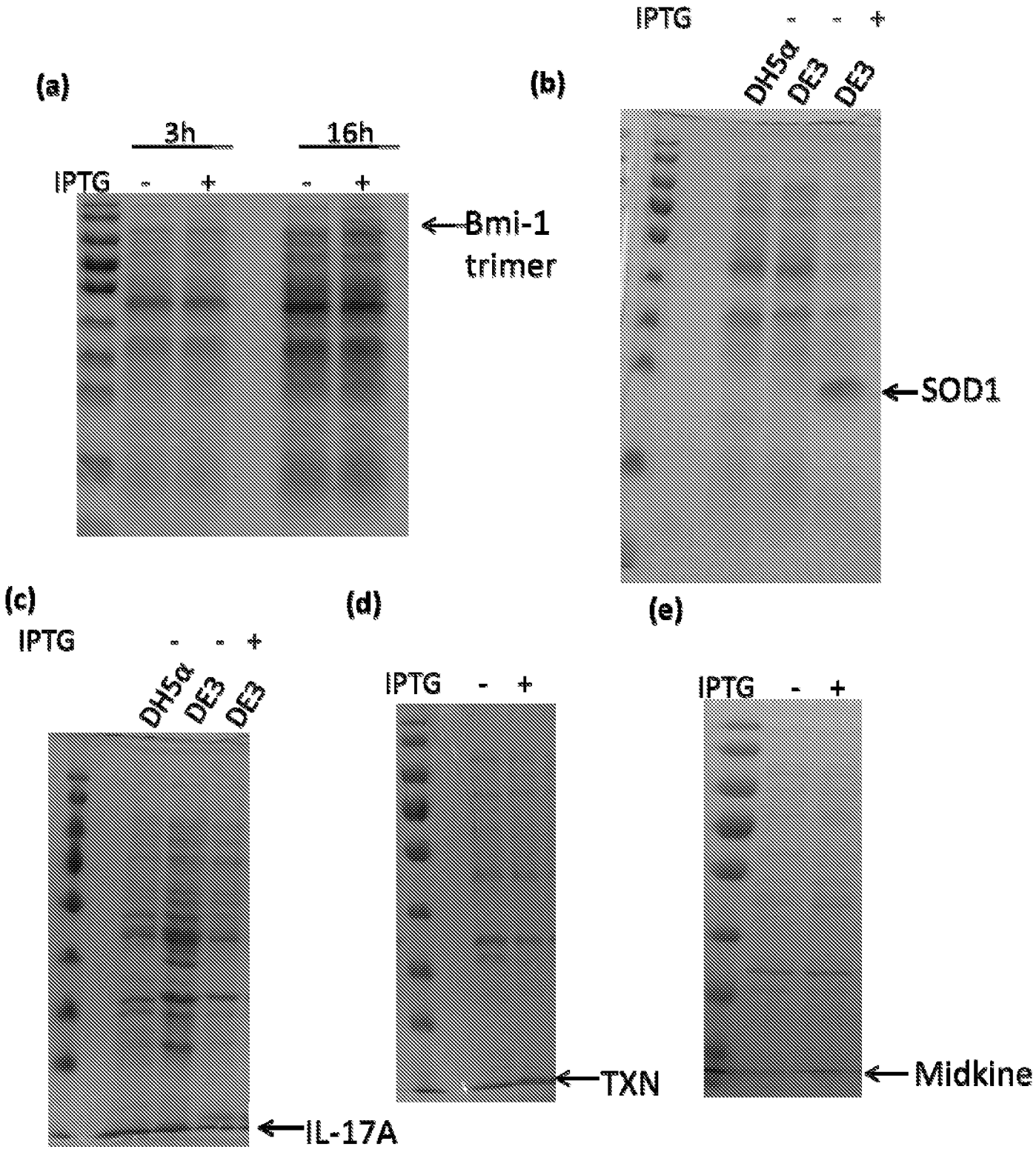

(a)
3h
16h
IPTG - + - +
Bmi-1
trimer
(b)
IPTG - - +
DH5α
DE3
DE3
SOD1
(c)
IPTG - - +
DH5α
DE3
DE3
IL-17A
(d)
IPTG - +
TXN
(e)
IPTG - +
Midkine

FIG. 9

(a)

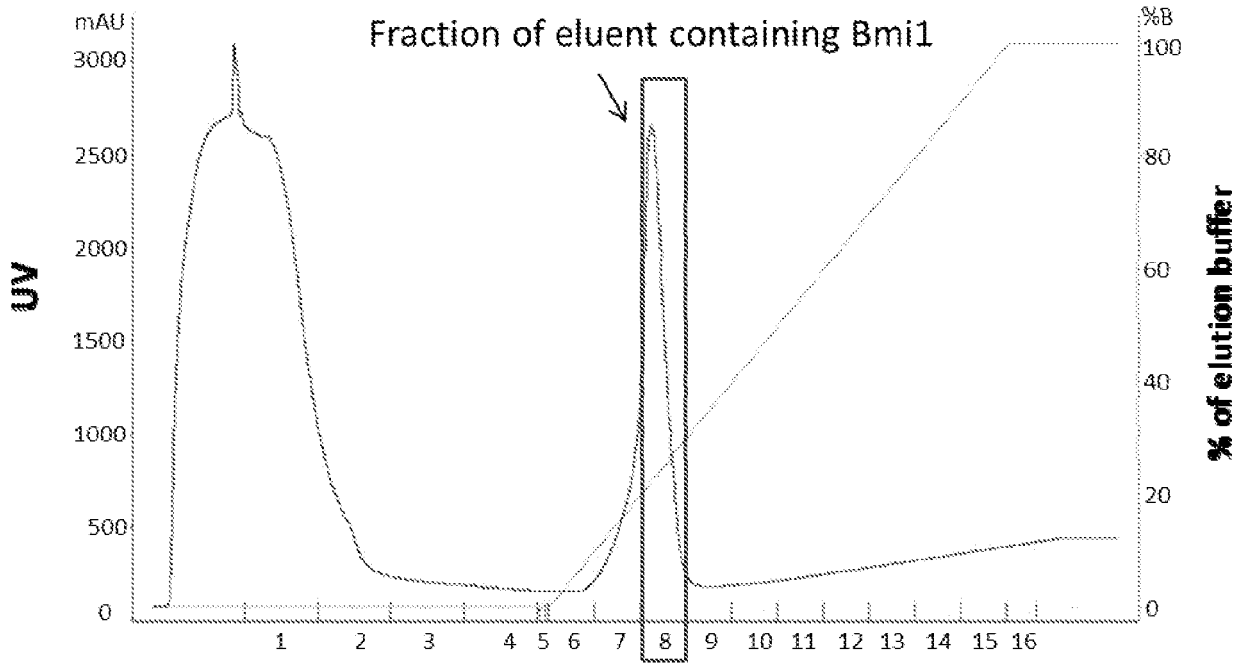

Fraction of eluent containing Bmi1
mAU
3000
2500
2000
1500
1000
500
0
UV
%B
100
80
60
40
20
0
% of elution buffer
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

FIG. 10

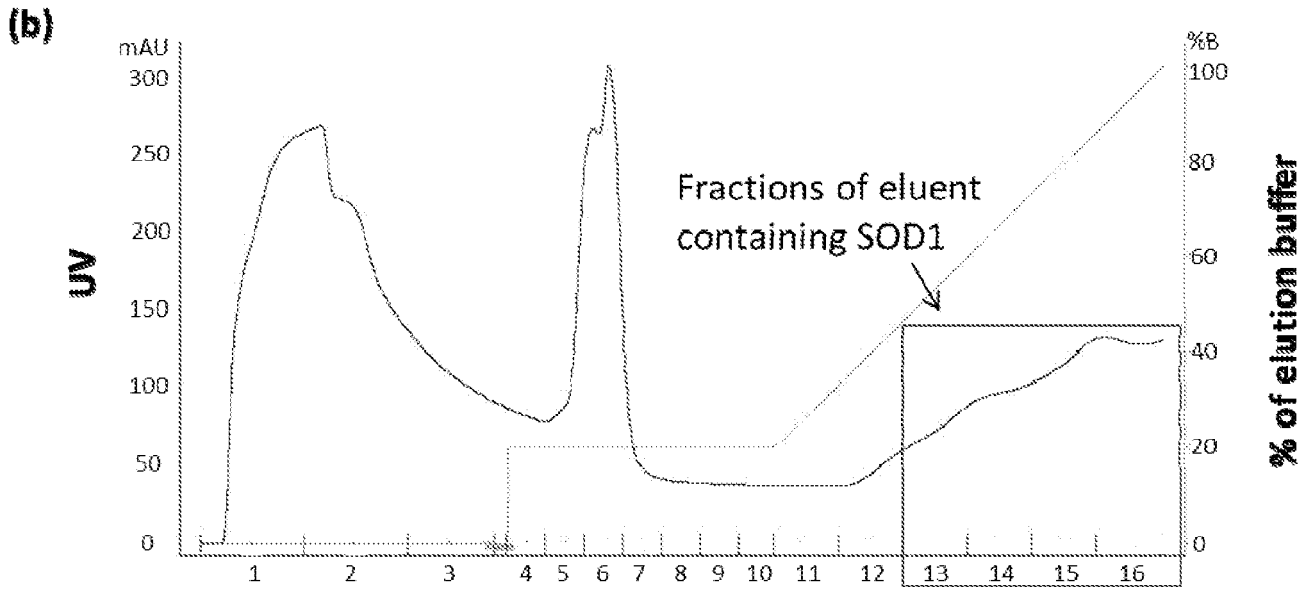

(b)
mAU
300
250
200
150
100
50
0
UV
%B
100
80
60
40
20
0
% of elution buffer
Fractions of eluent
containing SOD1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

FIG. 10 (cont'd)

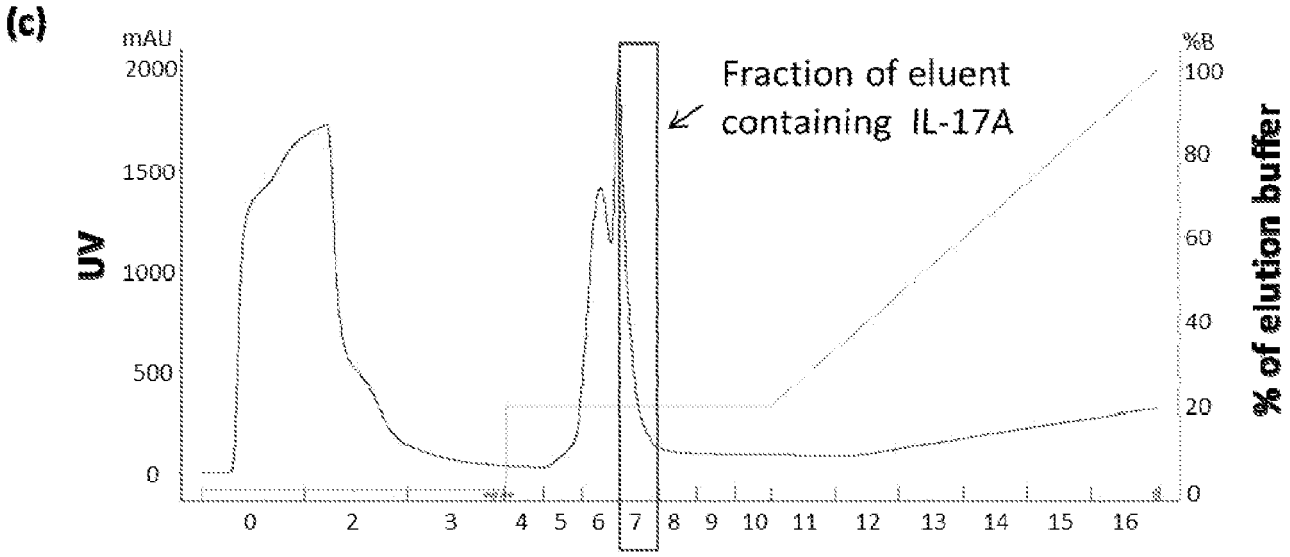

(c)
mAU
2000
1500
1000
500
0
UV
%B
100
80
60
40
20
0
% of elution buffer
Fraction of eluent
containing IL-17A
0 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

FIG. 10 (cont'd)

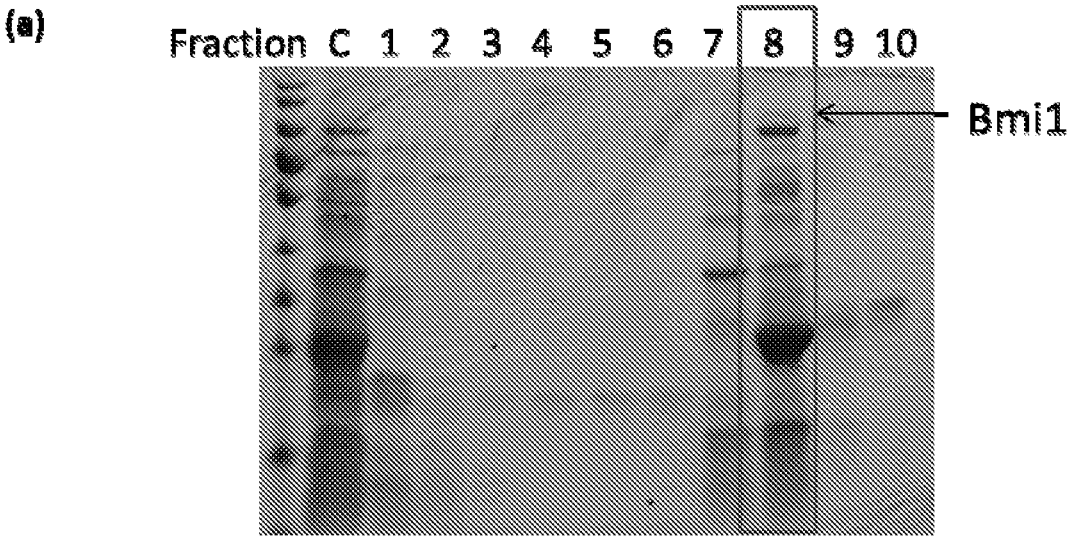


FIG. 11

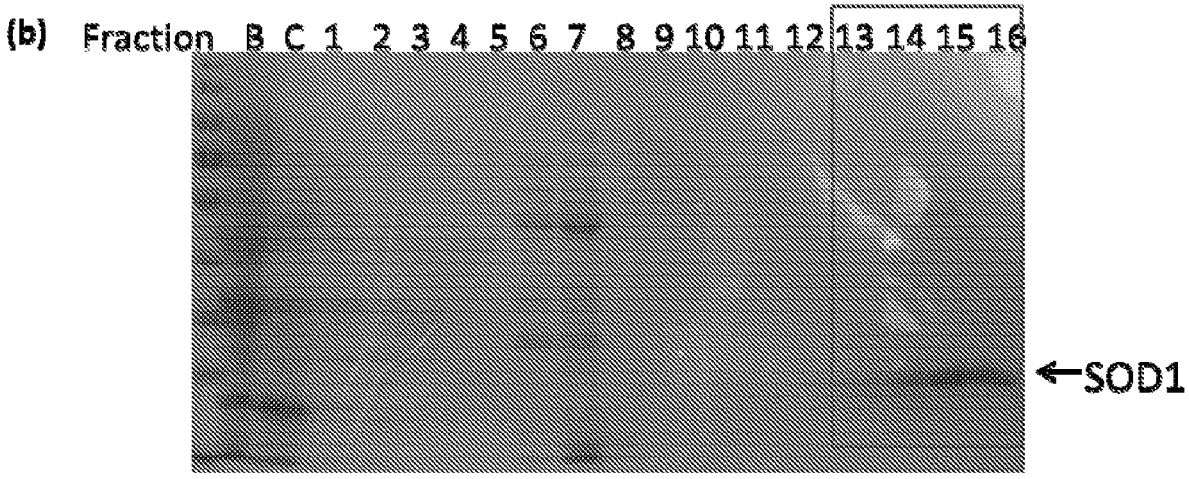


FIG. 11 (cont'd)

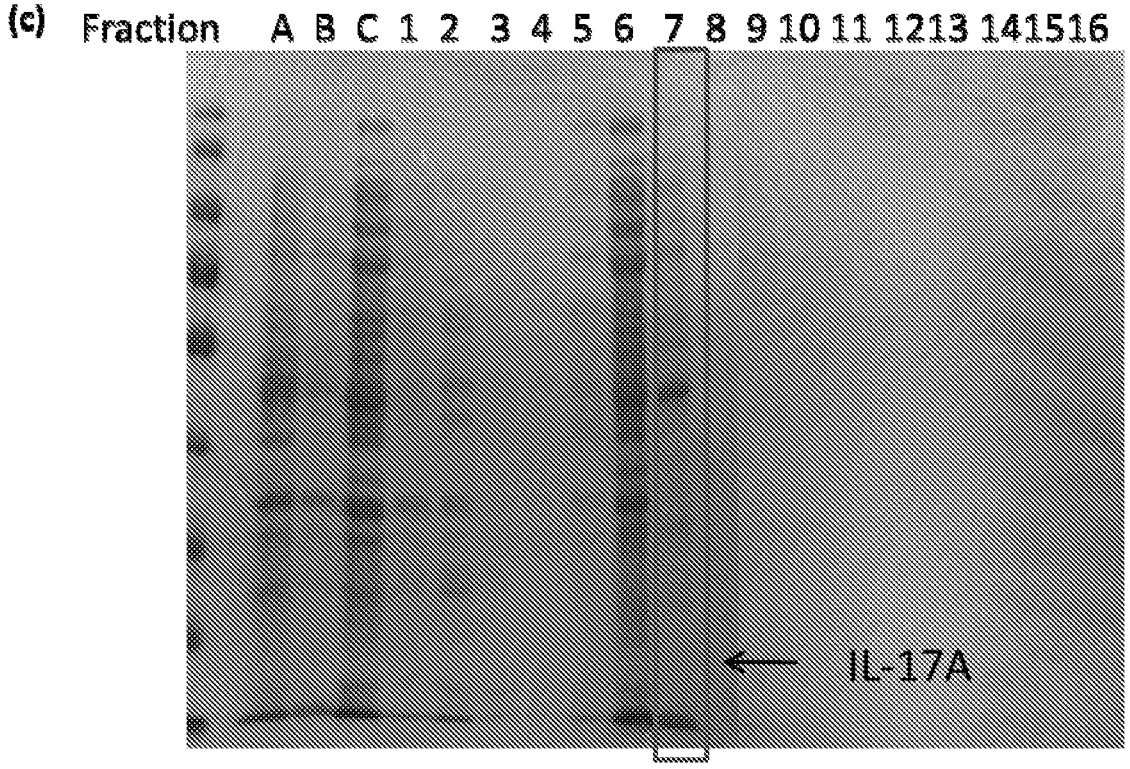


FIG. 11 (cont'd)

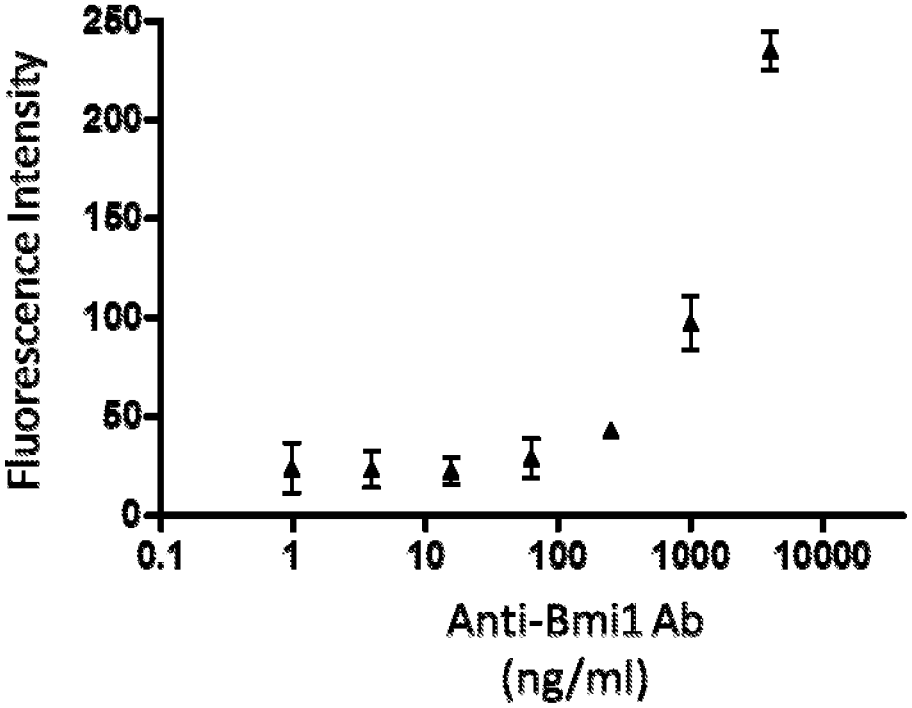

Fluorescence Intensity
250
200
150
100
50
0
0.1
1
10
100
1000
10000
Anti-Bmi1 Ab
(ng/ml)

FIG. 12

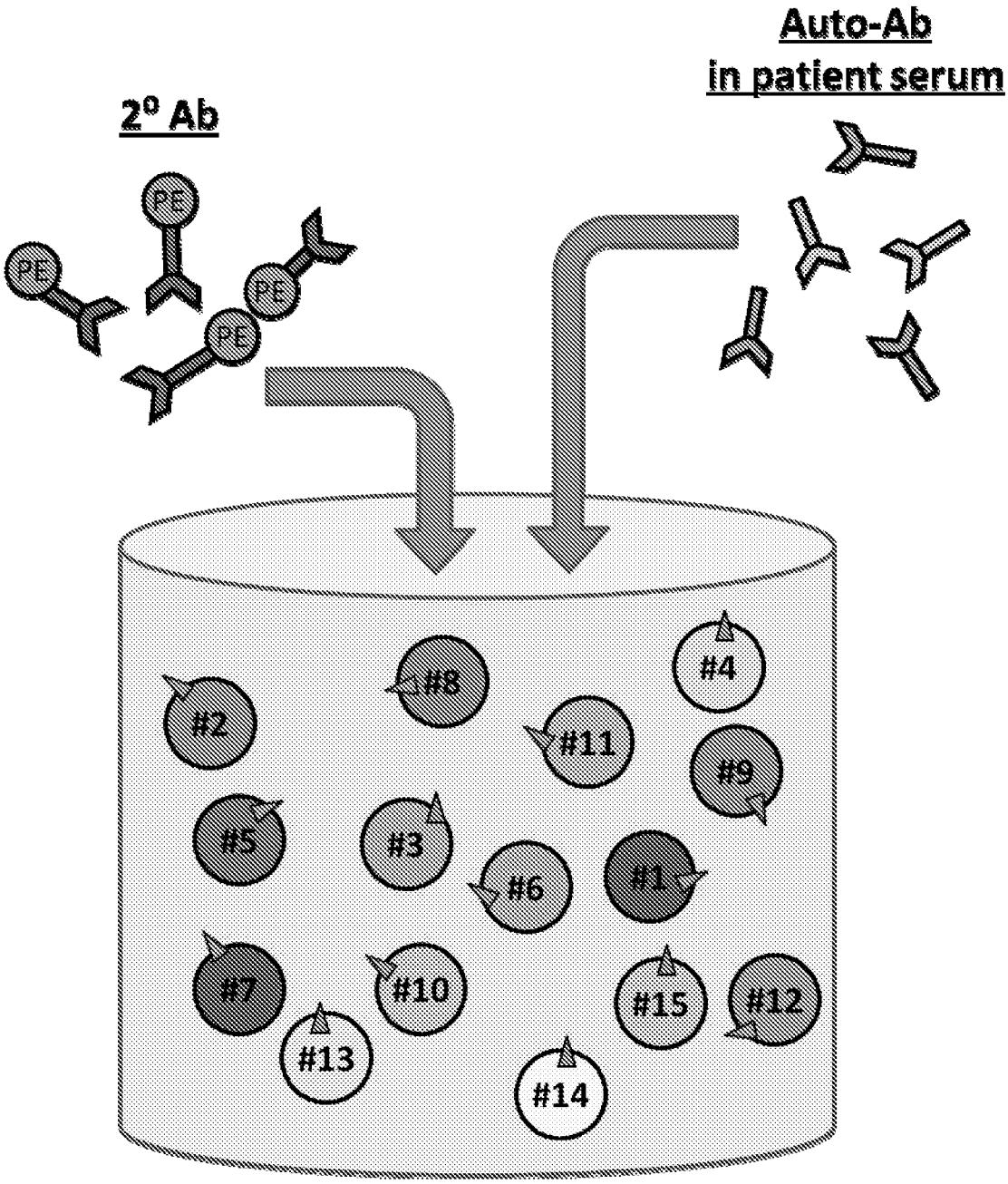

Auto-Ab
in patient serum
2° Ab
PE
PE
PE
PE
#8
#4
#2
#11
#9
#5
#3
#6
#1
#7
#10
#15
#12
#13
#14

FIG. 13