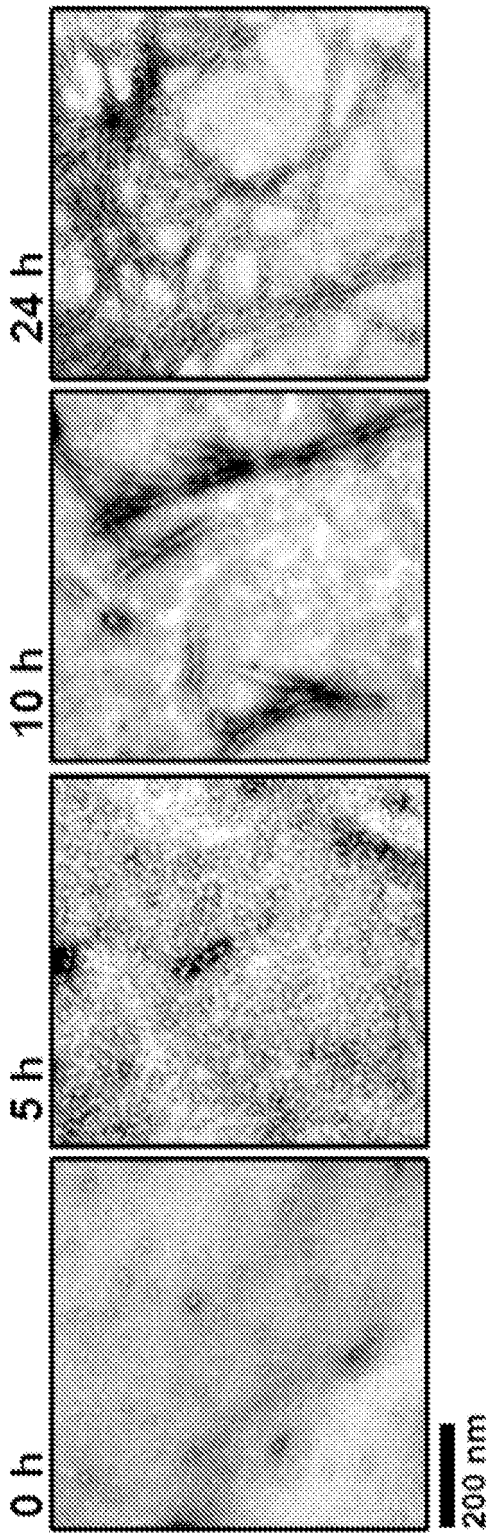

FIG. 1A

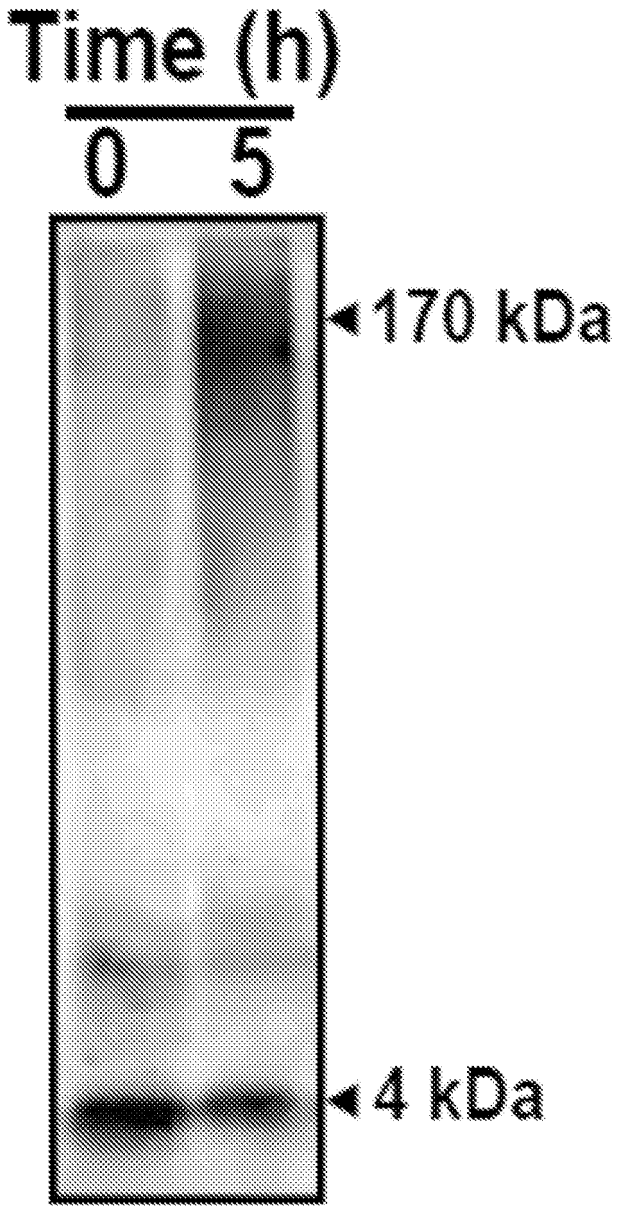

FIG. 1B

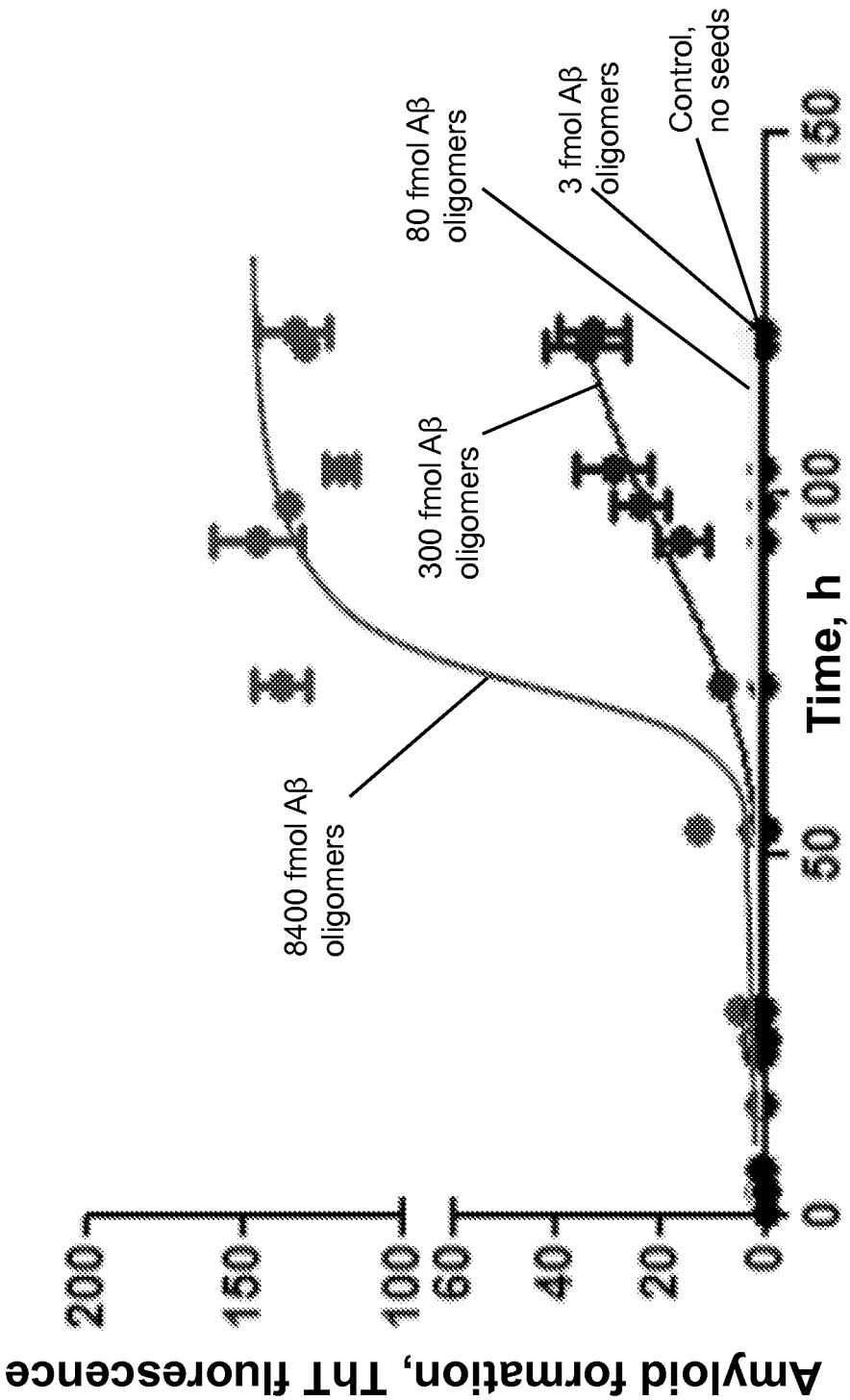
Amyloid formation, ThT fluorescence
200
150
100
60
40
20
0
0
50
100
150
Time, h
8400 fmol Aβ oligomers
300 fmol Aβ oligomers
80 fmol Aβ oligomers
3 fmol Aβ oligomers
Control, no seeds

FIG. 2A

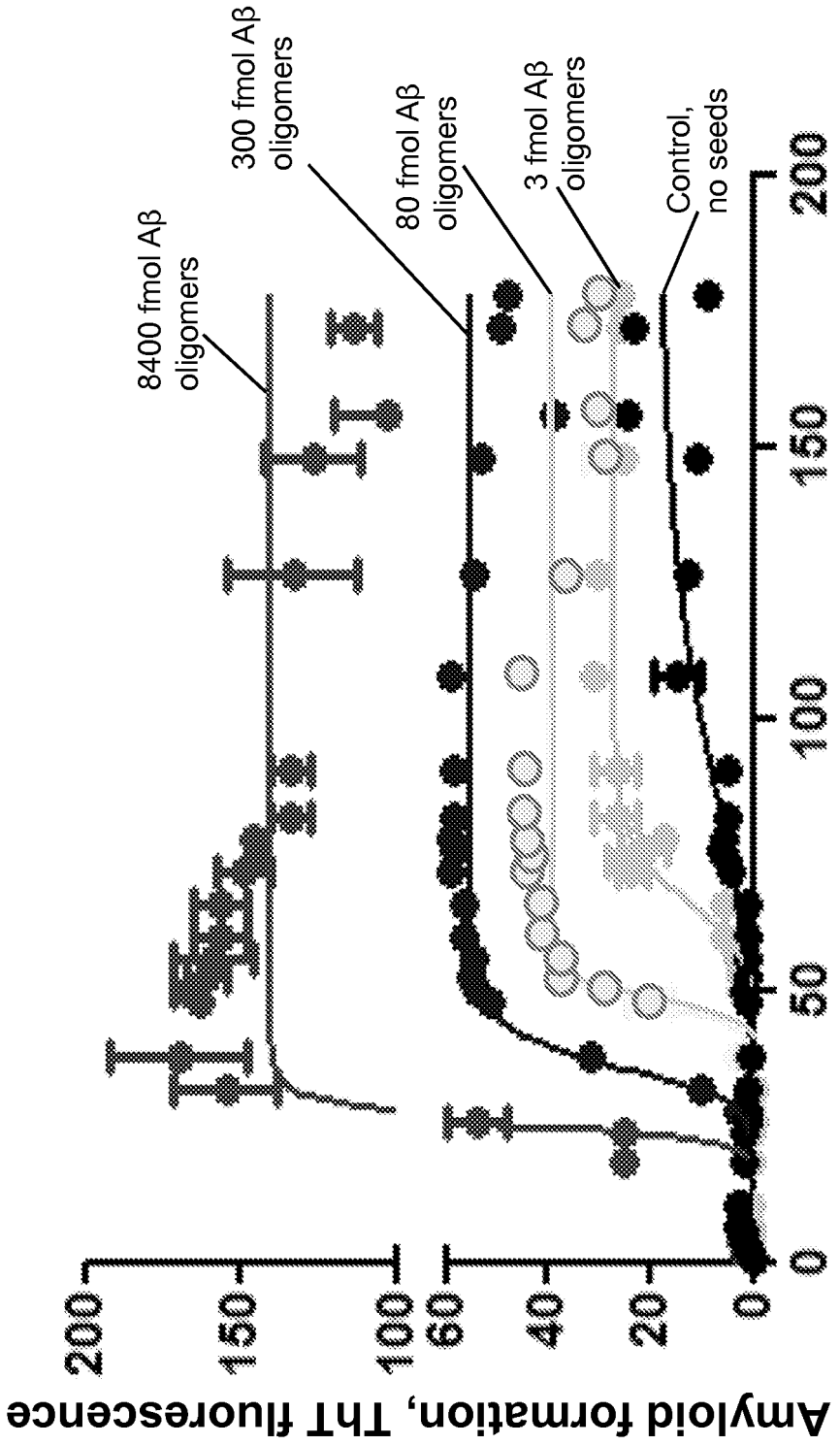
Amyloid formation, ThT fluorescence
200
150
100
60
40
20
0
0
50
100
150
200
Time, H
8400 fmol Aβ oligomers
300 fmol Aβ oligomers
80 fmol Aβ oligomers
3 fmol Aβ oligomers
Control, no seeds

FIG. 2B

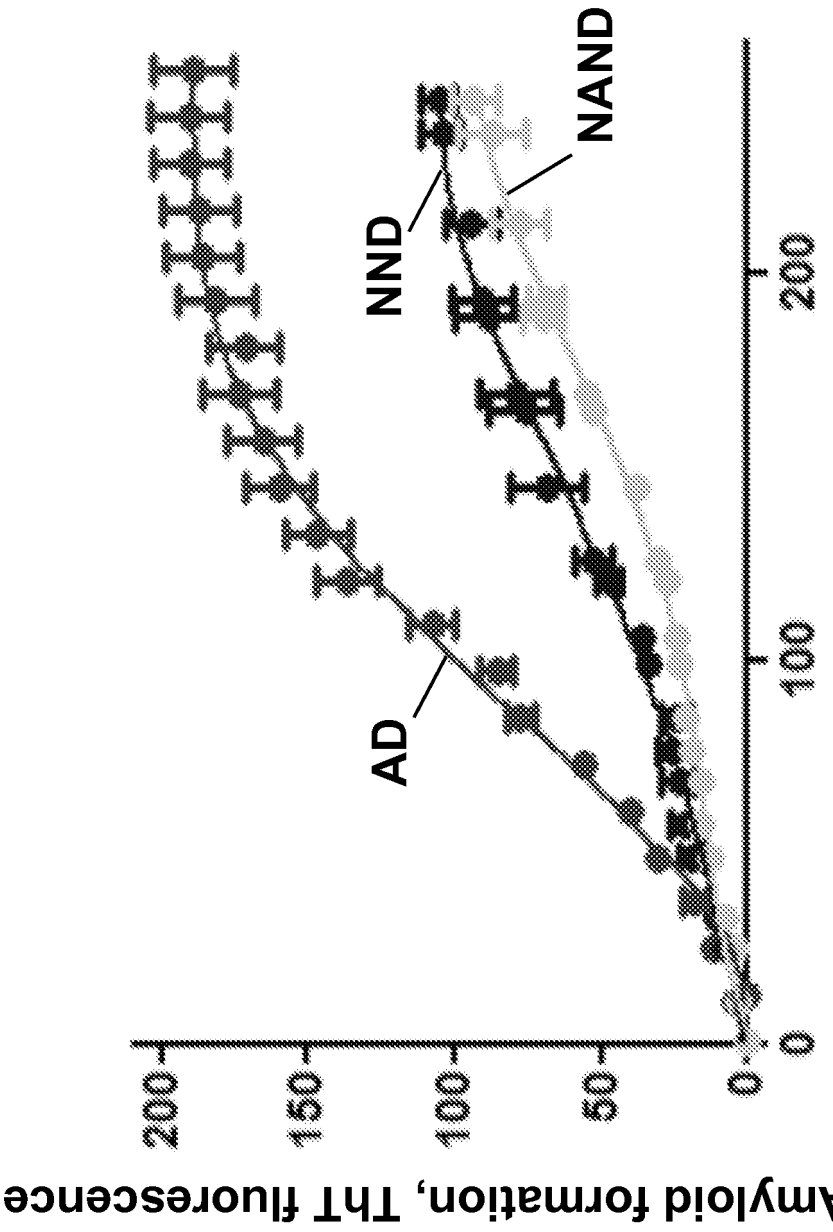
Amyloid formation, ThT fluorescence
200
150
100
50
0
0
100
200
Time, h
AD
NND
NAND

FIG. 3A

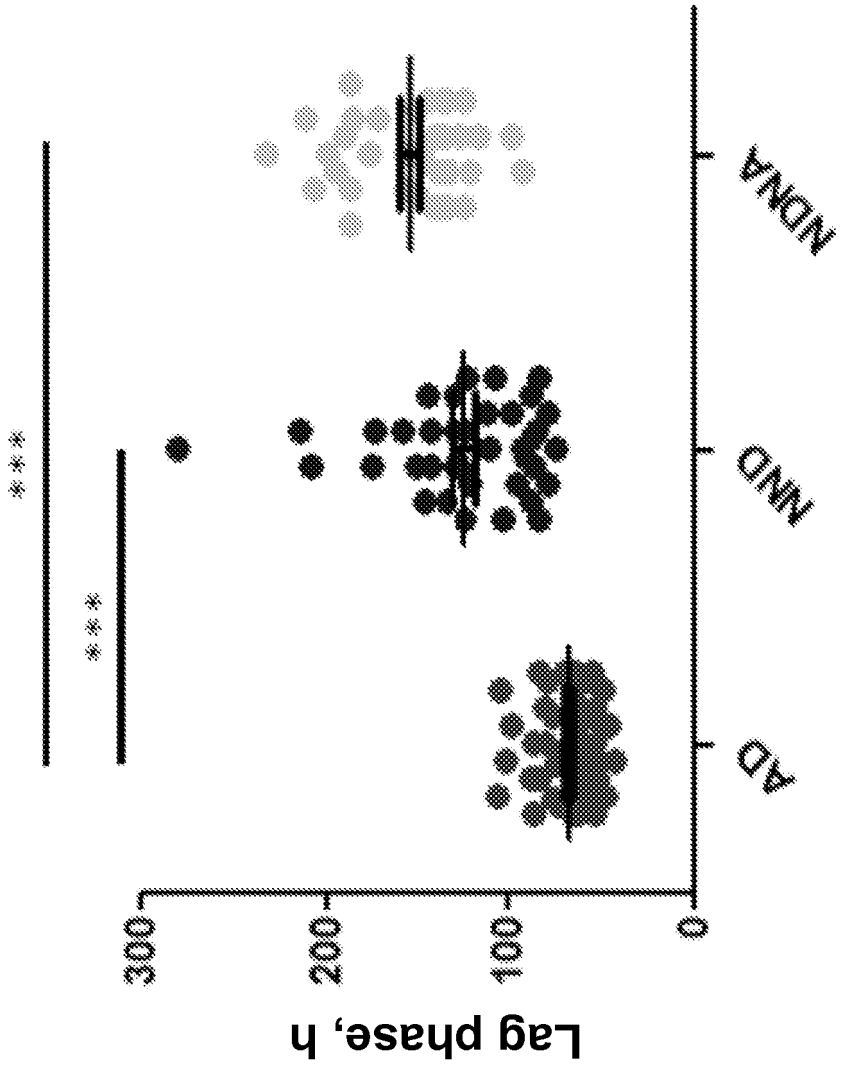

FIG. 3B

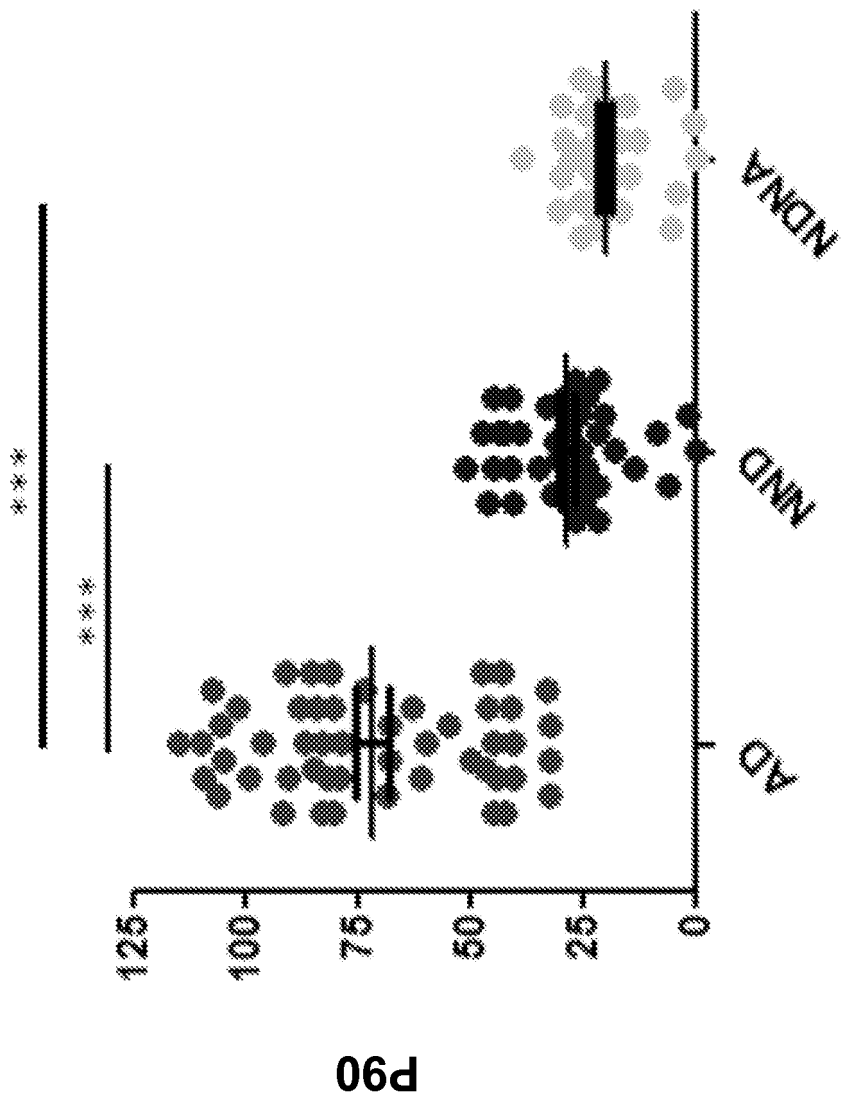

FIG. 3C

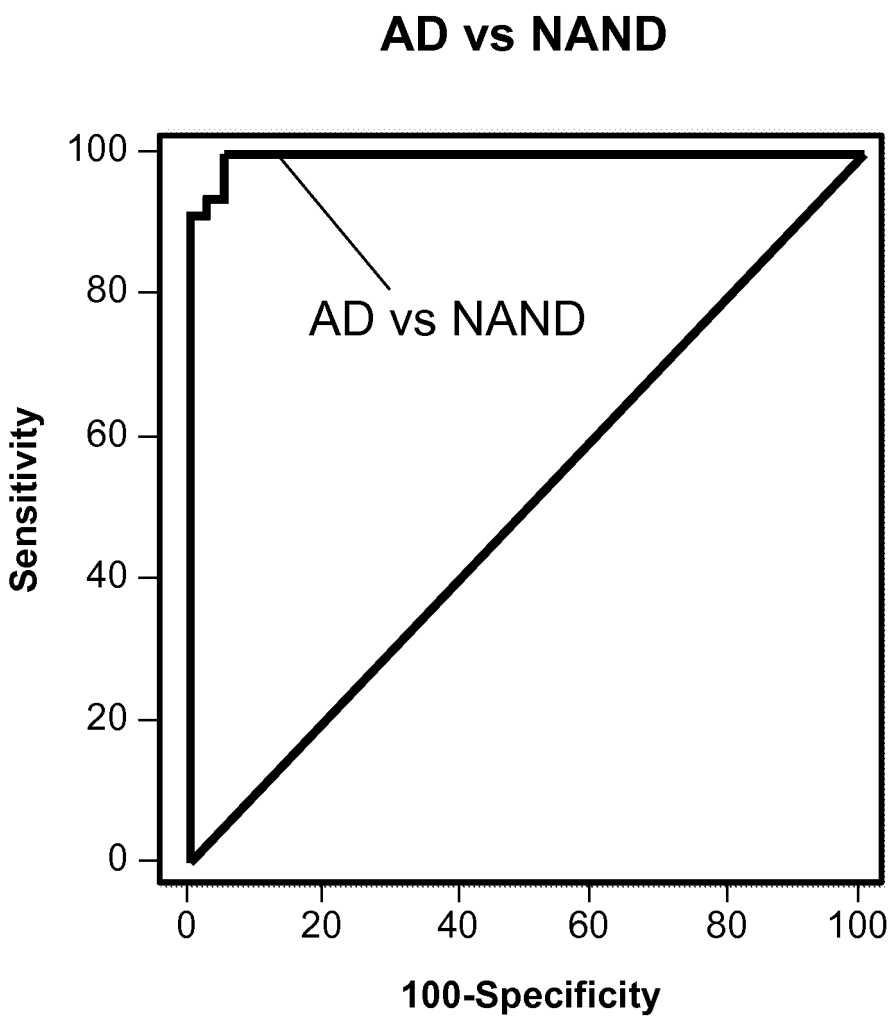
AD vs NAND
AD vs NAND
Sensitivity
100
80
60
40
20
0
0
20
40
60
80
100
100-Specificity

FIG. 4A

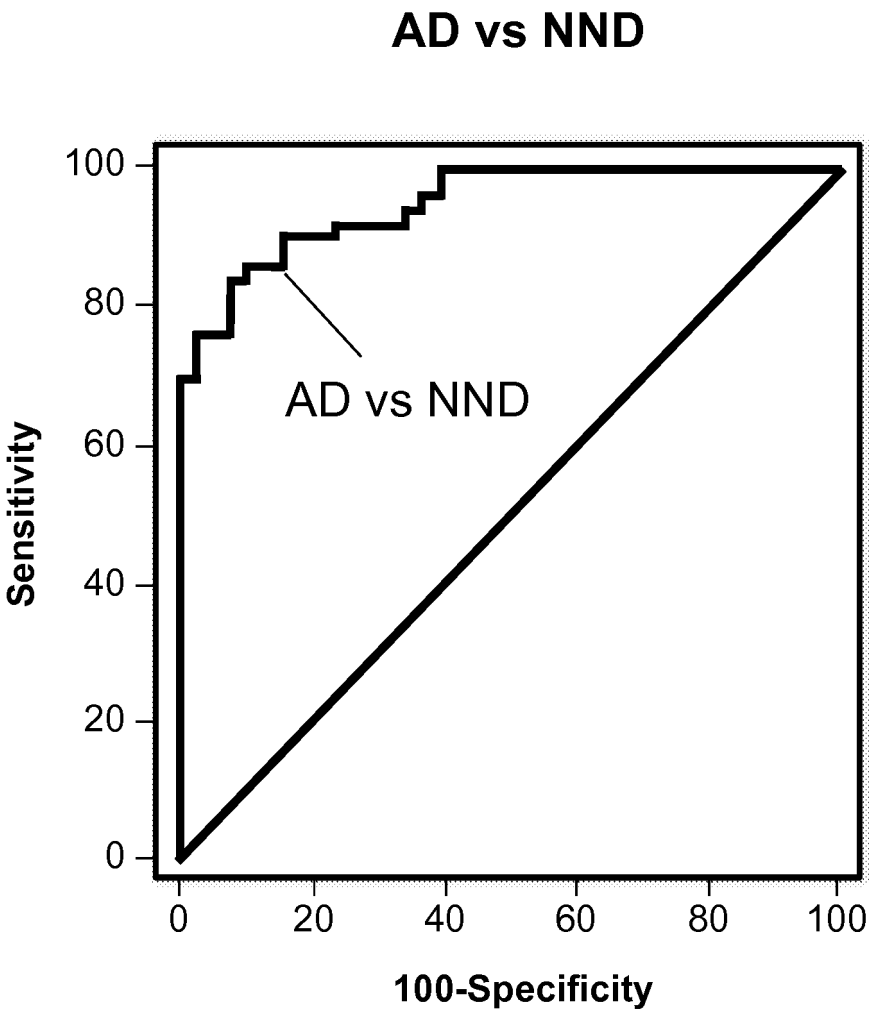
AD vs NND
100
80
60
40
20
0
Sensitivity
AD vs NND
0
20
40
60
80
100
100-Specificity

FIG. 4B

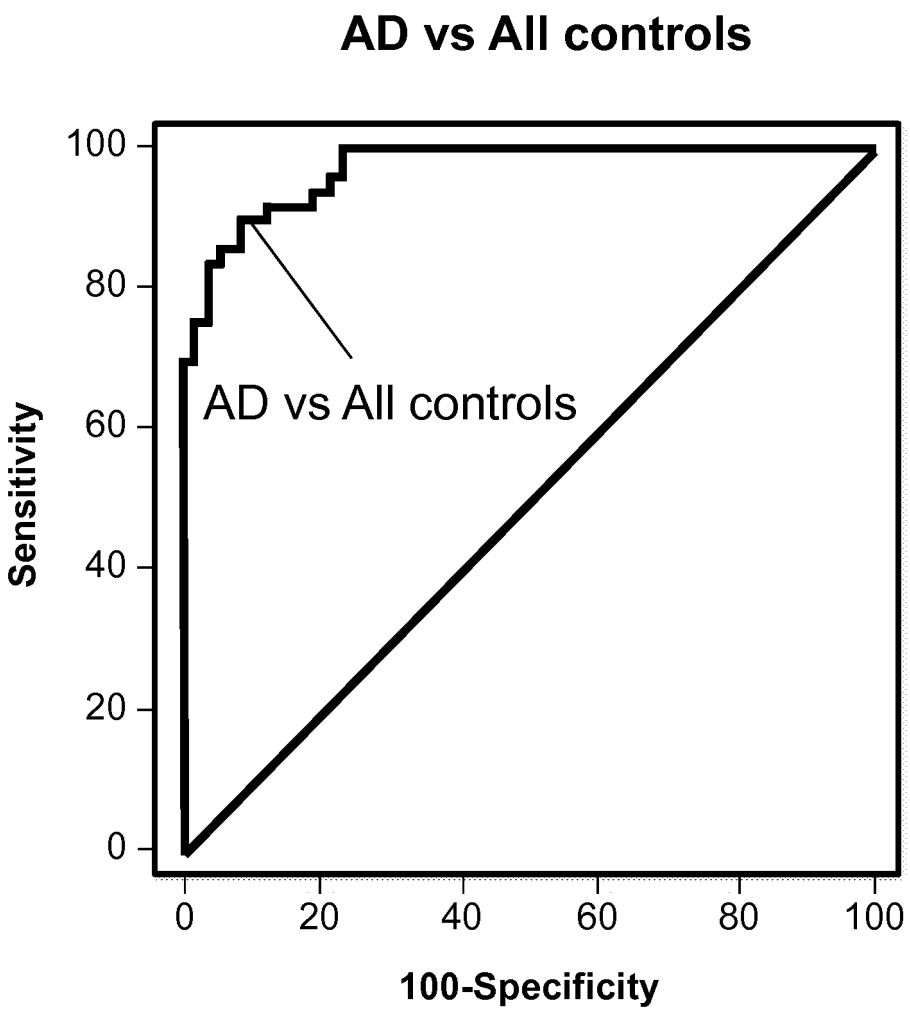
AD vs All controls
AD vs All controls
Sensitivity
100
80
60
40
20
0
0
20
40
60
80
100
100-Specificity

FIG. 4C

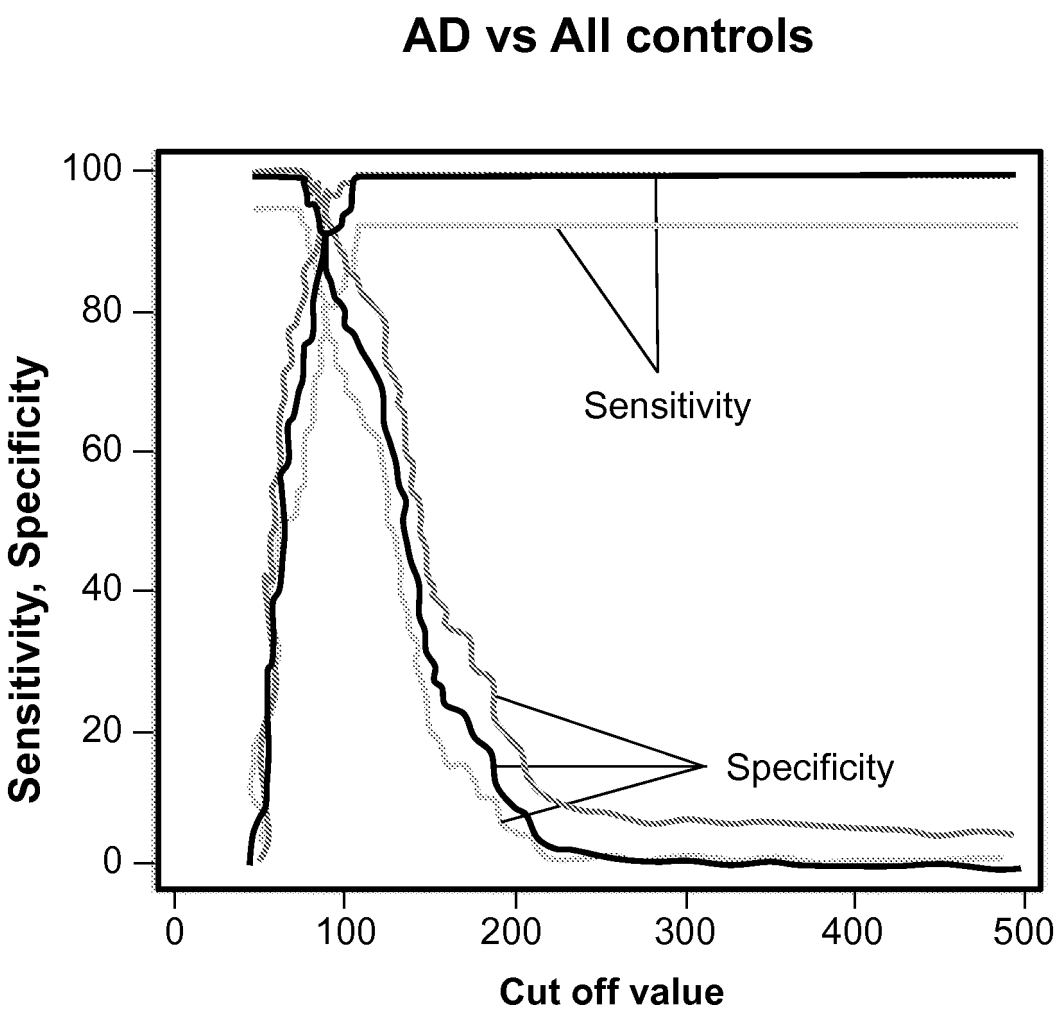
AD vs All controls
Sensitivity, Specificity
100
80
60
40
20
0
0
100
200
300
400
500
Cut off value
Sensitivity
Specificity

FIG. 4D

Table 1: Estimation of sensitivity, specificity, and predictive value for Aβ-PCMA in CSF[1]

GROUPS	SENSITIVITY[2]	SENSITIVITY[2]	POSITIVE PREDICTIVE VALUE[2]	NEGATIVE PREDICTIVE VALUE[2]
AD vs NAND	100.0%	94.6%	96.2%	100.0%
AD vs NND	90.0%	84.2%	88.2%	86.5%
AD vs controls[3]	90.0%	92.0%	88.2%	93.2%

1. For estimation of sensitivity, specificity and predictive value the results of the lag phase as shown in FIG. 9B were used. Cutoffs were estimated by Receiver Operating Characteristics (ROC) curve analysis using the MedCalc software.

2. Sensitivity was estimated by the formula: [True positives/(True positives + False negatives)] x 100; specificity was estimated by the formula: [True negatives/(False positives + True negatives)] x 100; positive predictive value was estimated by the formula: [True positives/(True positives + False positives)] x 100; negative predictive value was estimated by the formula: [True negatives/(True negatives + False negatives)] x 100

3. Controls refers to the samples from NND plus NAND.

FIG. 5

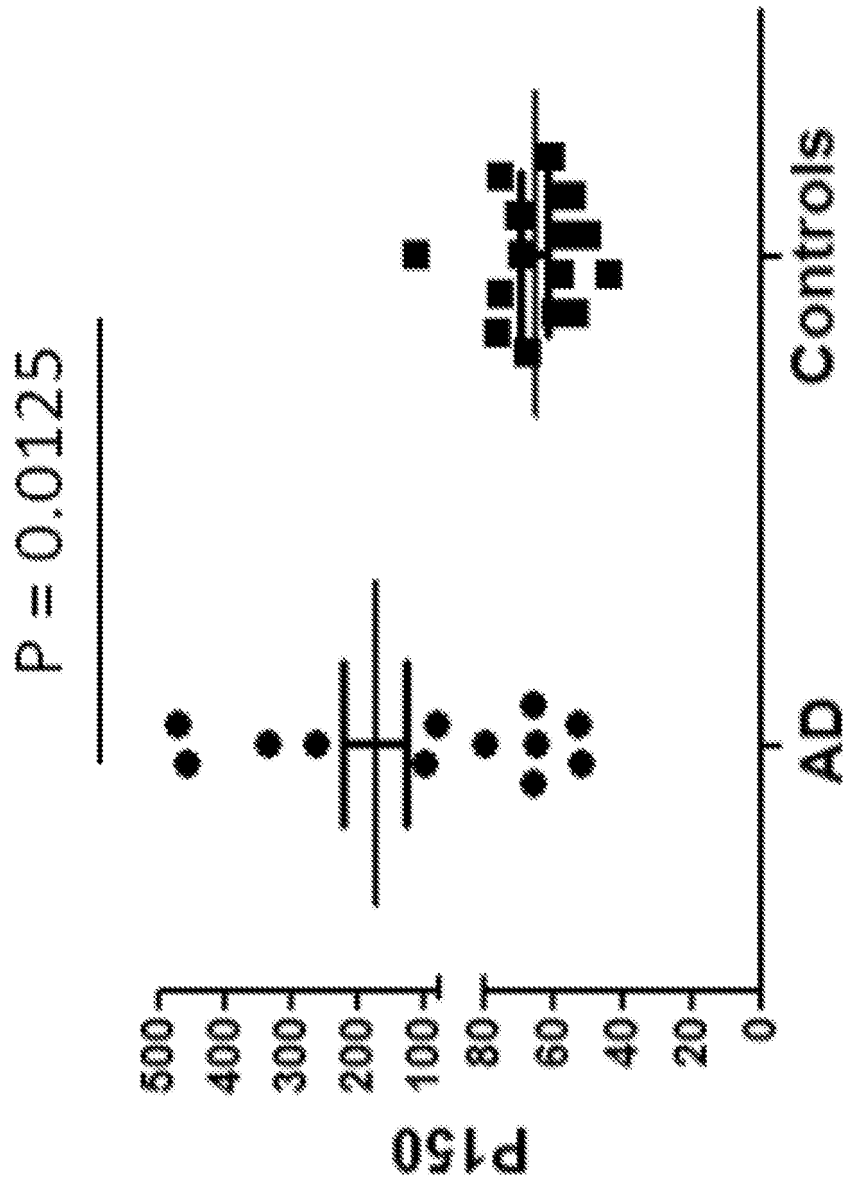

FIG. 6

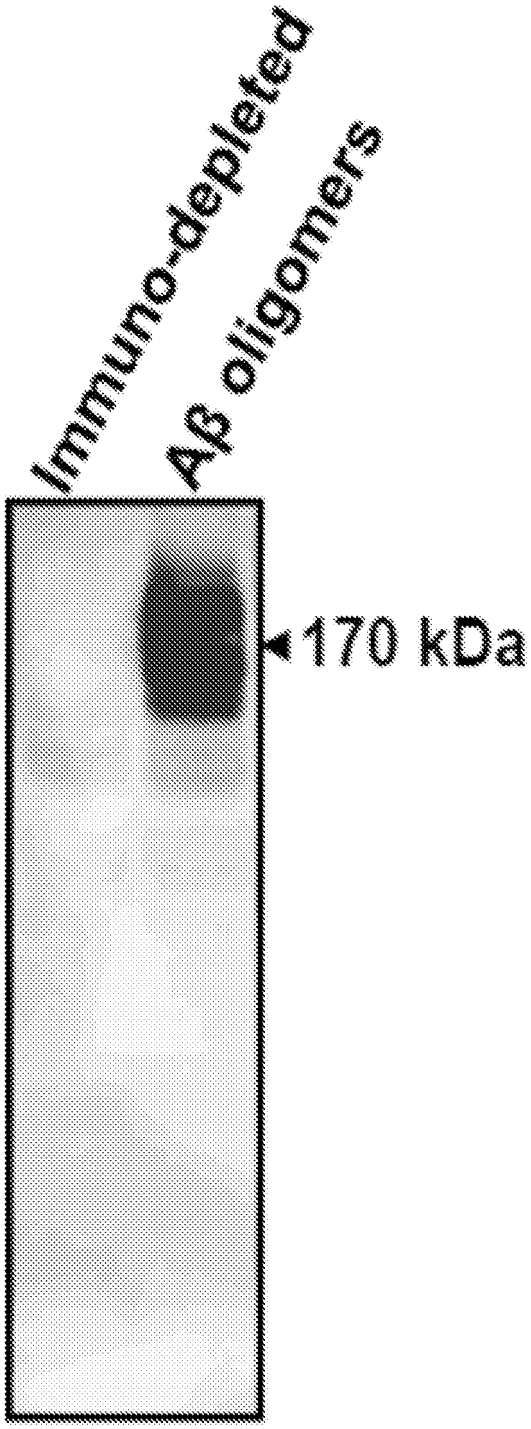

FIG. 7A

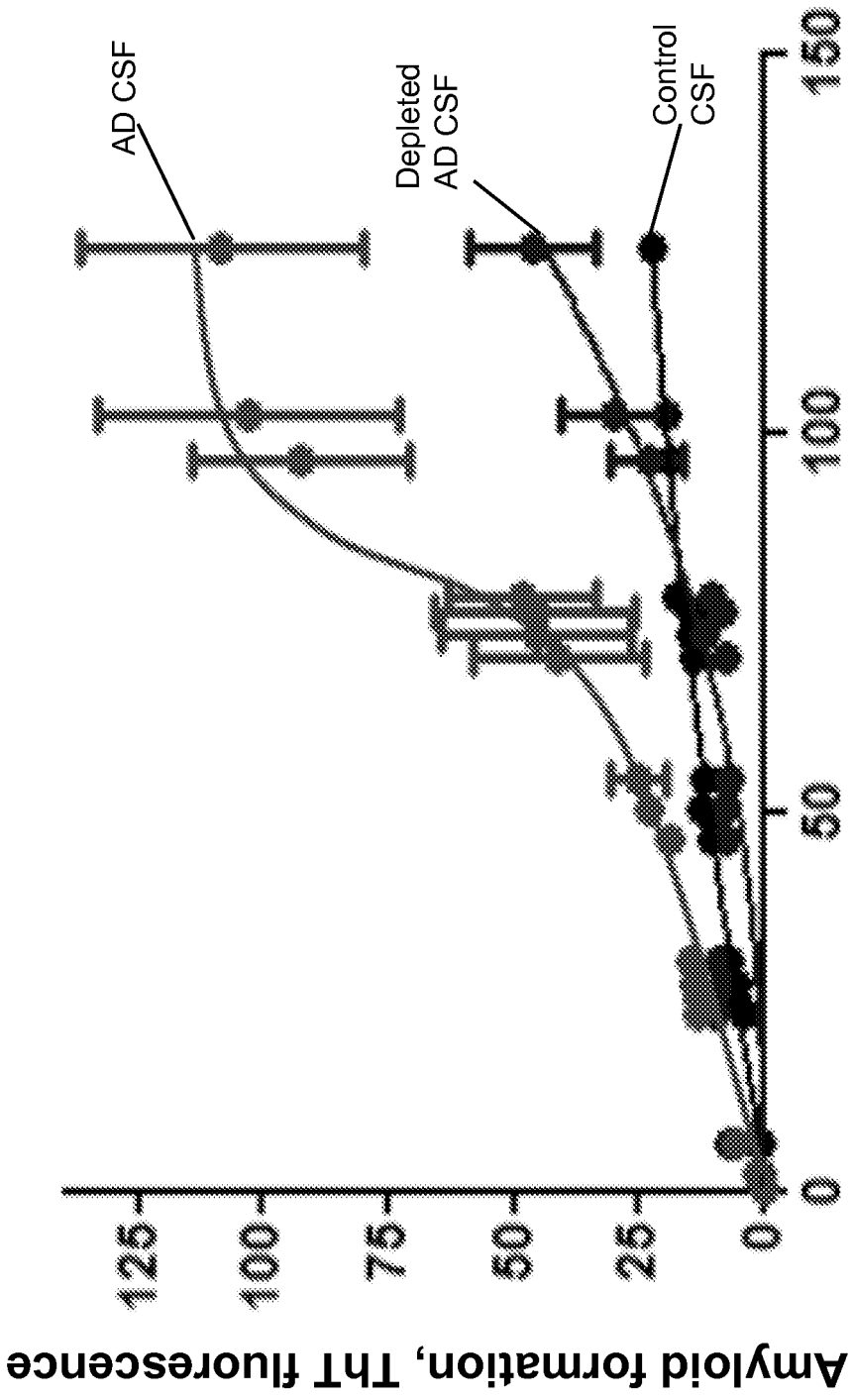
Amyloid formation, ThT fluorescence
125
100
75
50
25
0
0
50
100
150
Time, h
AD CSF
Depleted AD CSF
Control CSF

FIG. 7B

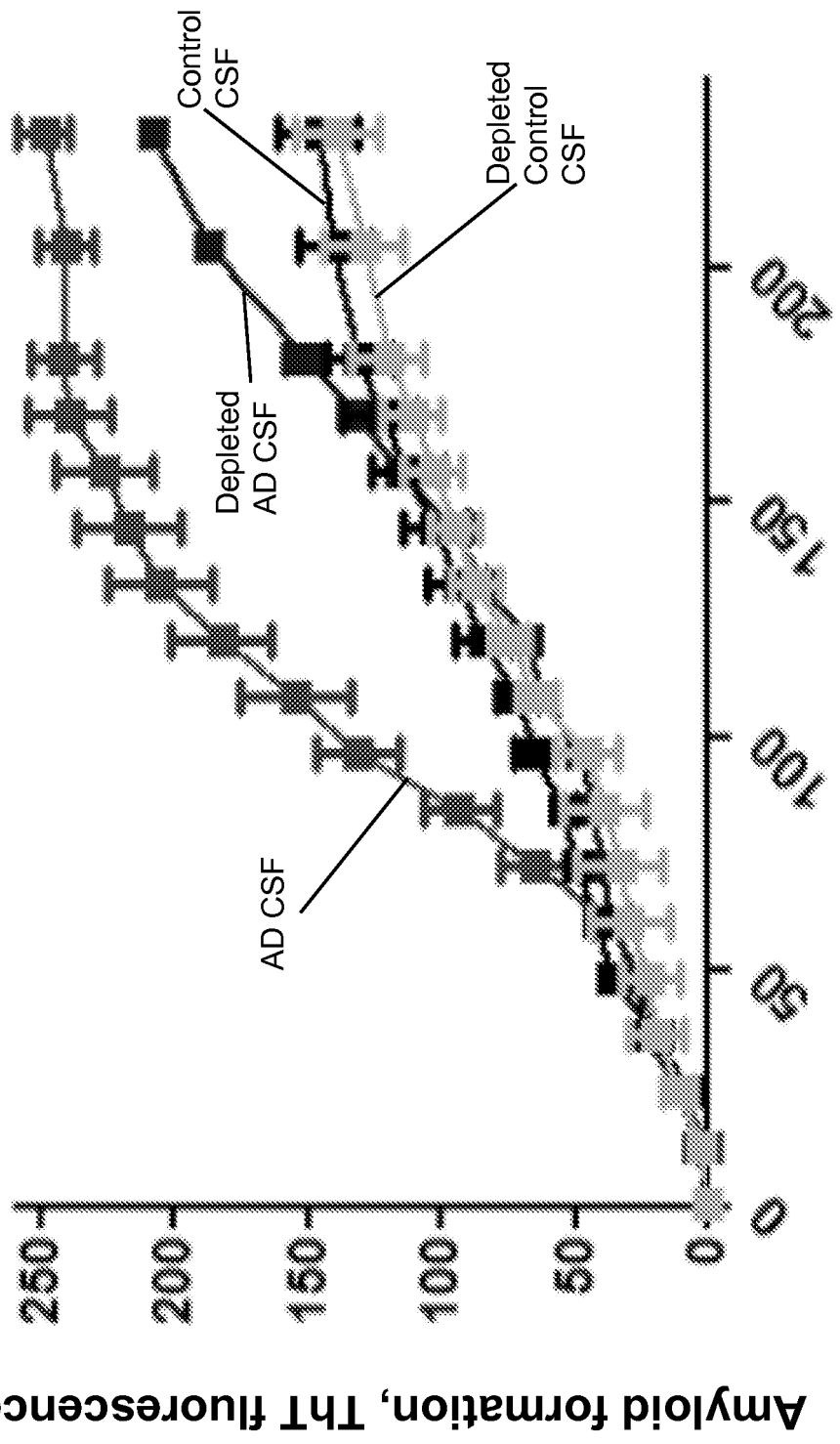
Amyloid formation, ThT fluorescence
250
200
150
100
50
0
0
50
100
150
200
Time, h
AD CSF
Depleted AD CSF
Control CSF
Depleted Control CSF

FIG. 7C

Strategy 1: Using antibody-coated plates

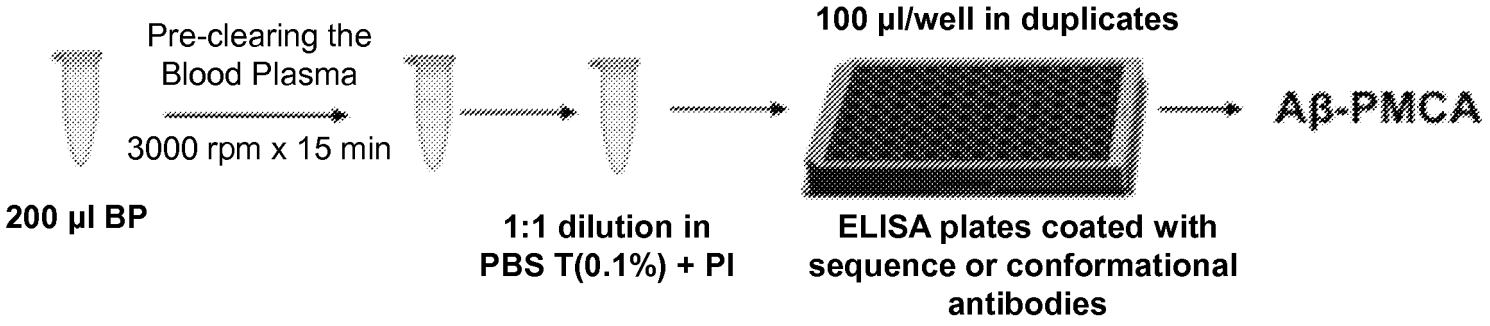

FIG. 8A

Strategy 2: Immuno-precipitation and concentration by antibody-coated beads

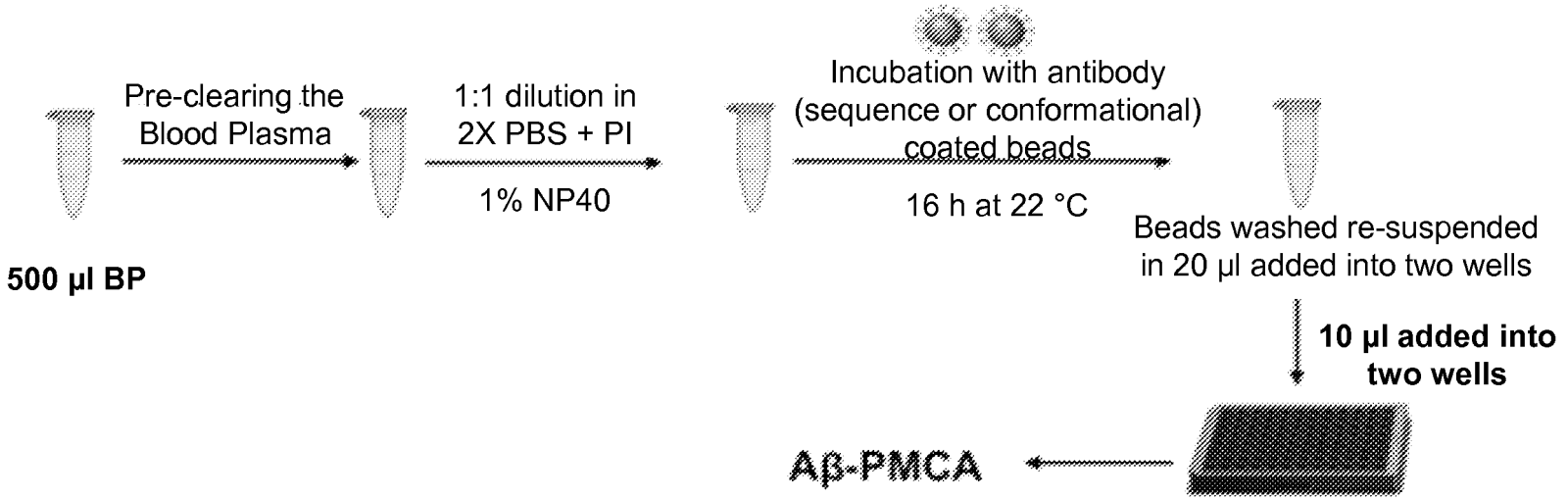

FIG. 8B

Table 2

Antibody	Epitope	Commercial source	Aβ oligomer capturing capacity
82E1	1-5	IBL America	+++
4G8	18-22	Covance	+
6E10	3-8	Covance	++
X·40/42	C·terminal	Mybiosource	-
16 ADV Mouse lgGl	Conformational	Acumen	++
A11	Conformational	Invitrogen	-

+++	***Best***
++	***very good***
+	***good***
-	***no result***

FIG. 9

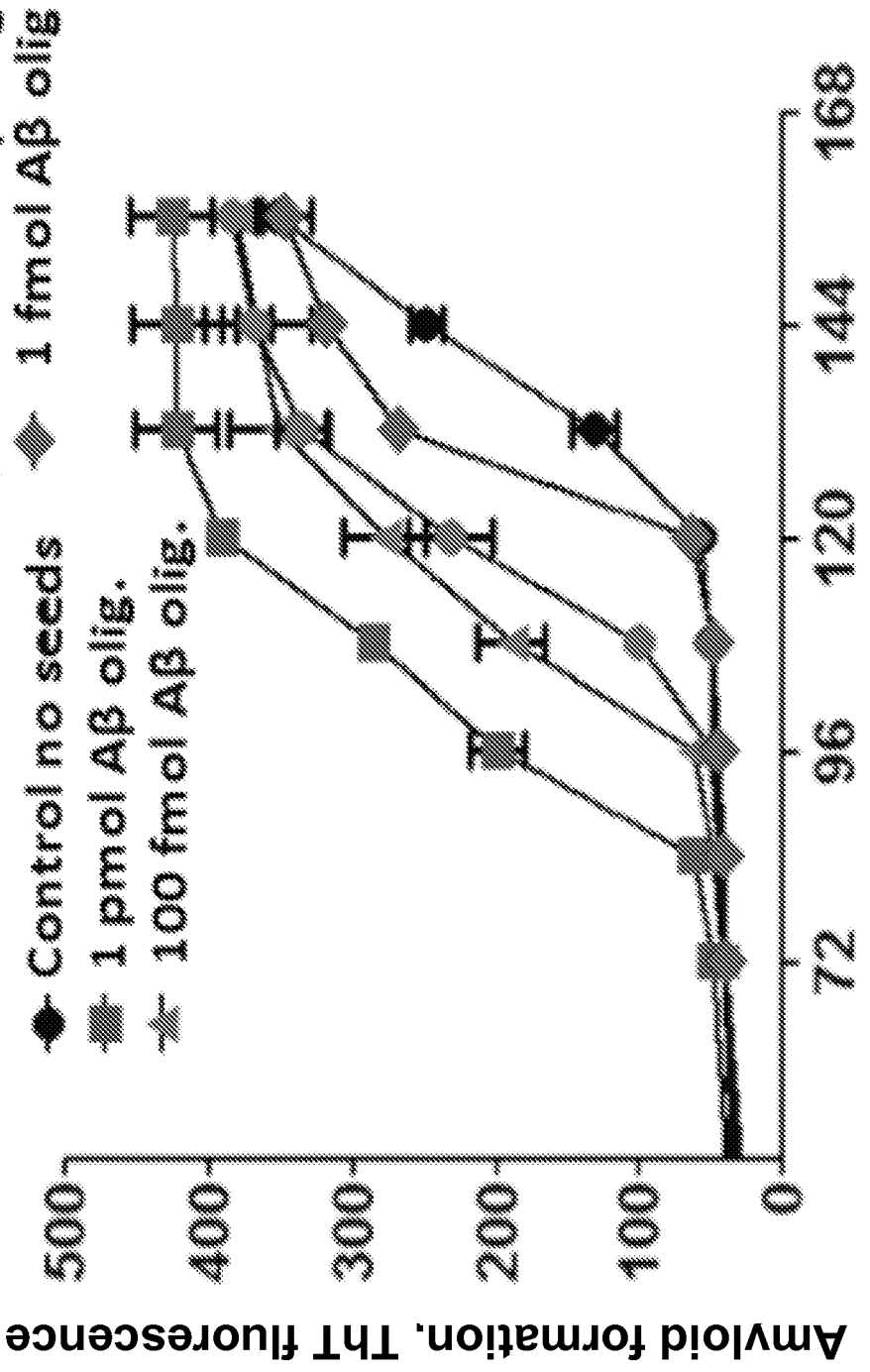
Amyloid formation. ThT fluorescence
500
400
300
200
100
0
72
96
120
144
168
Time, h
Control no seeds
1 pmol Aβ olig.
100 fmol Aβ olig.
10 fmol Aβ olig
1 fmol Aβ olig

FIG. 10

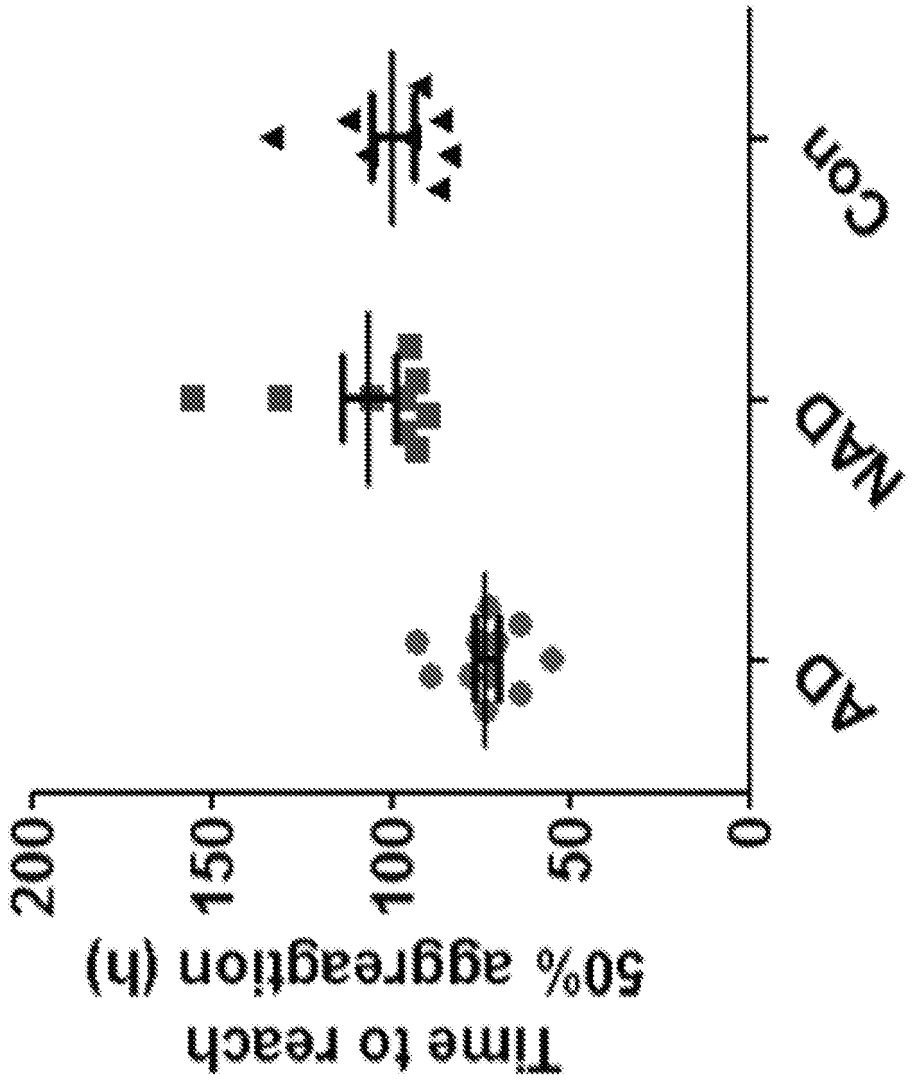

FIG. 11

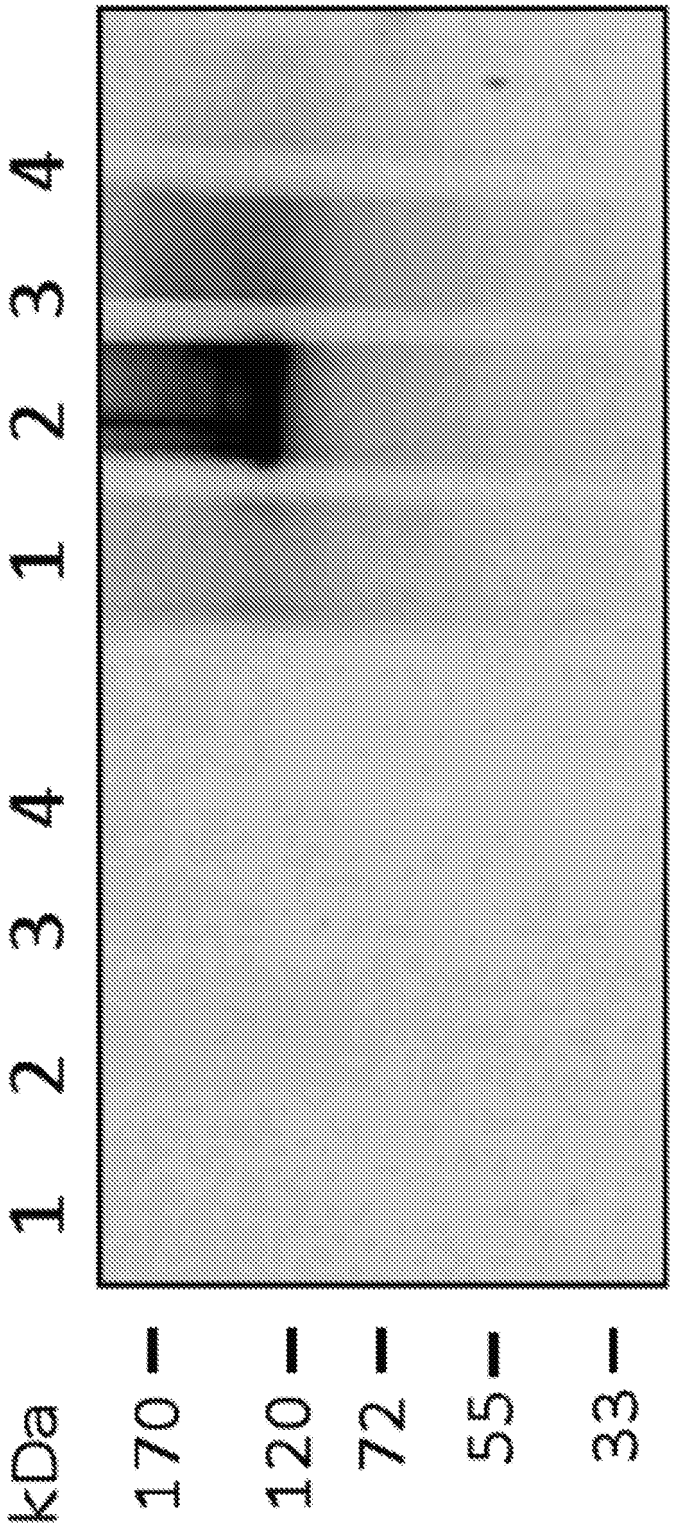

FIG. 12

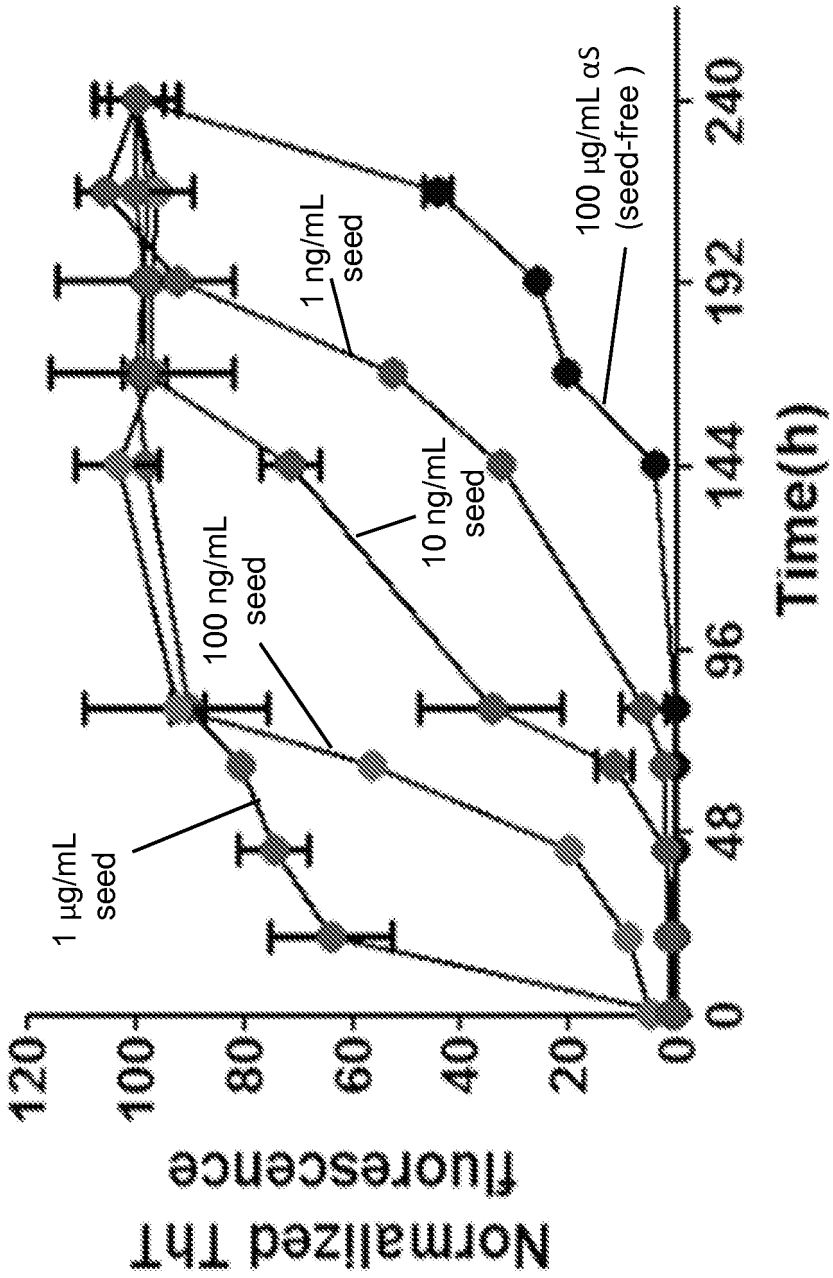
Normalized ThT fluorescence
120
100
80
60
40
20
0
0
48
96
144
192
240
Time(h)
1 µg/mL seed
100 ng/mL seed
10 ng/mL seed
1 ng/mL seed
100 µg/mL αS (seed-free)

FIG. 13A

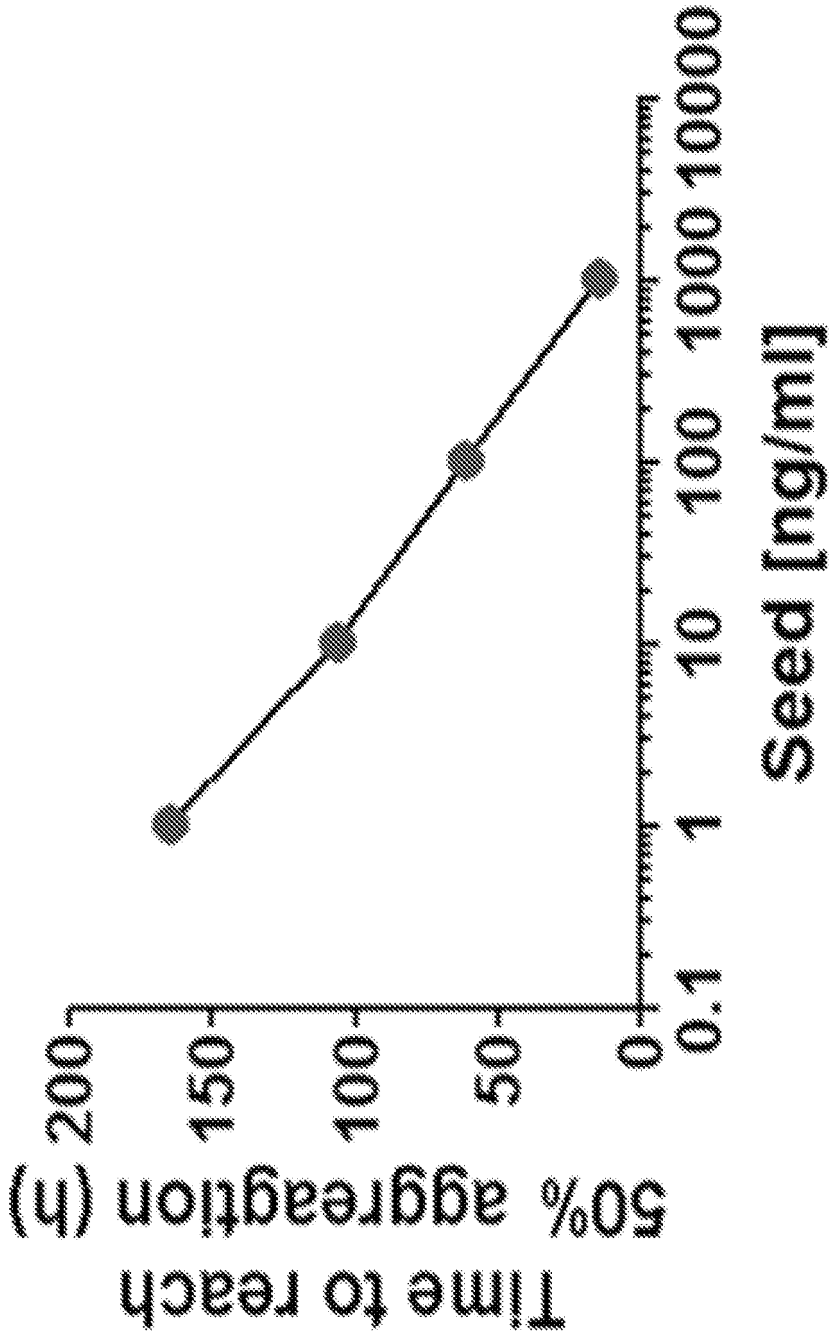
Time to reach
50% aggreagtion (h)
200
150
100
50
0
0.1
1
10
100
1000
10000
Seed [ng/ml]

FIG. 13B

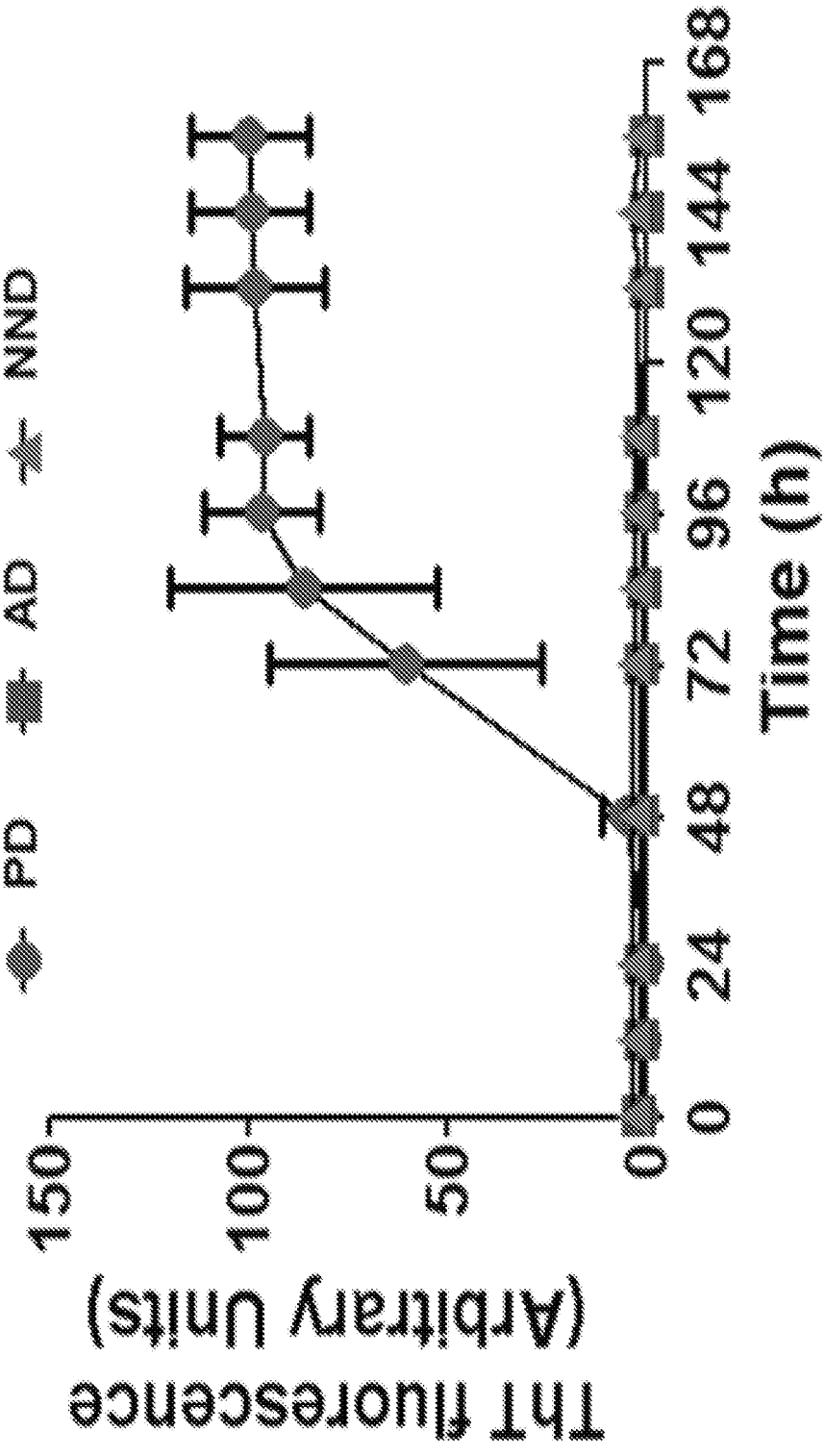
PD
AD
NND
ThT fluorescence
(Arbitrary Units)
150
100
50
0
0
24
48
72
96
120
144
168
Time (h)

FIG. 14

Table 3

Antibodies (Company)	Epitope	Alpha-Synuclein oligomer capturing capacity
Alpha/beta-Synuclein Antibody N-19 (Santa Cruz Biotechnology)	N-terminal	+++
Alpha-Synuclein Antibody C-20-R (Santa Cruz Biotechnology)	C-terminal	+++
Alpha-Synuclein Antibody 211 (Santa Cruz Biotechnology)	Amino acids 121-125	++
Alpha-synuclein Antibody Syn-204 (Santa Cruz Biotechnology)	Fragment 1-130	+
16 ADV Mouse IgG1 (Acumen)	Conformational	-

+++ ***Best***
++ ***very good***
+ ***good***
- ***no result***

FIG. 15

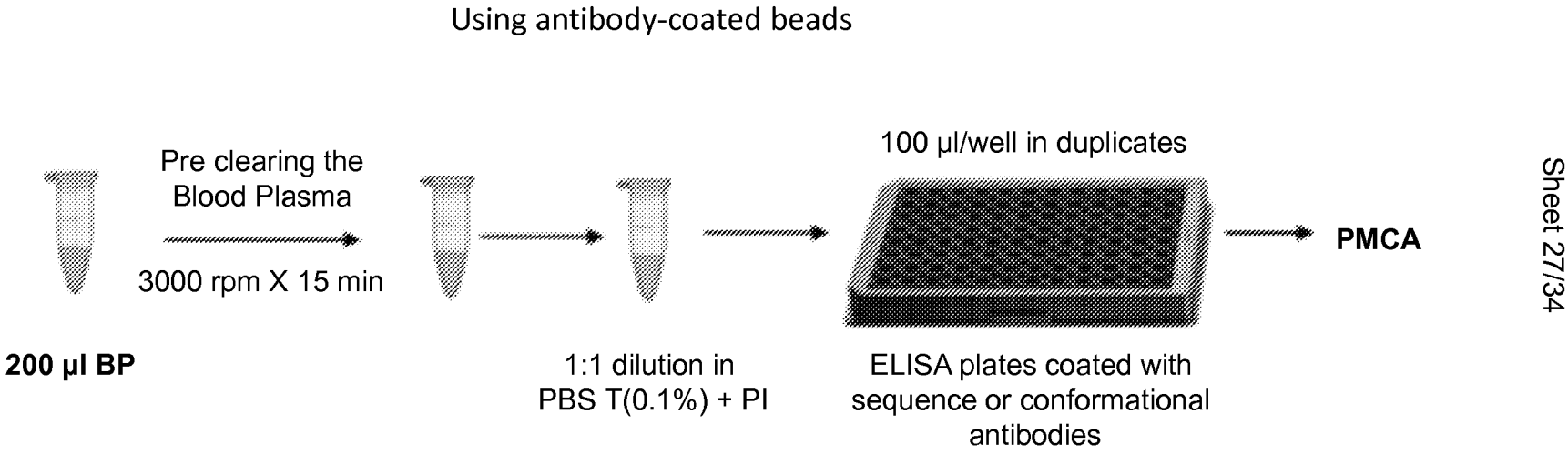
Using antibody-coated beads
Pre clearing the
Blood Plasma
3000 rpm X 15 min
100 µl/well in duplicates
PMCA
200 µl BP
1:1 dilution in
PBS T(0.1%) + PI
ELISA plates coated with
sequence or conformational
antibodies

FIG. 16A

Detection of α-Synuclein oligomers spiked in human blood plasma

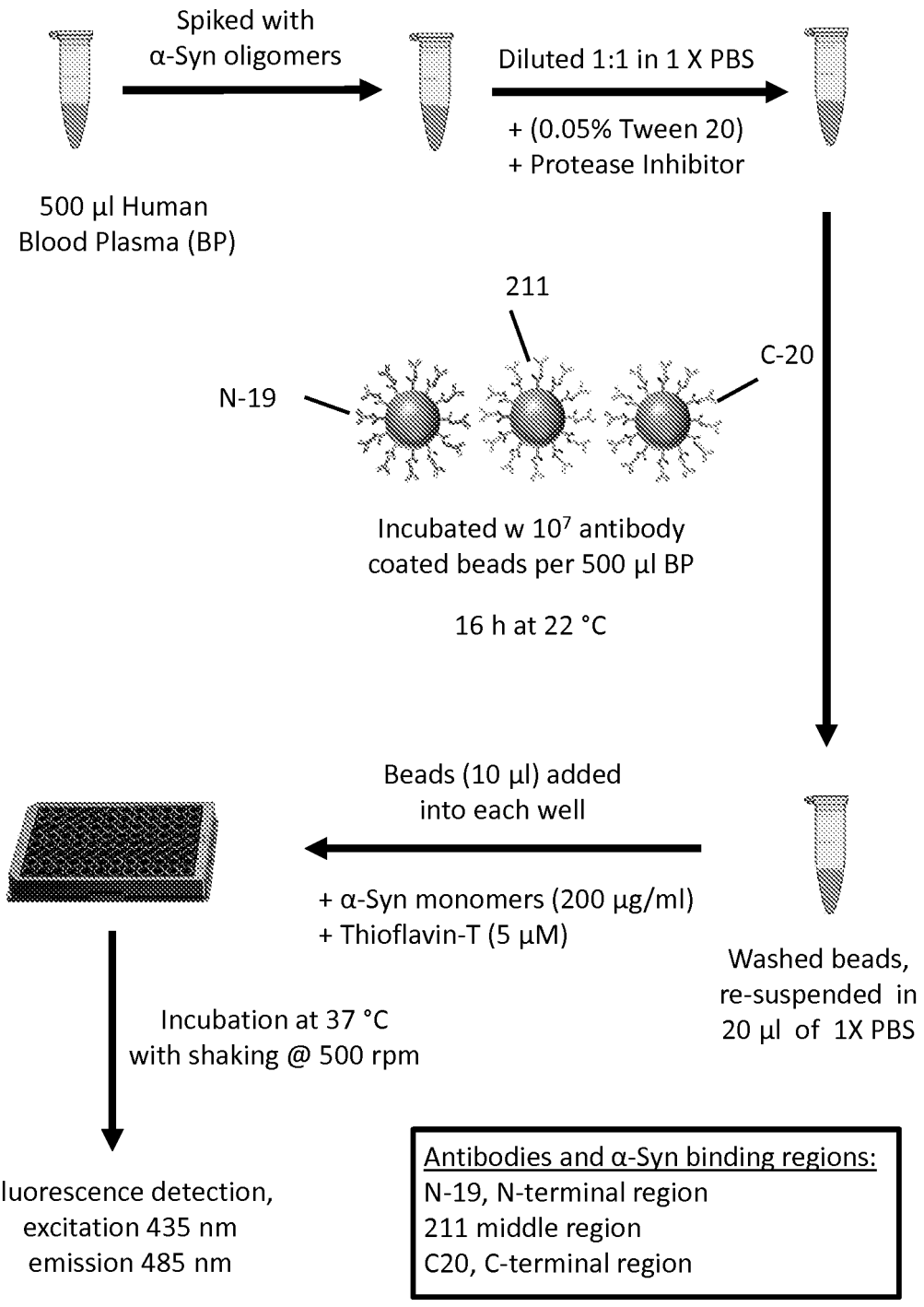

FIG. 16B

Immunoprecipitation with N-19 antibody

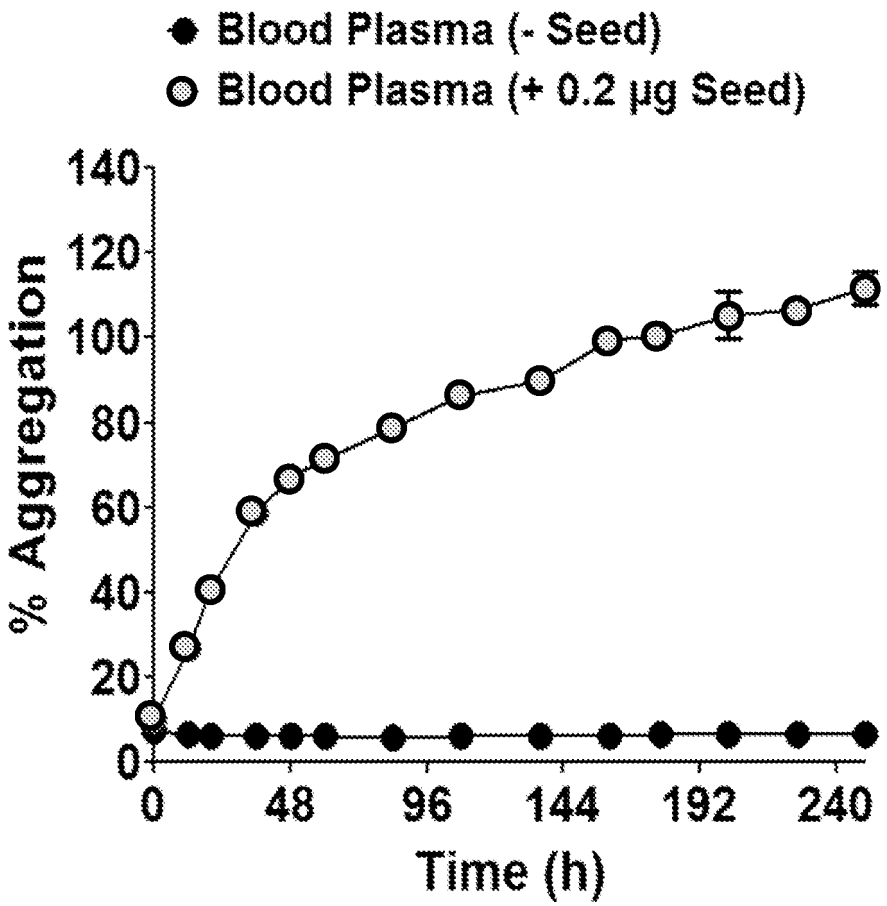

FIG. 17A

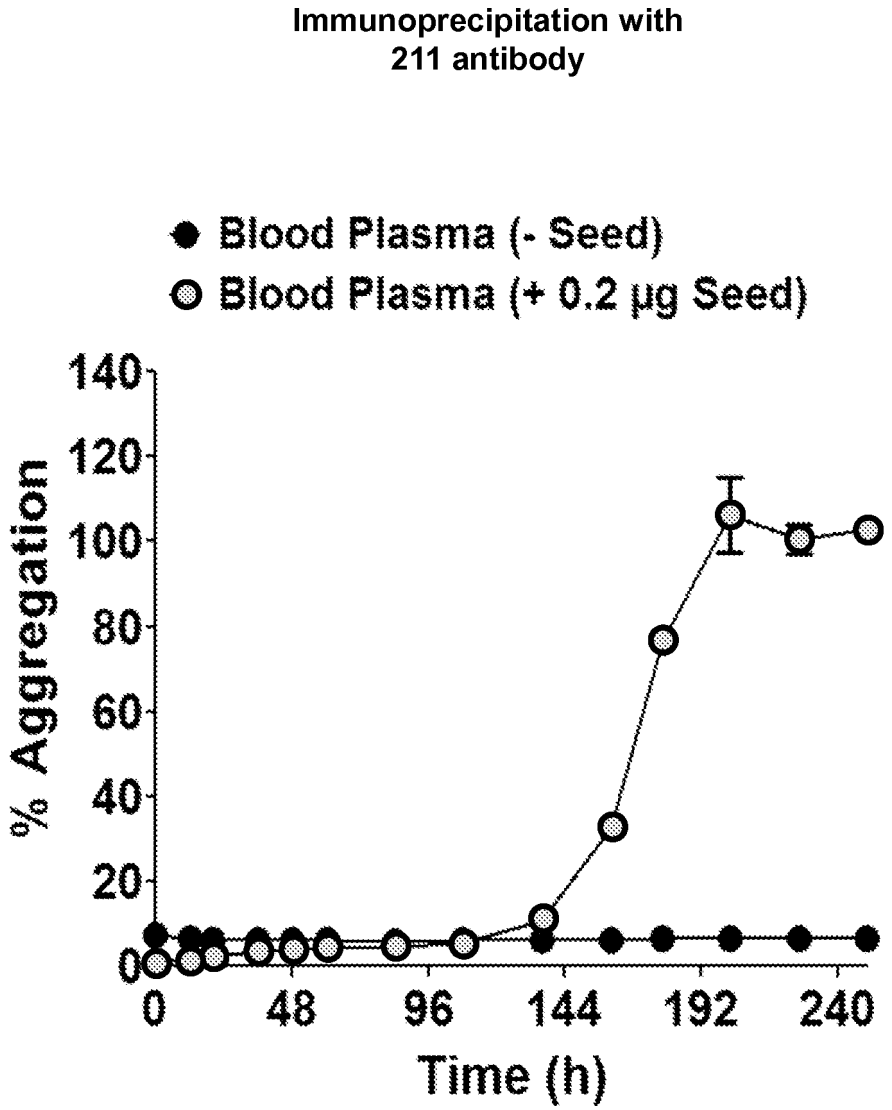
Immunoprecipitation with
211 antibody
Blood Plasma (- Seed)
Blood Plasma (+ 0.2 µg Seed)
% Aggregation
140
120
100
80
60
40
20
0
0
48
96
144
192
240
Time (h)

FIG. 17B

Immunoprecipitation with C-20 antibody

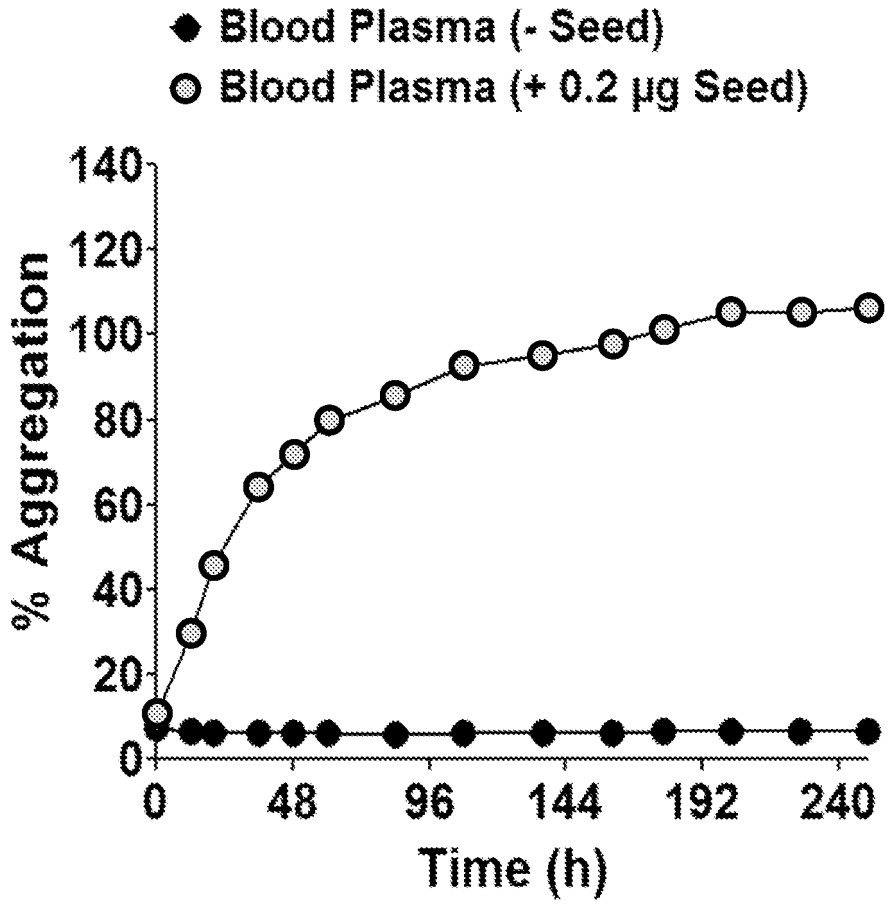

FIG. 17C

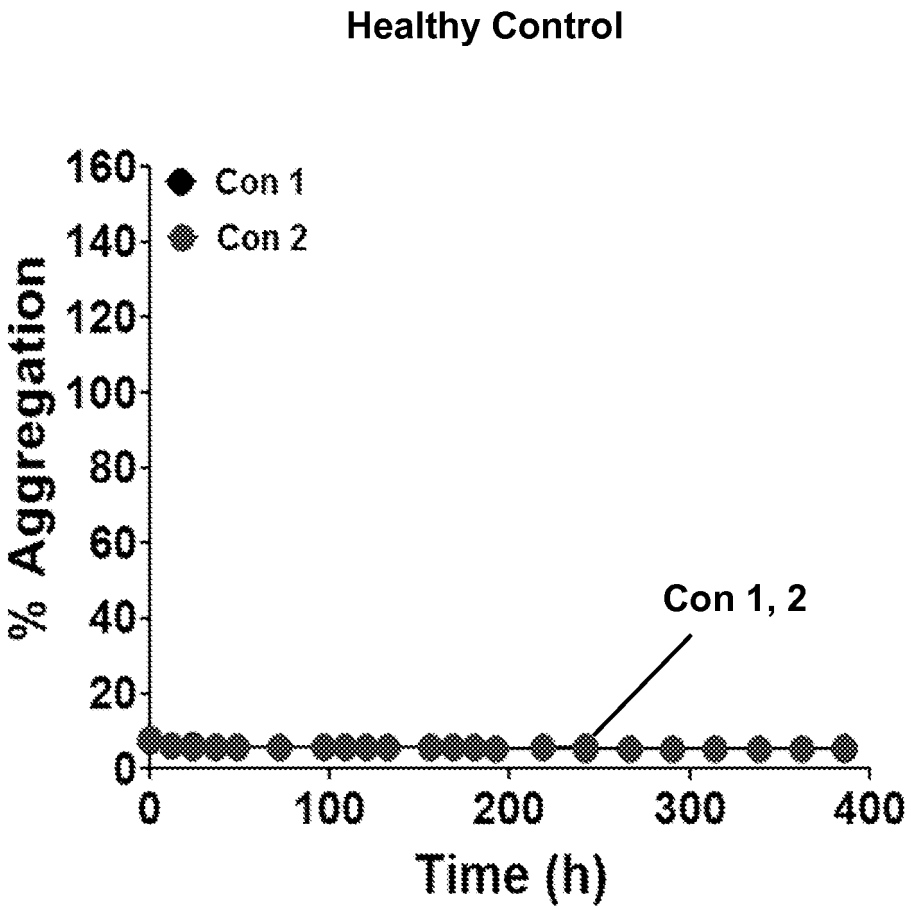
Healthy Control
Con 1
Con 2
% Aggregation
160
140
120
100
80
60
40
20
0
Con 1, 2
0
100
200
300
400
Time (h)

FIG. 18A

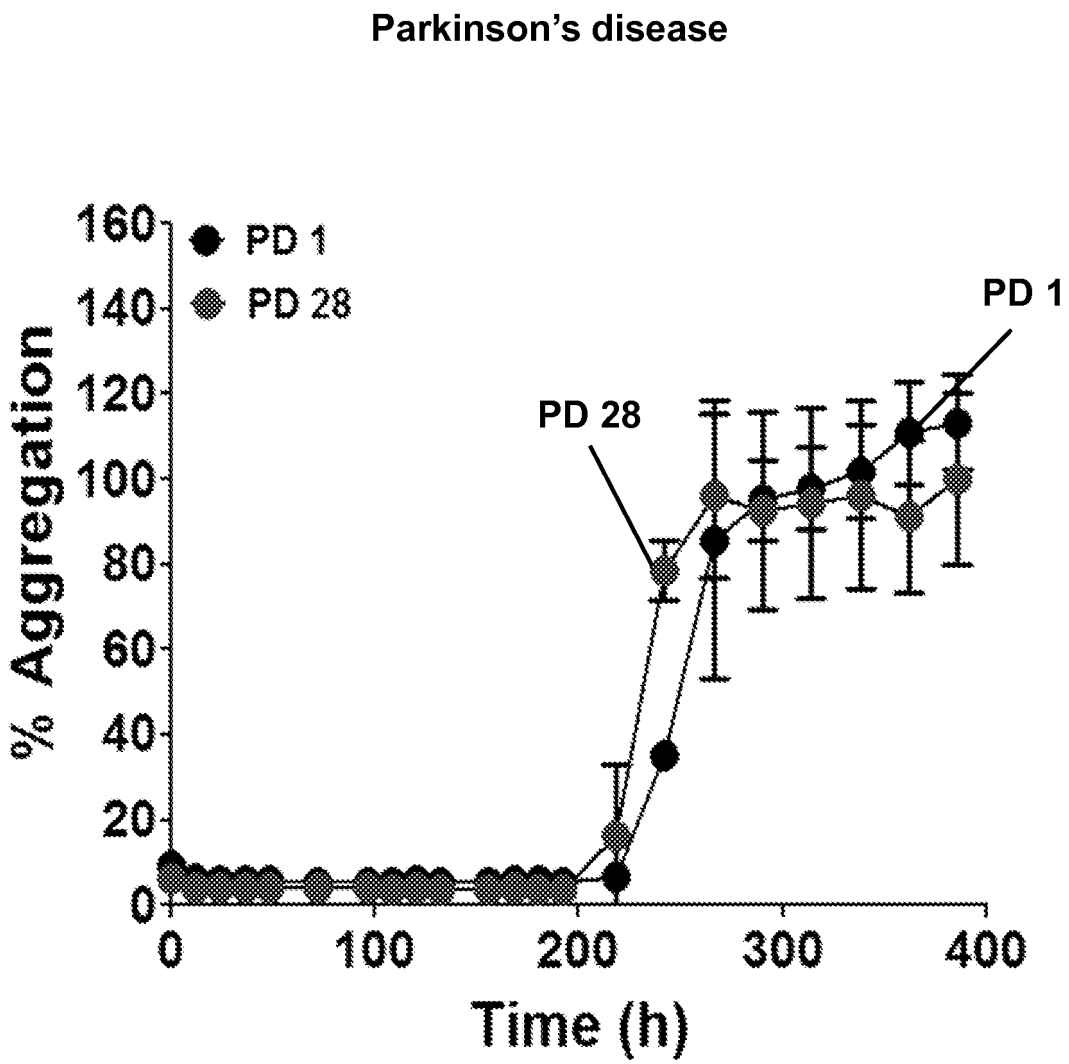
Parkinson's disease
PD 1
PD 28
% Aggregation
Time (h)
0
20
40
60
80
100
120
140
160
0
100
200
300
400
PD 1
PD 28

FIG. 18B

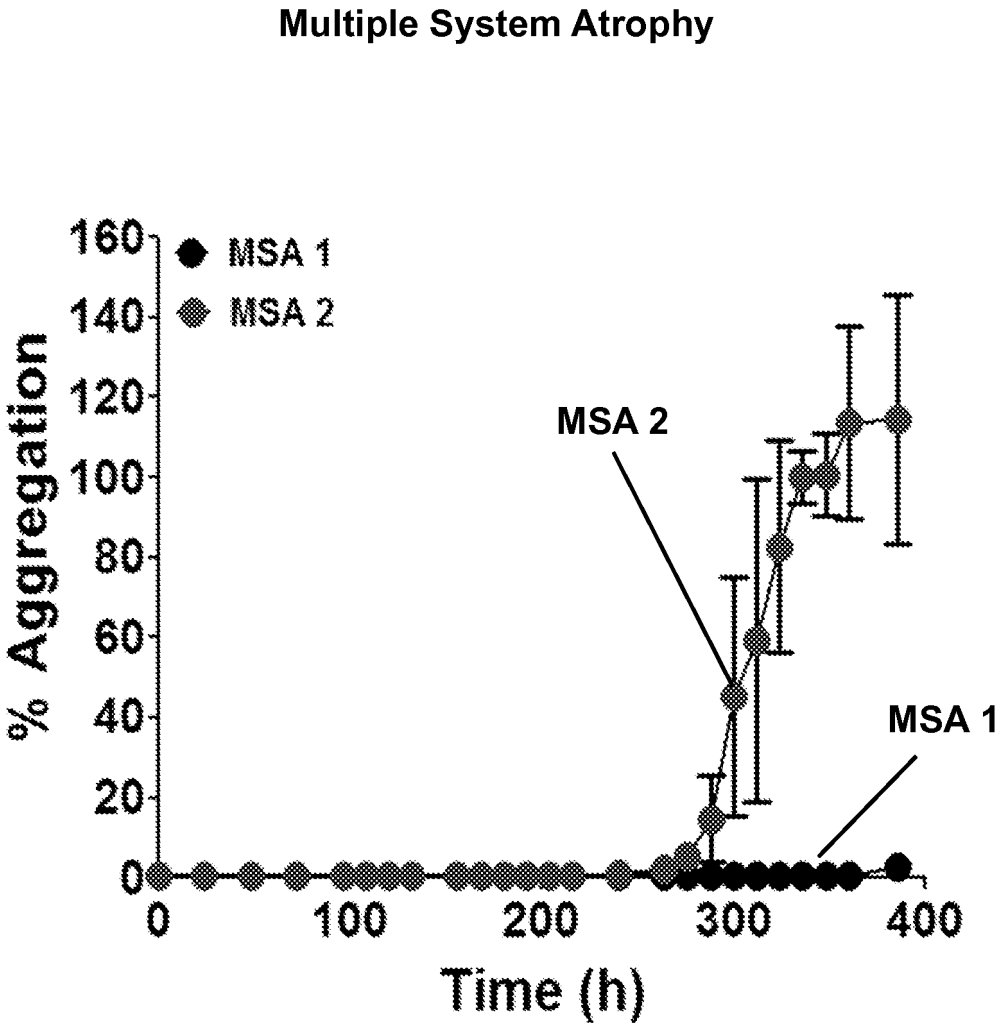
Multiple System Atrophy
MSA 1
MSA 2
% Aggregation
160
140
120
100
80
60
40
20
0
0
100
200
300
400
Time (h)
MSA 2
MSA 1

FIG. 18C