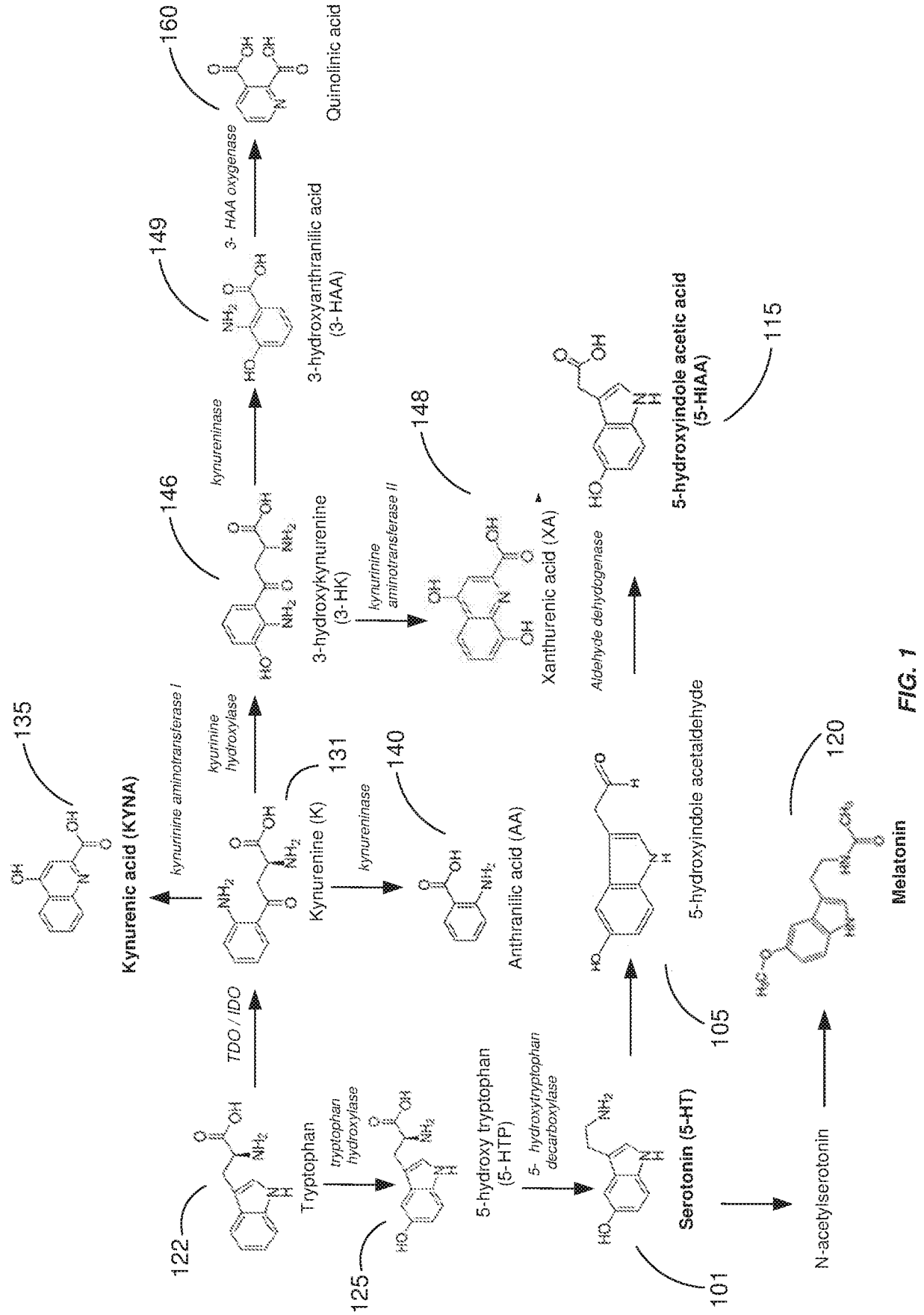

122
Tryptophan
tryptophan hydroxylase
125
5-hydroxy tryptophan (5-HTP)
5- hydroxytryptophan decarboxylase
101
Serotonin (5-HT)
N-acetylserotonin
Melatonin
120
105
5-hydroxyindole acetaldehyde
Aldehyde dehydrogenase
5-hydroxyindole acetic acid (5-HIAA)
115
TDO / IDO
Kynurenine (K)
131
kynureninase
Anthranilic acid (AA)
140
kynurinine aminotransferase I
Kynurenic acid (KYNA)
135
kyurinine hydroxylase
3-hydroxykynurenine (3-HK)
146
kynurinine aminotransferase II
Xanthurenic acid (XA)
148
kynureninase
3-hydroxyanthranilic acid (3-HAA)
149
3- HAA oxygenase
Quinolinic acid
160

FIG. 1

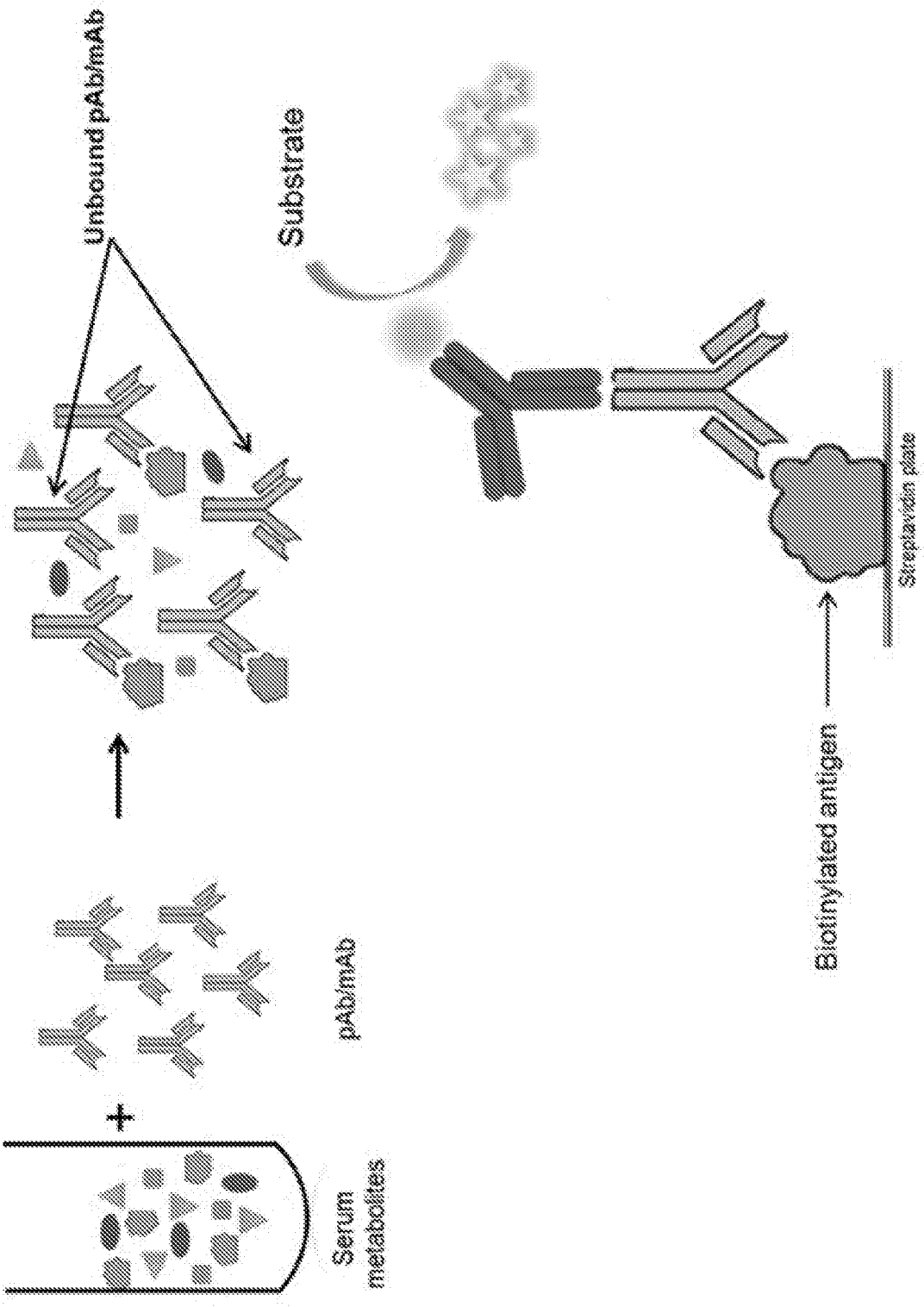

Serum metabolites
+
pAb/mAb
Unbound pAb/mAb
Substrate
Biotinylated antigen
Streptavidin plate

FIG. 2

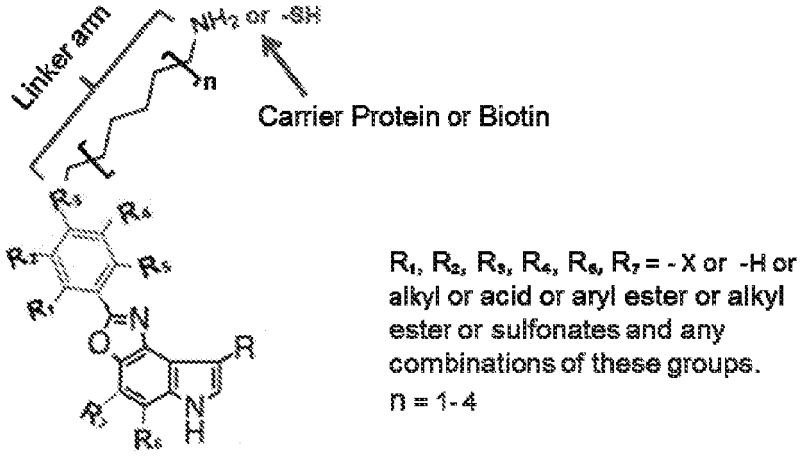
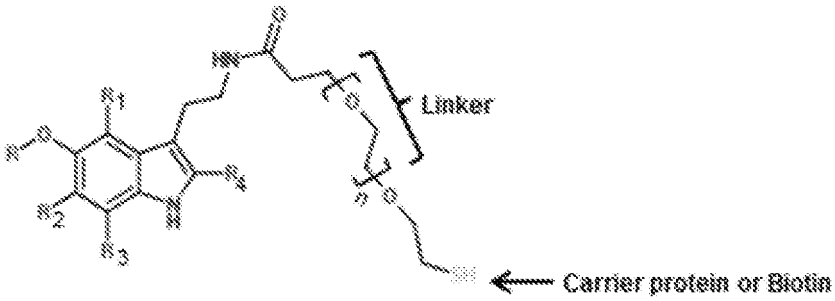
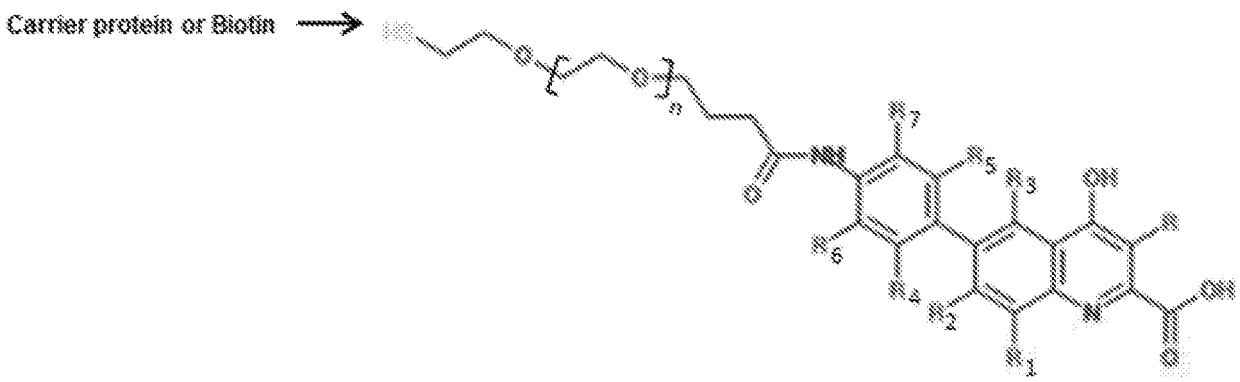


FIG. 3A



R = CH_3 or CH_2 CH_3 or $CH(CH_3)_2$ or $C(CH_3)_3$,
R_1, R_2, R_3, R_4 = H or –X or alkyl or acid or aryl ester or sulfonate and any combination of theses groups
N= 1-8

FIG. 3B



R, R_1, R_2, R_3, R_4 = H or –X or alkyl or acid or aryl ester or sulfonate and any combination of theses groups
N= 1-8

FIG. 3C

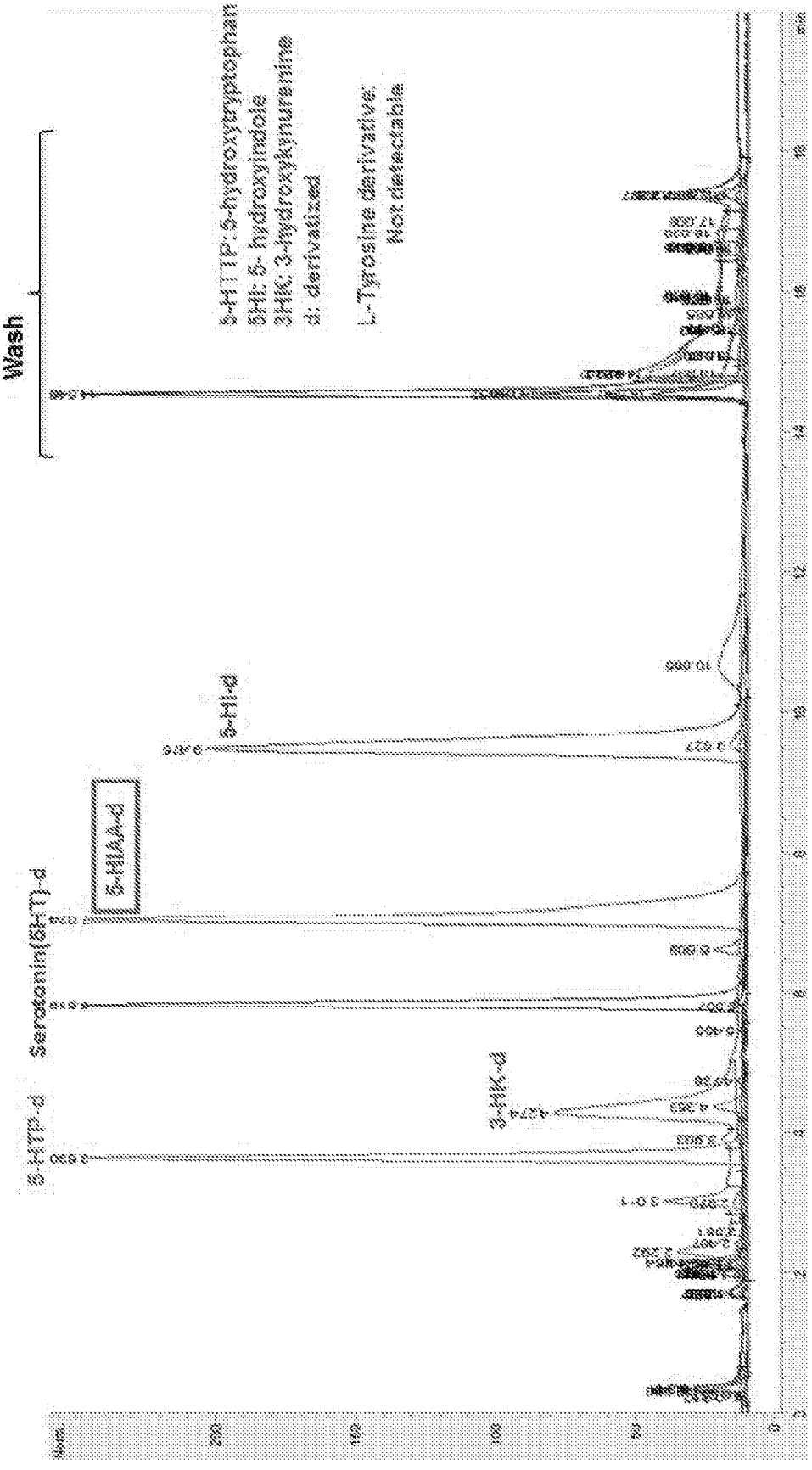

Wash
5-HTTP: 5-hydroxytryptophan
5HI: 5- hydroxyindole
3HK: 3-hydroxykynurenine
d: derivatized
L-Tyrosine derivative:
Not detectable
5-HTP-d
Serotonin(5HT)-d
5-HIAA-d
5-HI-d
3-HK-d

FIG. 3D

FIG. 4A

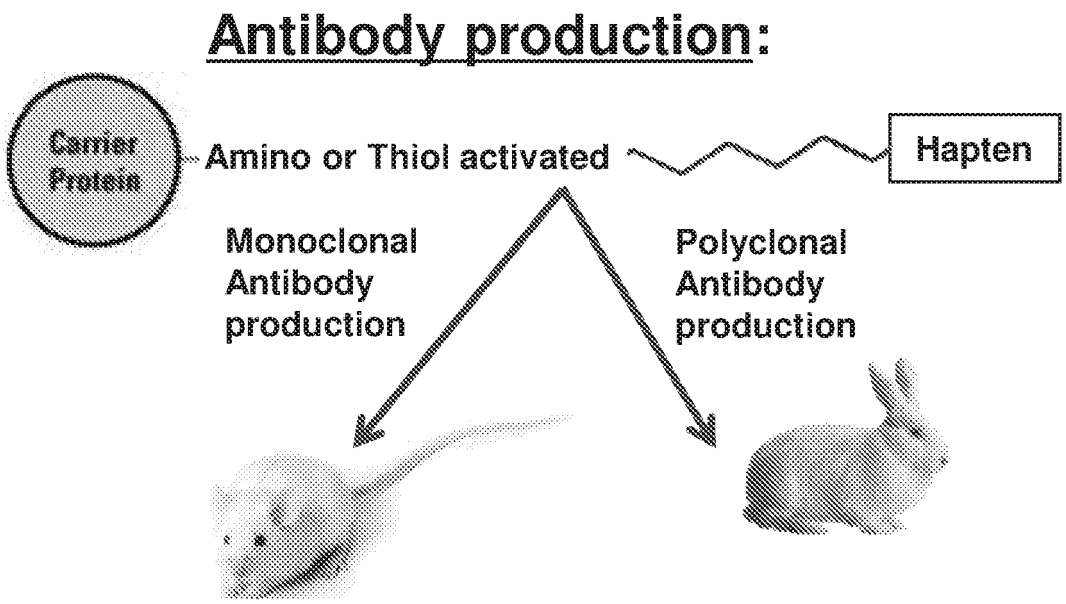
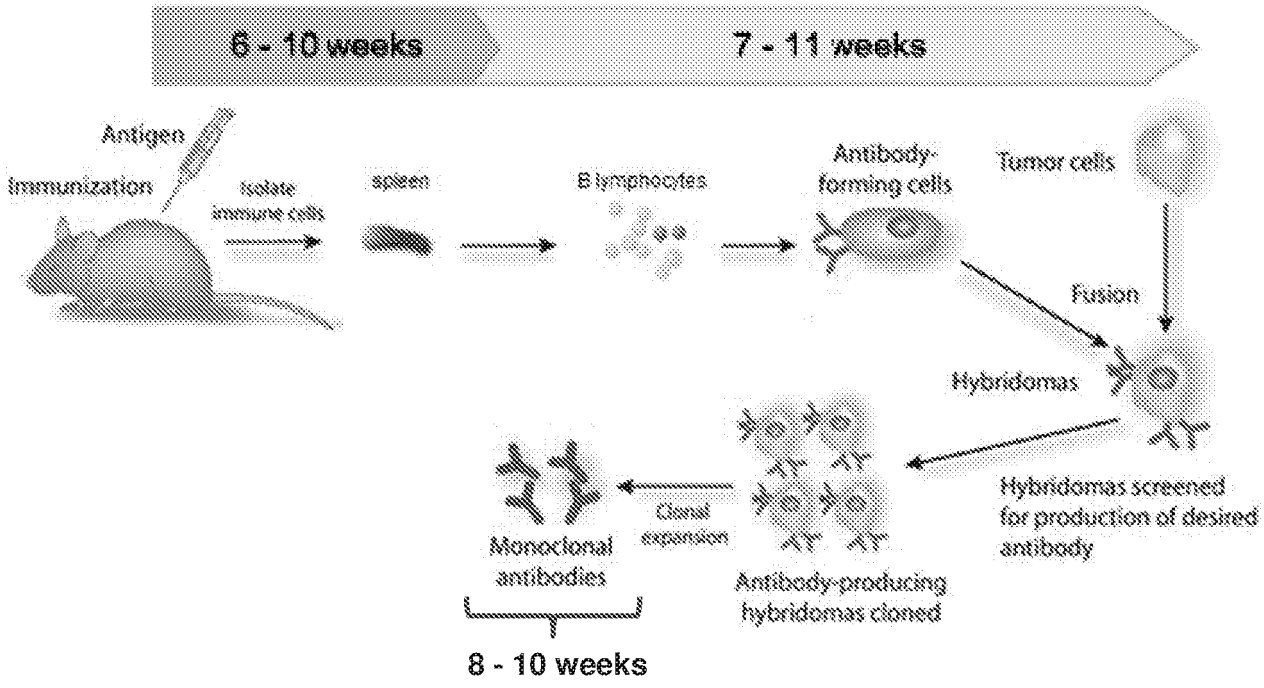

Antibody production:
Carrier Protein
Amino or Thiol activated
Hapten
Monoclonal Antibody production
Polyclonal Antibody production

FIG. 4B


6 - 10 weeks
7 - 11 weeks
Antigen
Immunization
Isolate immune cells
spleen
B lymphocytes
Antibody-forming cells
Tumor cells
Fusion
Hybridomas
Hybridomas screened for production of desired antibody
Clonal expansion
Monoclonal antibodies
Antibody-producing hybridomas cloned
8 - 10 weeks

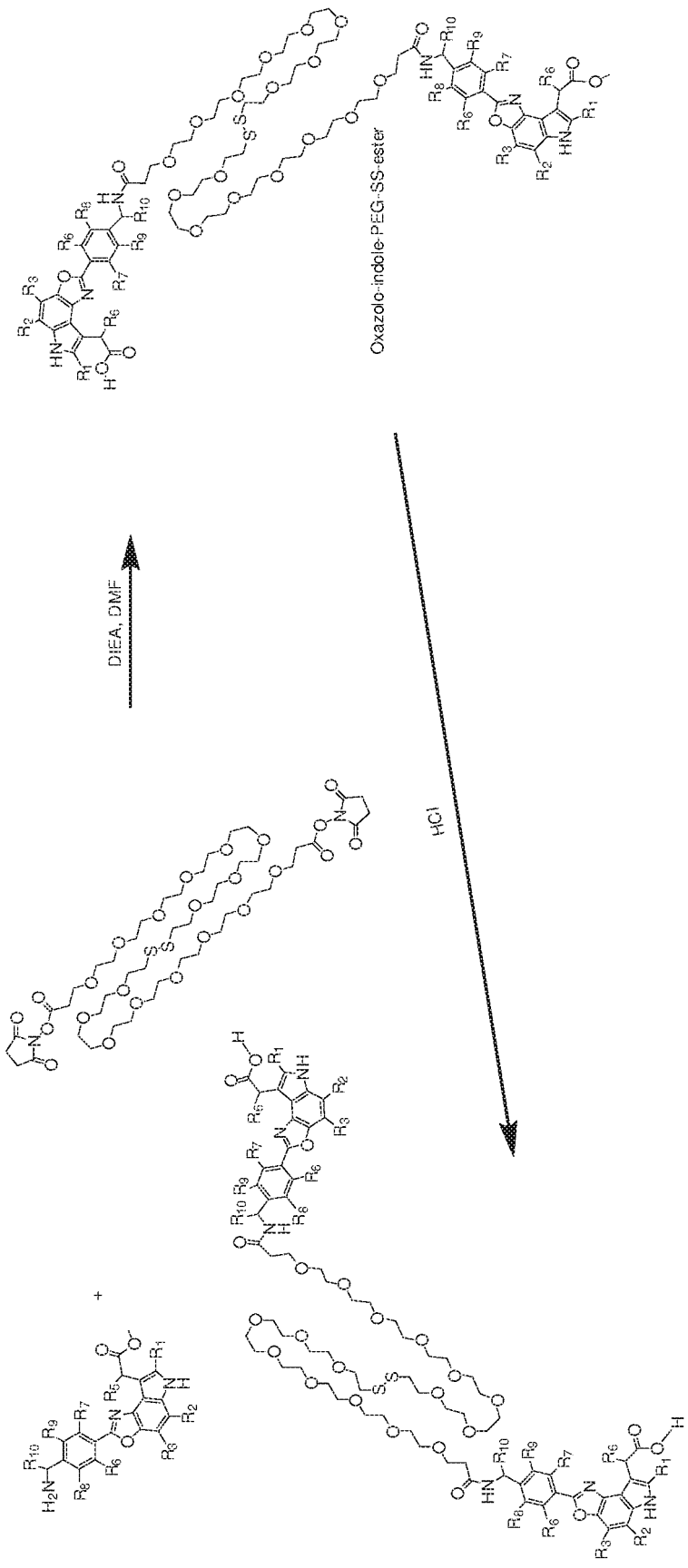


FIG. 5A

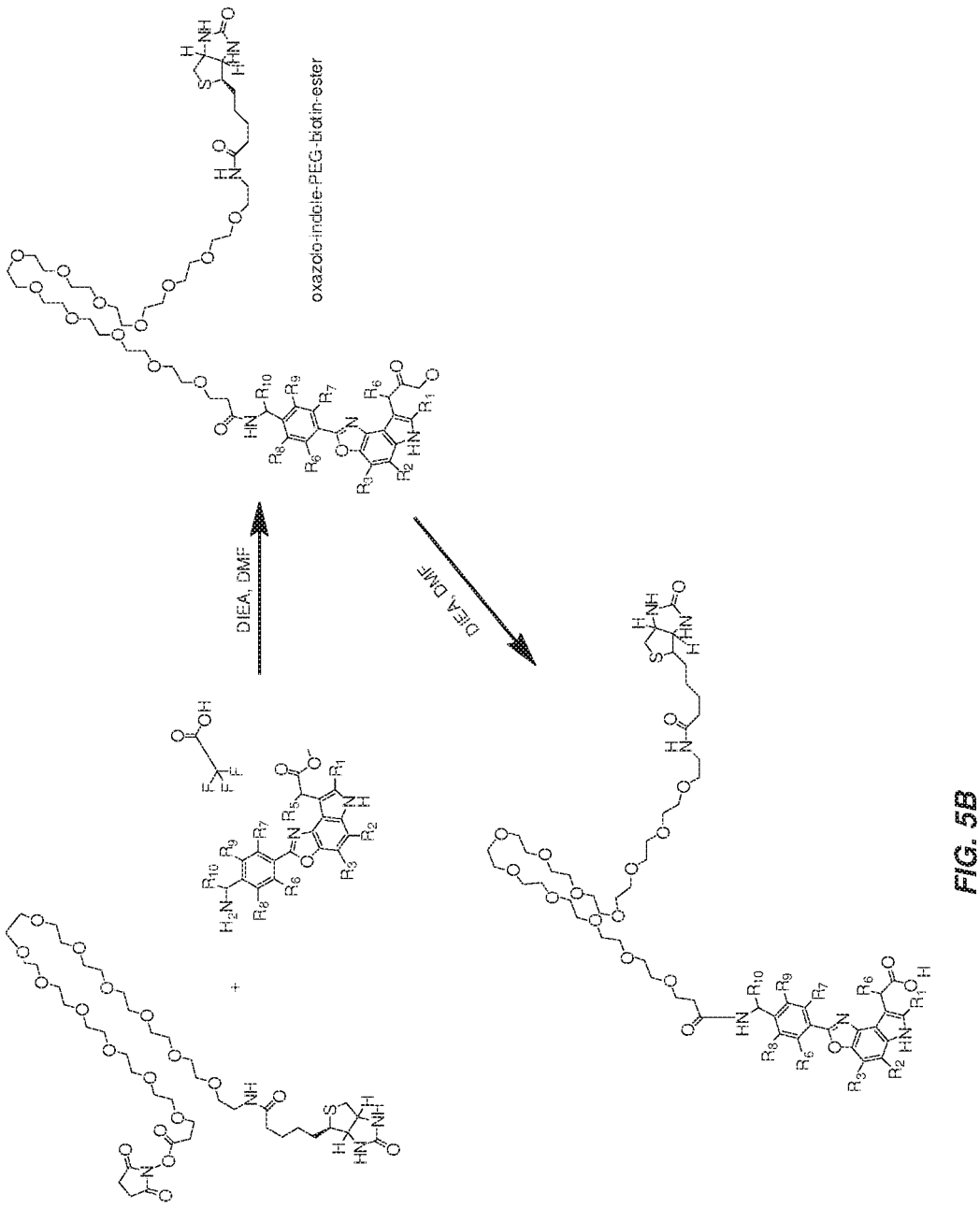


FIG. 5B

FIG. 6A

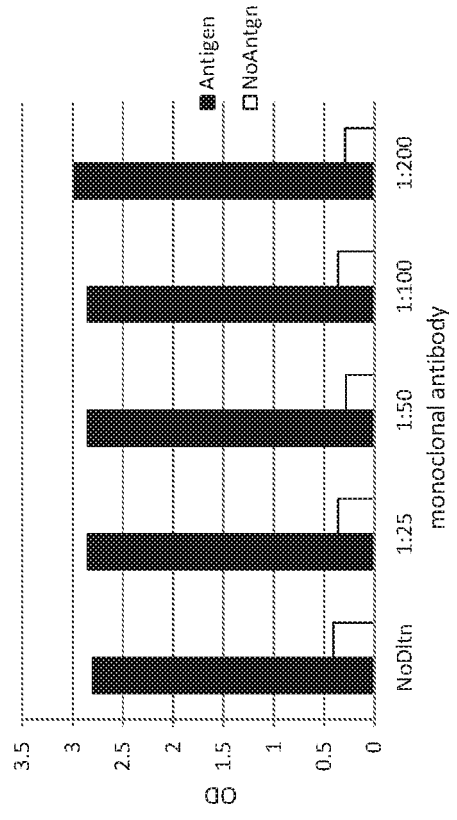


FIG. 6B

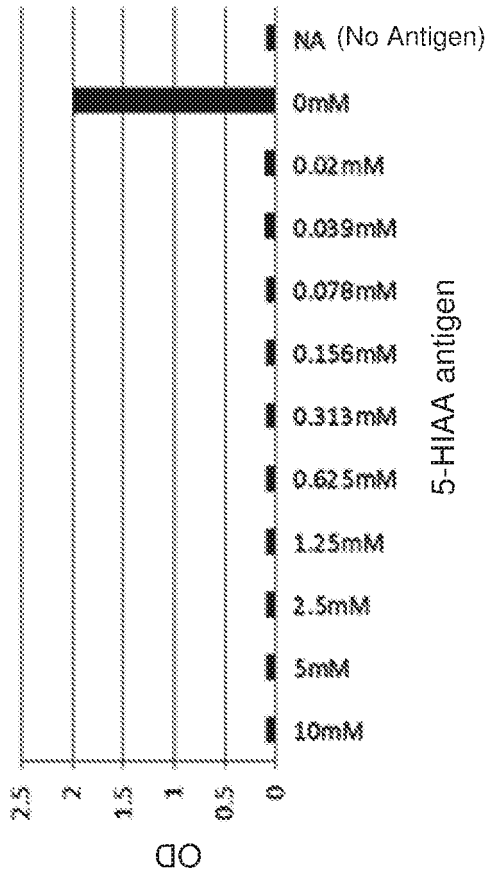


FIG. 6C

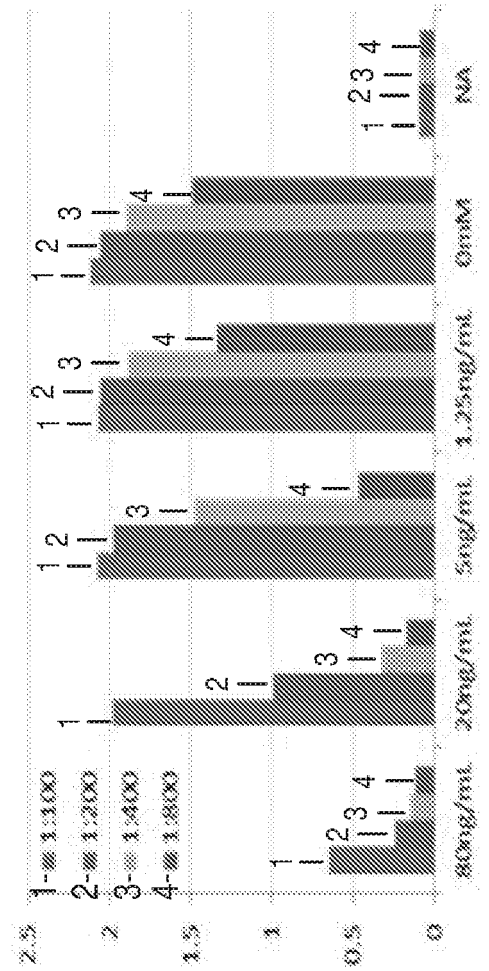
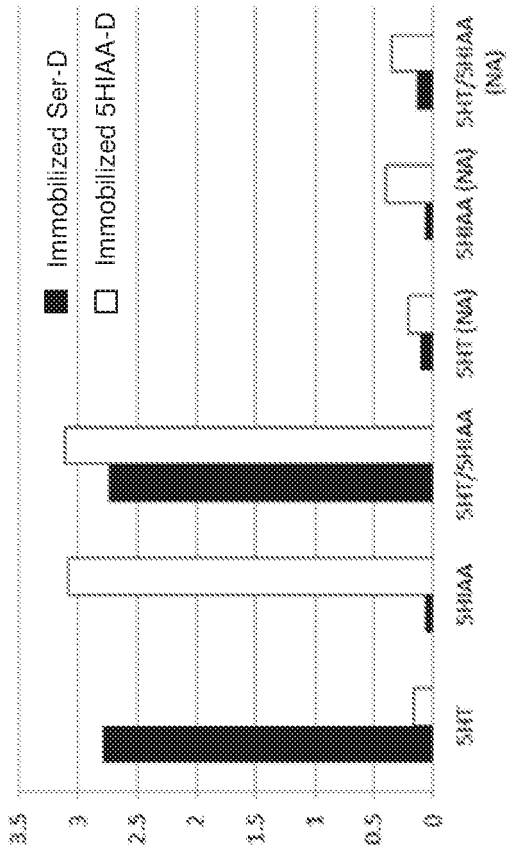


FIG. 6D



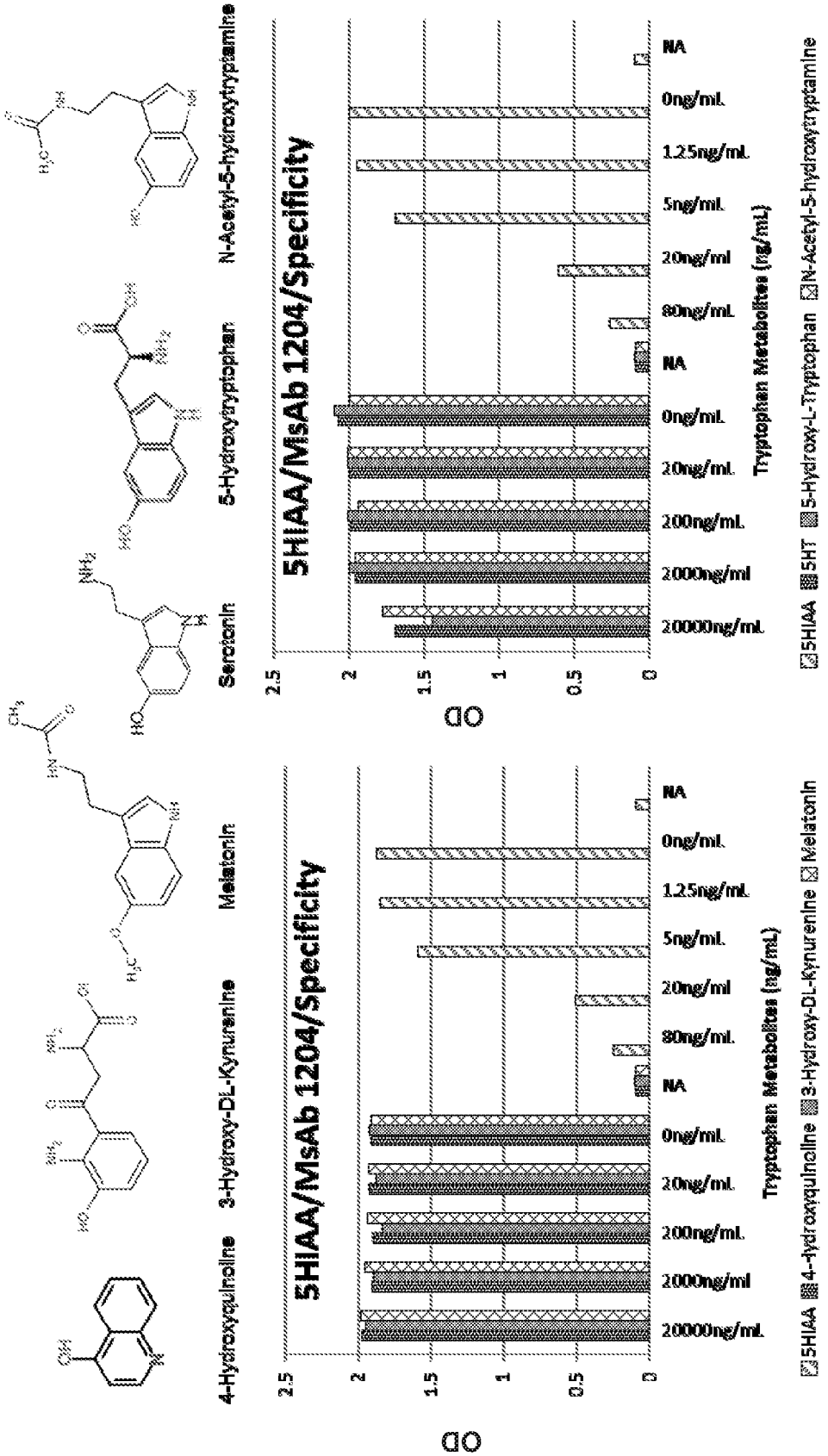

4-Hydroxyquinoline
3-Hydroxy-DL-Kynurenine
Melatonin
Serotonin
5-Hydroxytryptophan
N-Acetyl-5-hydroxytryptamine
5HIAA/MsAb 1204/Specificity
OD
Tryptophan Metabolites (ng/mL)
NA
0ng/mL
1.25ng/mL
5ng/mL
20ng/ml
80ng/mL
NA
0ng/mL
20ng/mL
200ng/mL
2000ng/ml
20000ng/mL
5HIAA
4-hydroxyquinoline
3-Hydroxy-DL-Kynurenine
Melatonin
5HT
5-Hydroxy-L-Tryptophan
N-Acetyl-5-hydroxytryptamine

FIG. 7A

FIG. 7B

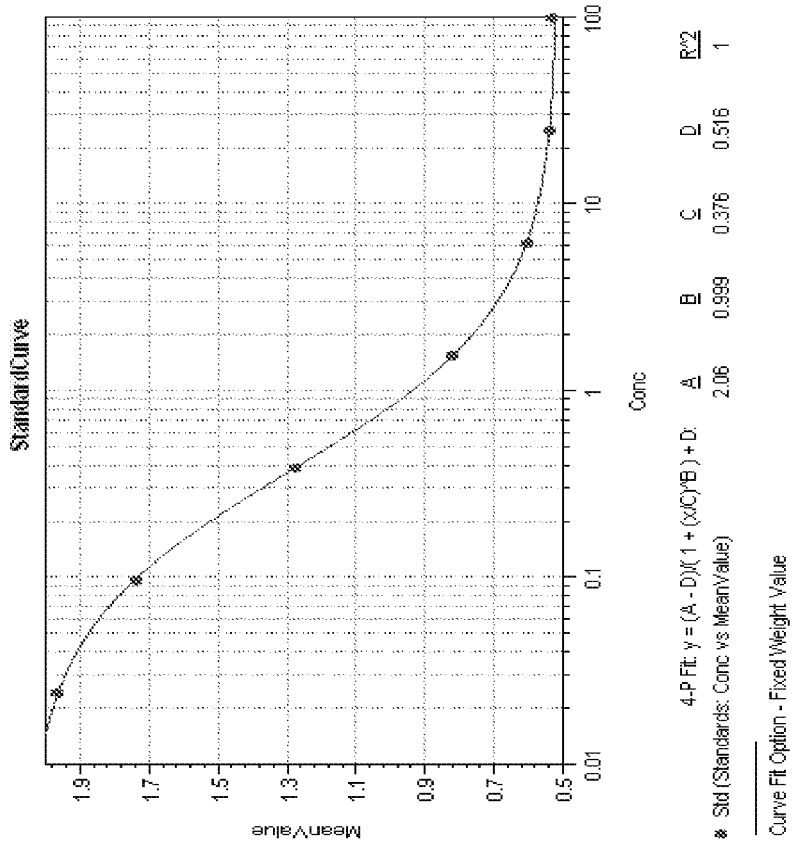

StandardCurve
MeanValue
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.01
0.1
1
10
100
Conc
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A
B
C
D
R^2
2.06
0.999
0.376
0.516
1
Std (Standards: Conc vs MeanValue)
Curve Fit Option - Fixed Weight Value

FIG. 8

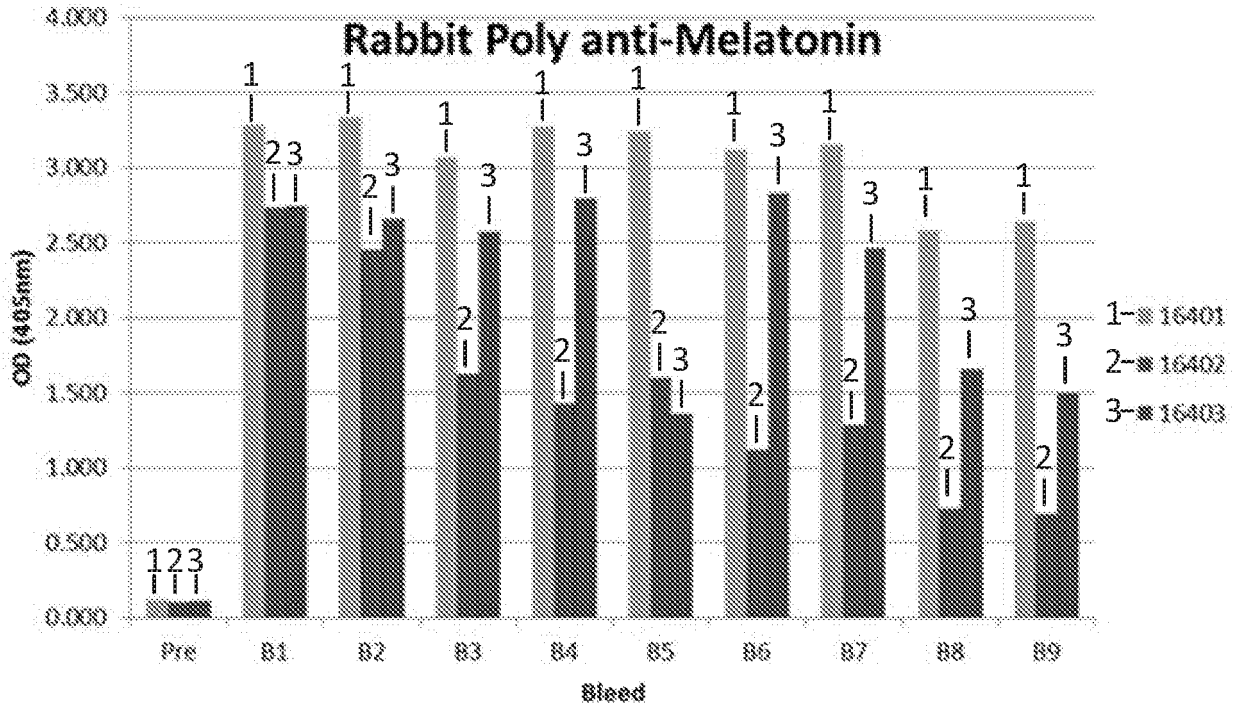

Rabbit Poly anti-Melatonin
OD (405nm)
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Pre
B1
B2
B3
B4
B5
B6
B7
B8
B9
Bleed
1-■ 16401
2-■ 16402
3-■ 16403

FIG. 9

FIG. 10A

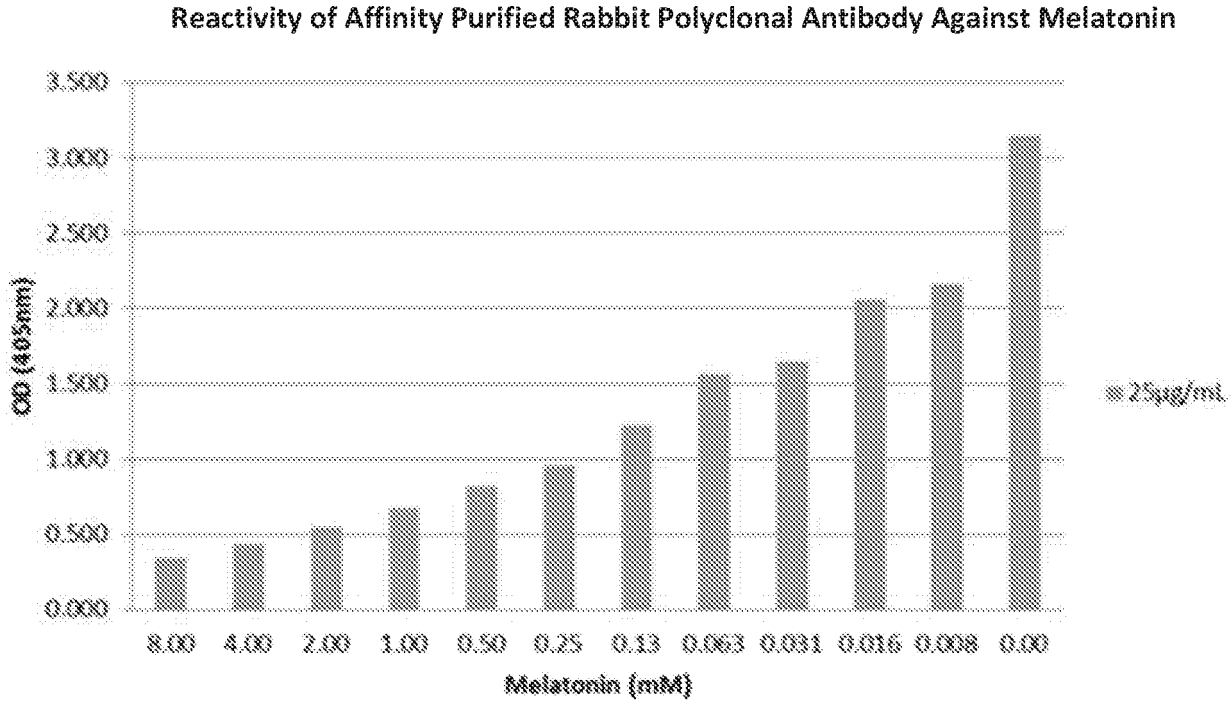
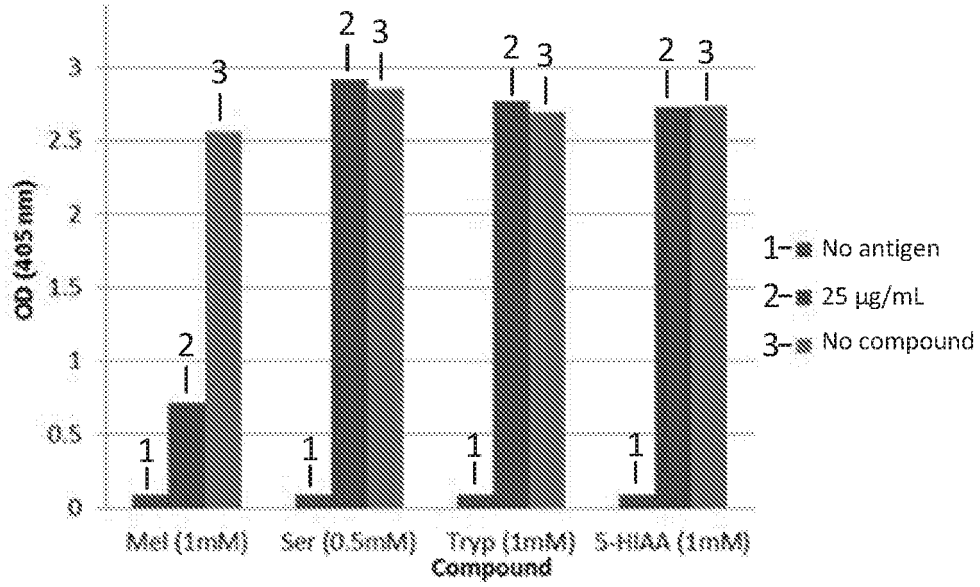


FIG. 10B



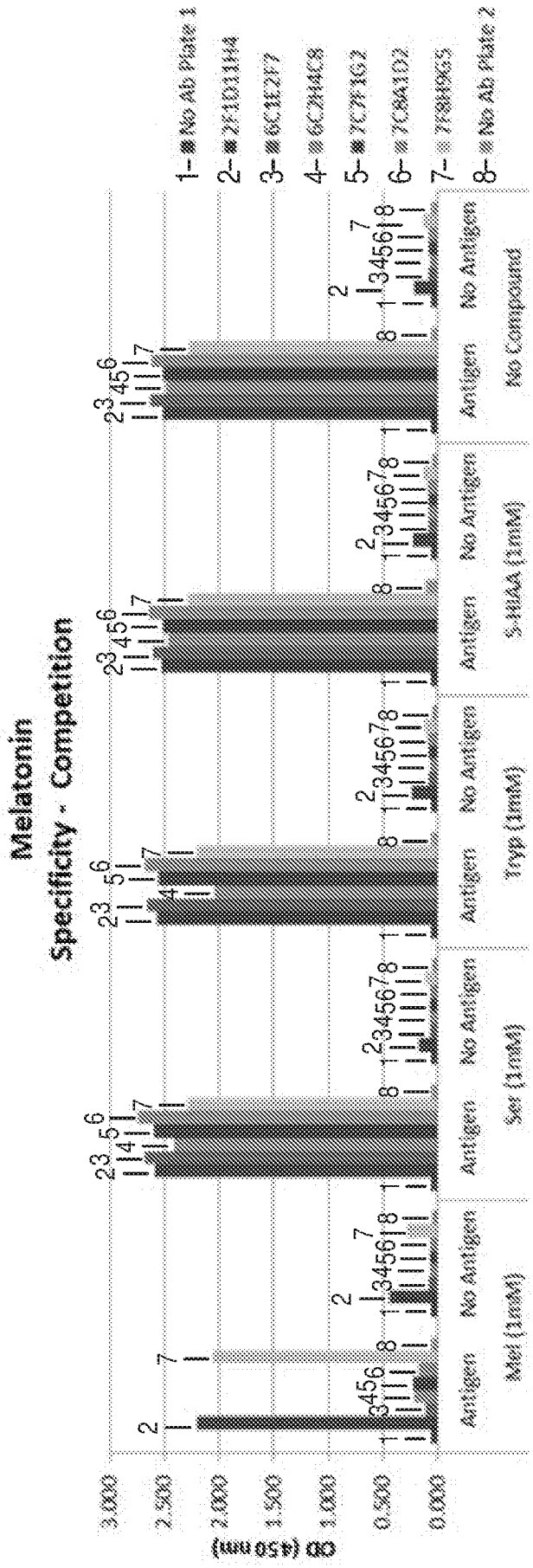

Melatonin
Specificity - Competition
OD (450 nm)
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Antigen
No Antigen
Mel (1mM)
Ser (1mM)
Tryp (1mM)
5-HIAA (1mM)
No Compound
Compound
1- No Ab Plate 1
2- 2F1D11H4
3- 6C1E2F7
4- 6C2H4C8
5- 7C7F1G2
6- 7C8A1D2
7- 7F8H5G5
8- No Ab Plate 2

FIG. 11

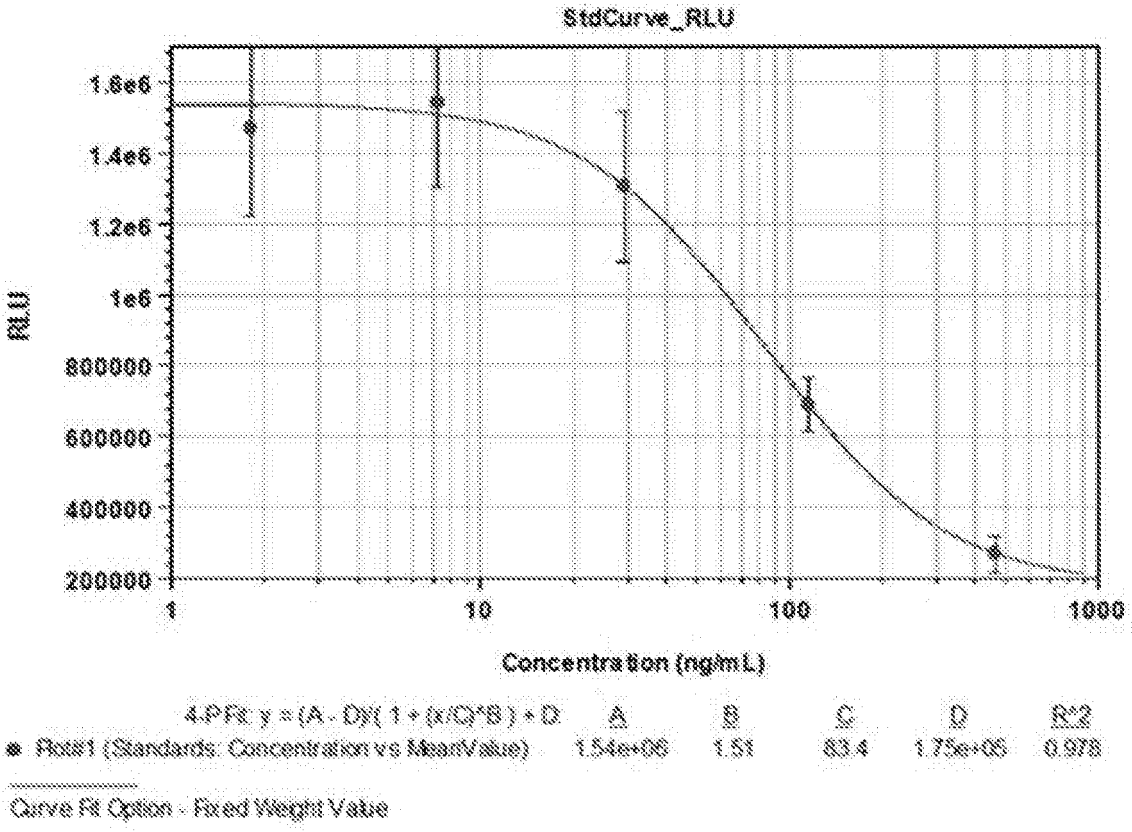

StdCurve_RLU
RLU
1.6e6
1.4e6
1.2e6
1e6
800000
600000
400000
200000
1
10
100
1000
Concentration (ng/mL)
4-PRL: y = (A - D)/(1 + (x/C)^B) + D
A
B
C
D
R^2
Plot#1 (Standards: Concentration vs MeanValue)
1.54e+06
1.51
83.4
1.75e+05
0.978
Curve Fit Option - Fixed Weight Value

FIG. 12

FIG. 13A

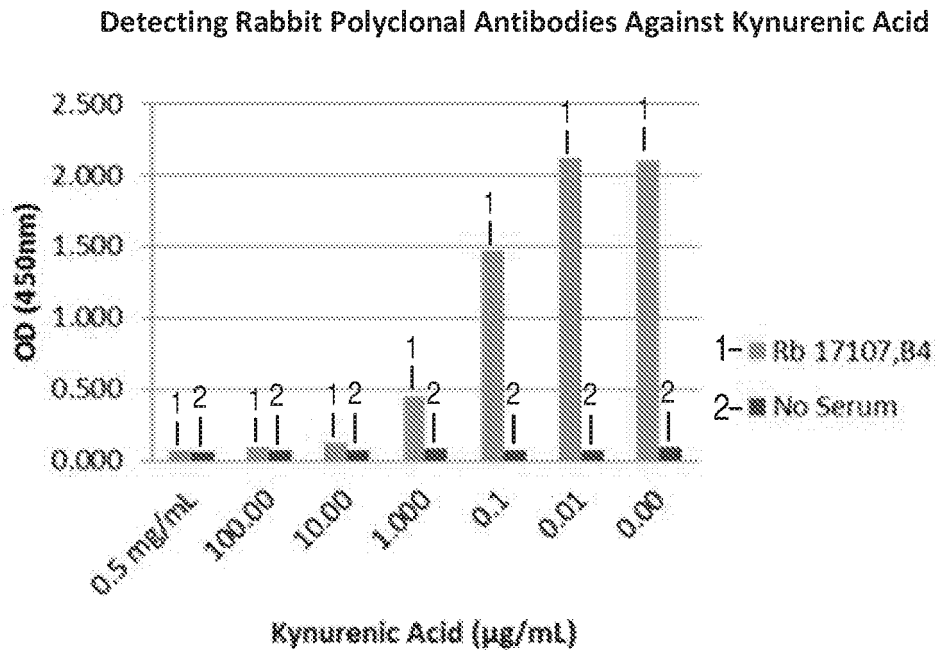


FIG. 13B

Reactivity of Affinity Purified Rabbit Polyclonal Antibodies Against Kynurenic Acid
Competitive ELISA

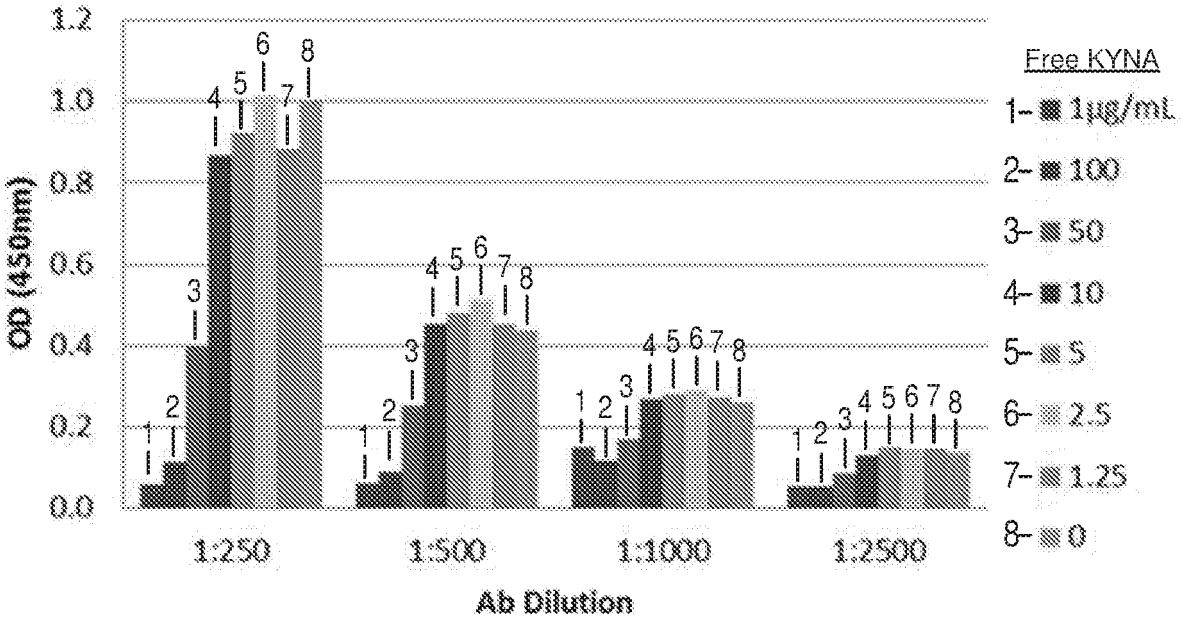


FIG. 14A

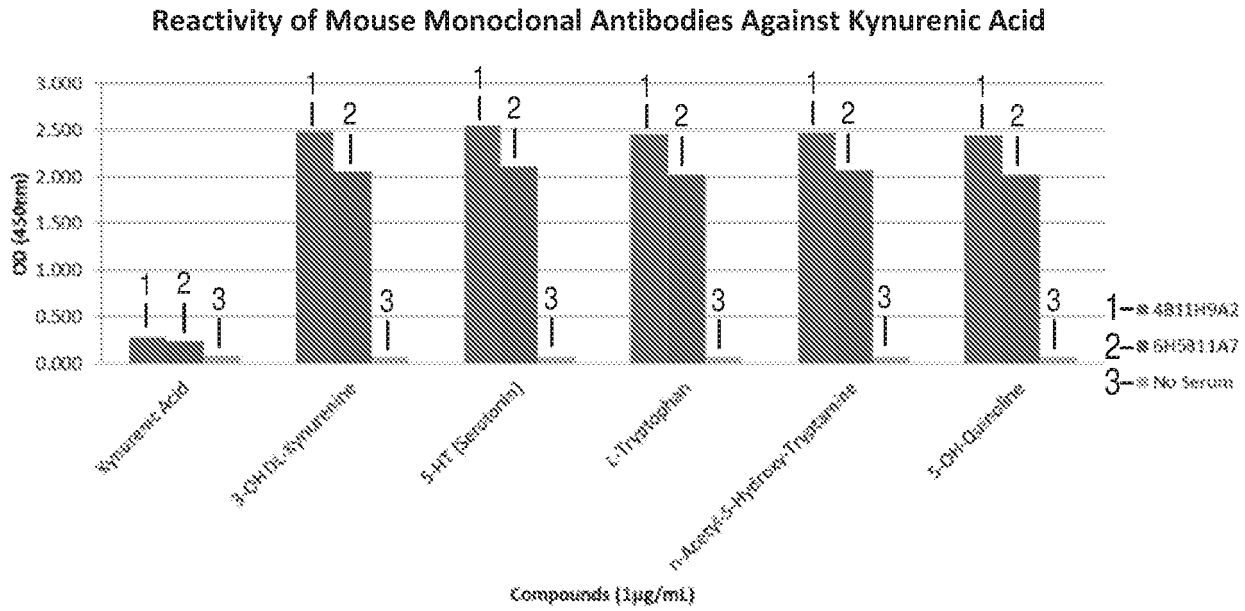


FIG. 14B

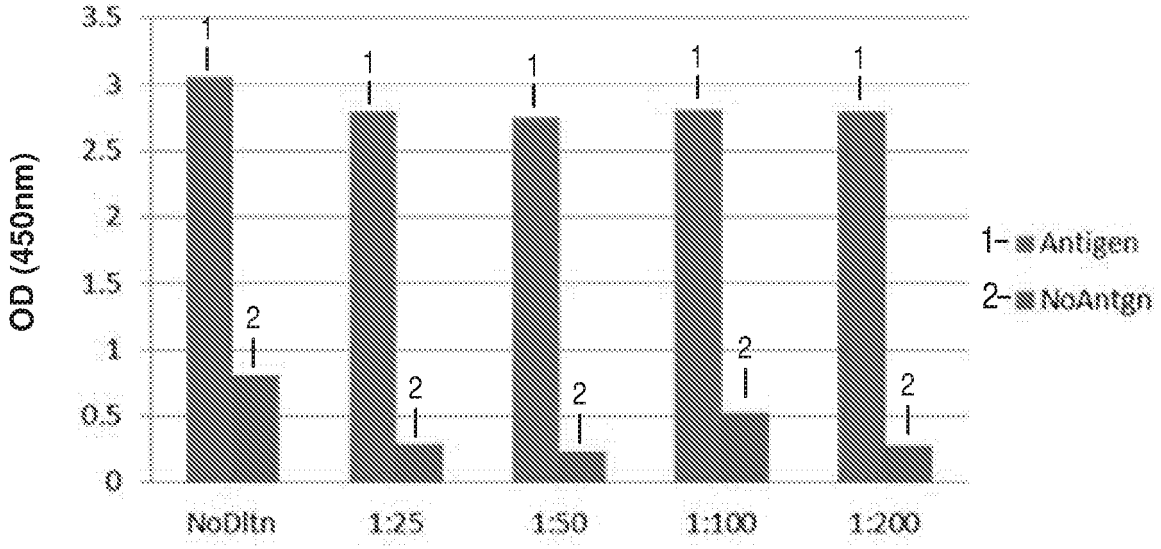


FIG. 15A

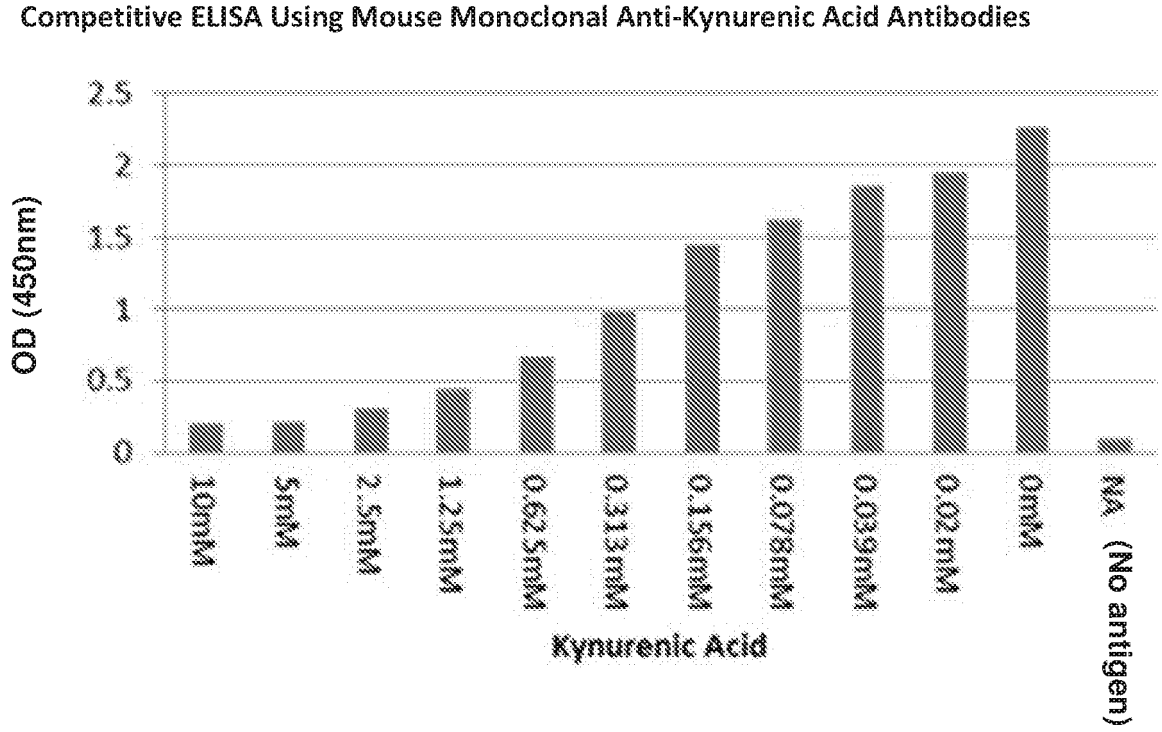


FIG. 15B

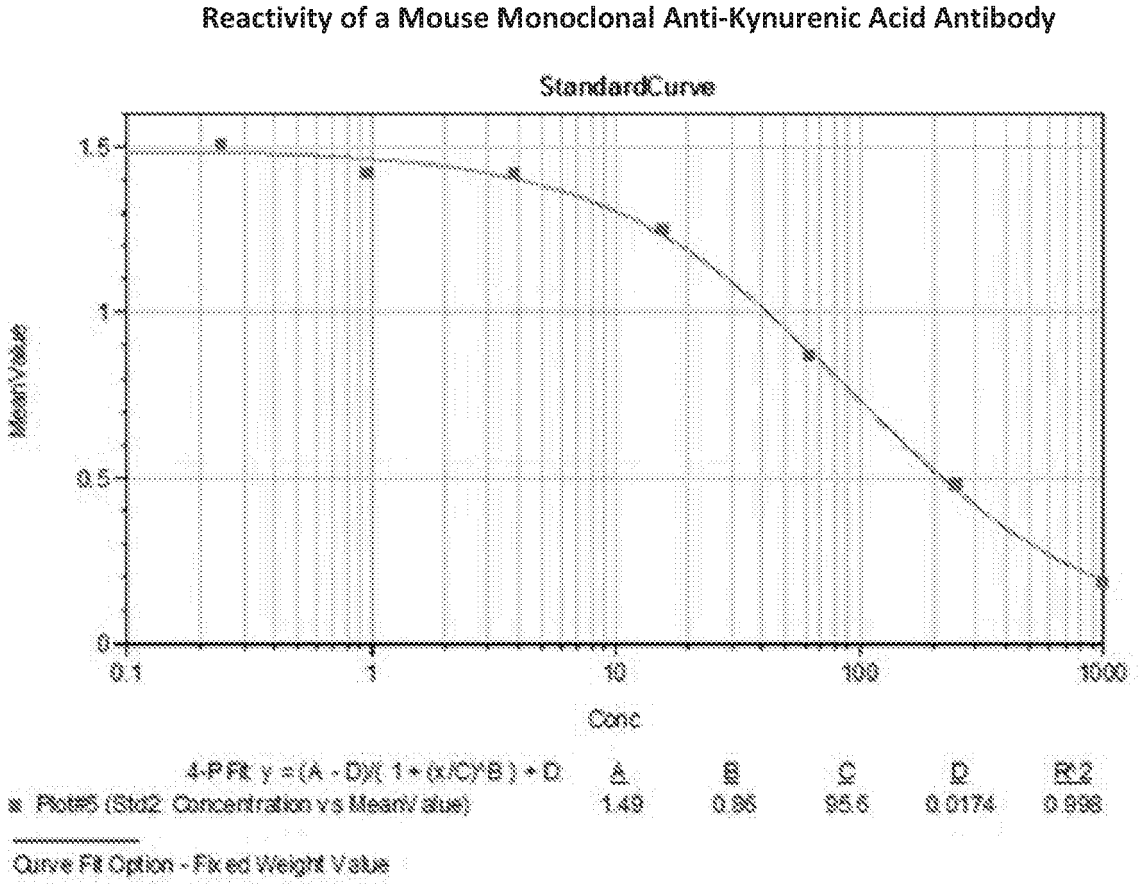


FIG. 16A

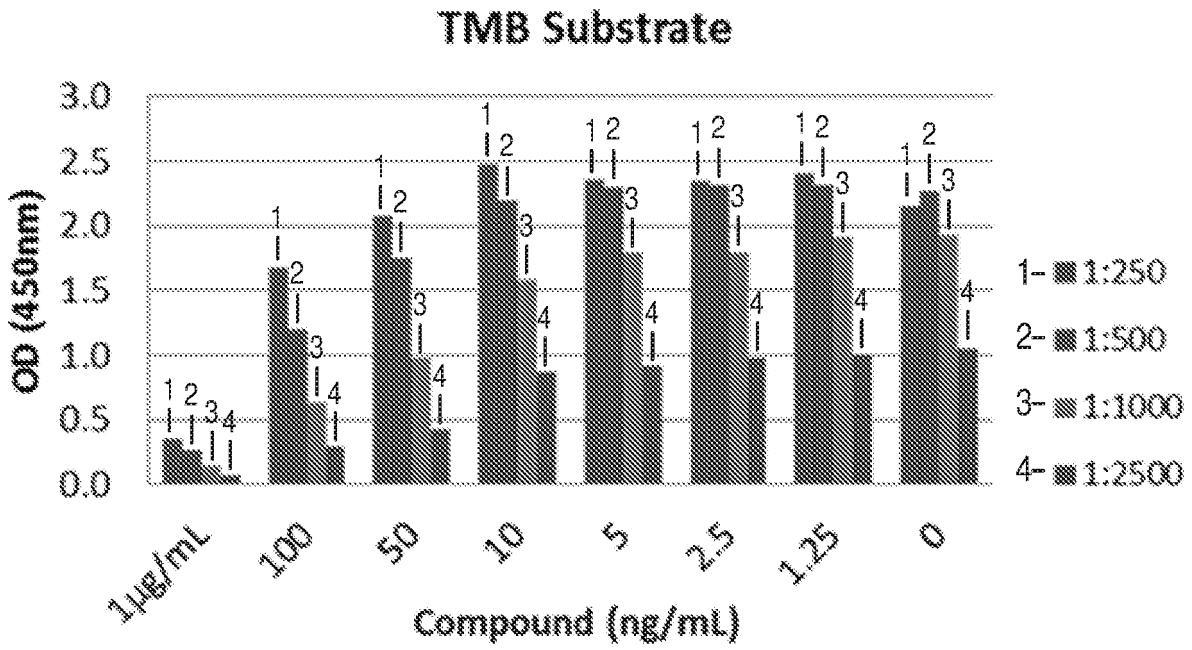
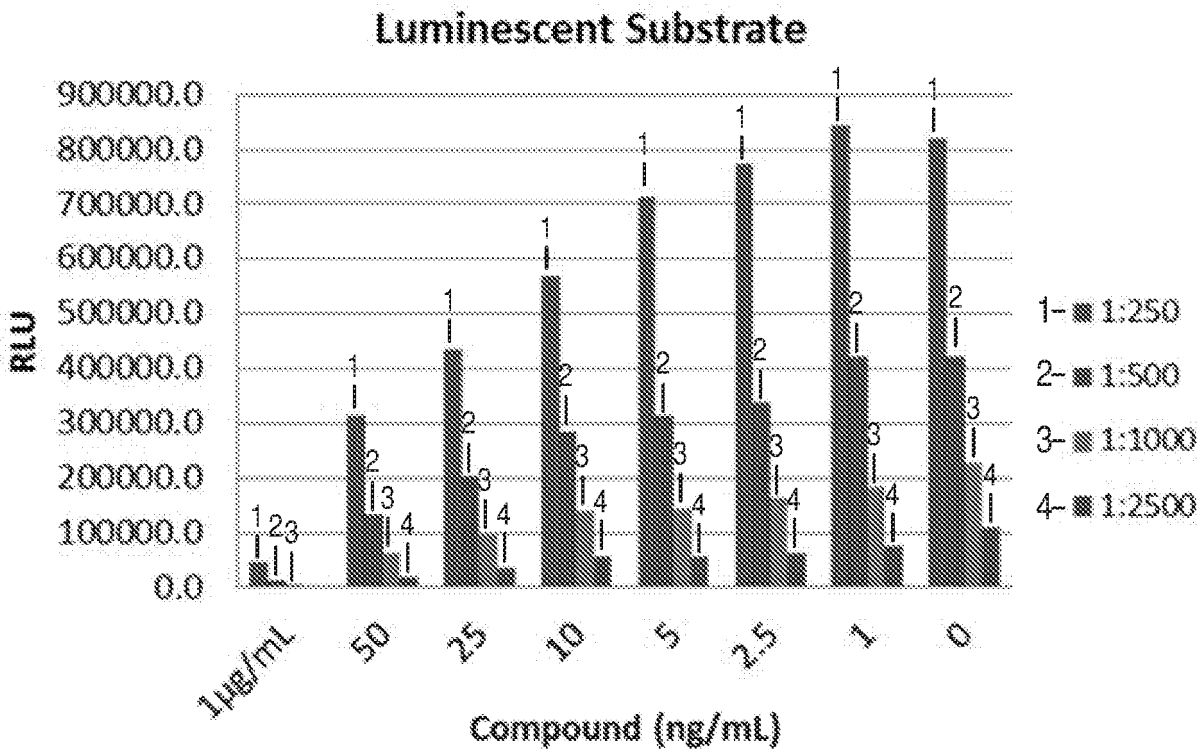



FIG. 16B