E25 light chain (V_L and C_L domains)

```
        10         20         30         40         50         60         70         80
DIQLTQSPSS LSASVGDRVT ITC[RASQSVD YDGDSYMN]WY QQKPGKAPKL LIY[AASYLES] GVPSRFSGSG SGTDFTLTIS

        90         100        110     CL starts
SLQPEDFATY YC[QQSHEDPY T]FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD


SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

Figure 1A

E25 heavy chain (V_H and C_H domains)

```
        10         20         30          40         50 a       60          70         80          90
EVQLVESGGG LVQPGGSLRL SCAVSGYSIT S[GYSWNW]IRQ APGKGLEWVA [SITYDGSTNY NPSVKG]RITI SRDDSKNTFY LQMNSLRAED

        100        110ab        120 CH starts
TAVYYCAR[GS HYFGHWHFAV]  WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS

VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE

VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE

SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
```

Figure 1B

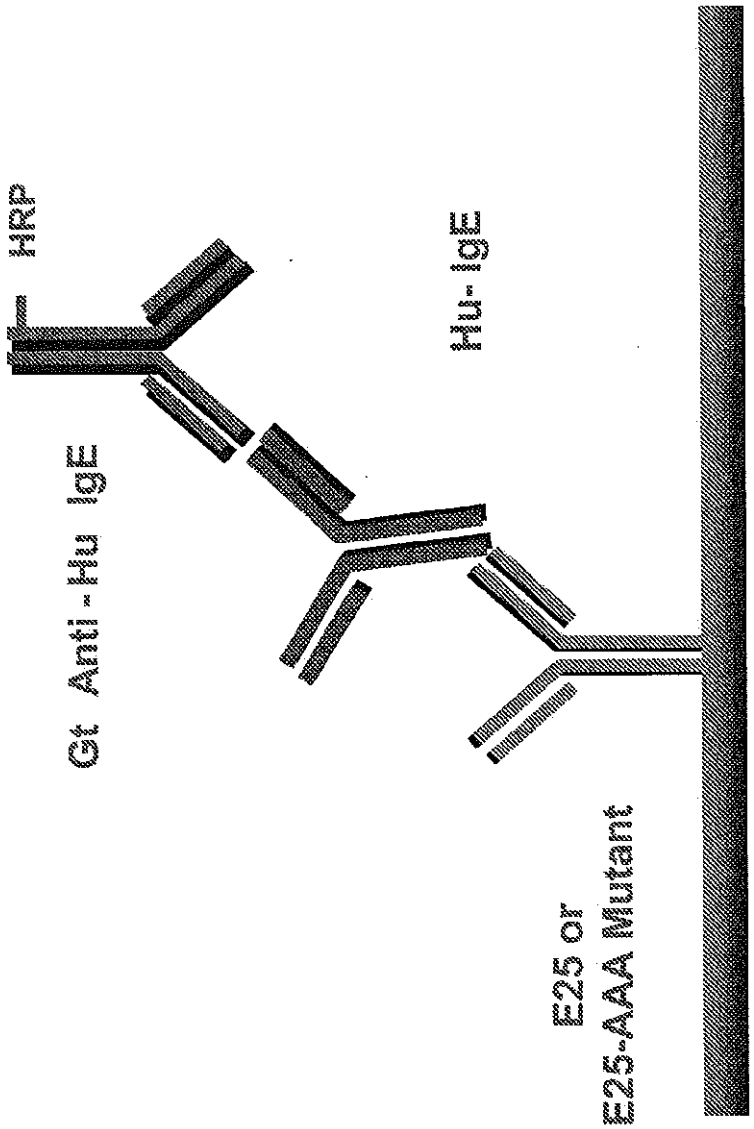


Figure 2A

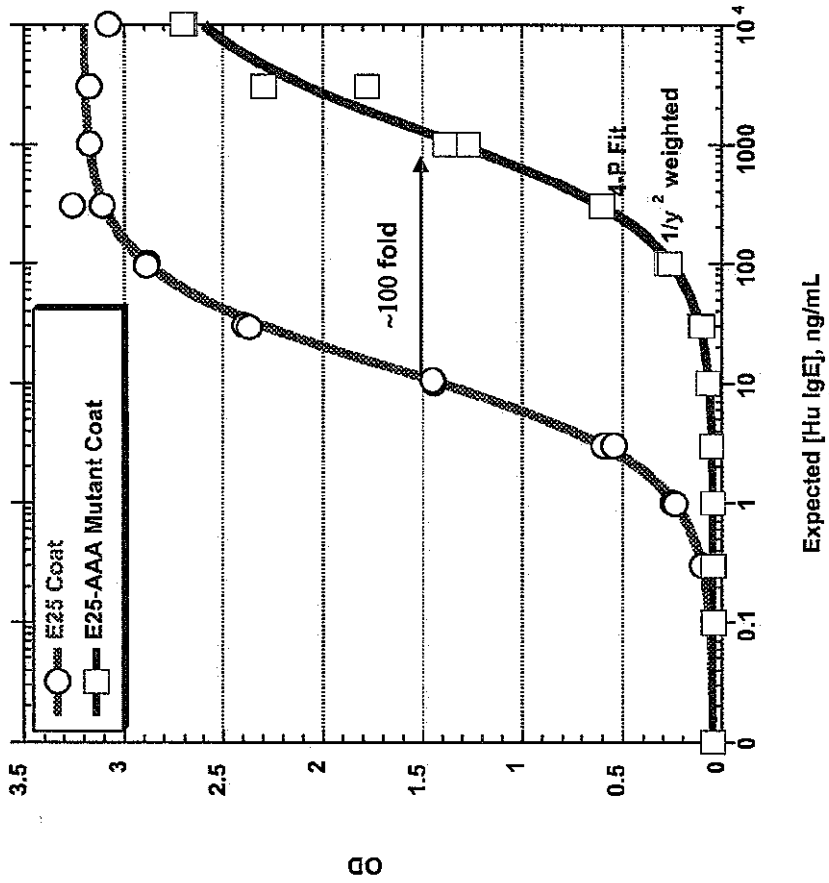

E25 Coat
E25-AAA Mutant Coat
~100 fold
4 P Fit
1/y ² weighted
OD
Expected [Hu IgE], ng/mL
0
0.5
1
1.5
2
2.5
3
3.5
0
0.1
1
10
100
1000
10 ⁴

Figure 2B

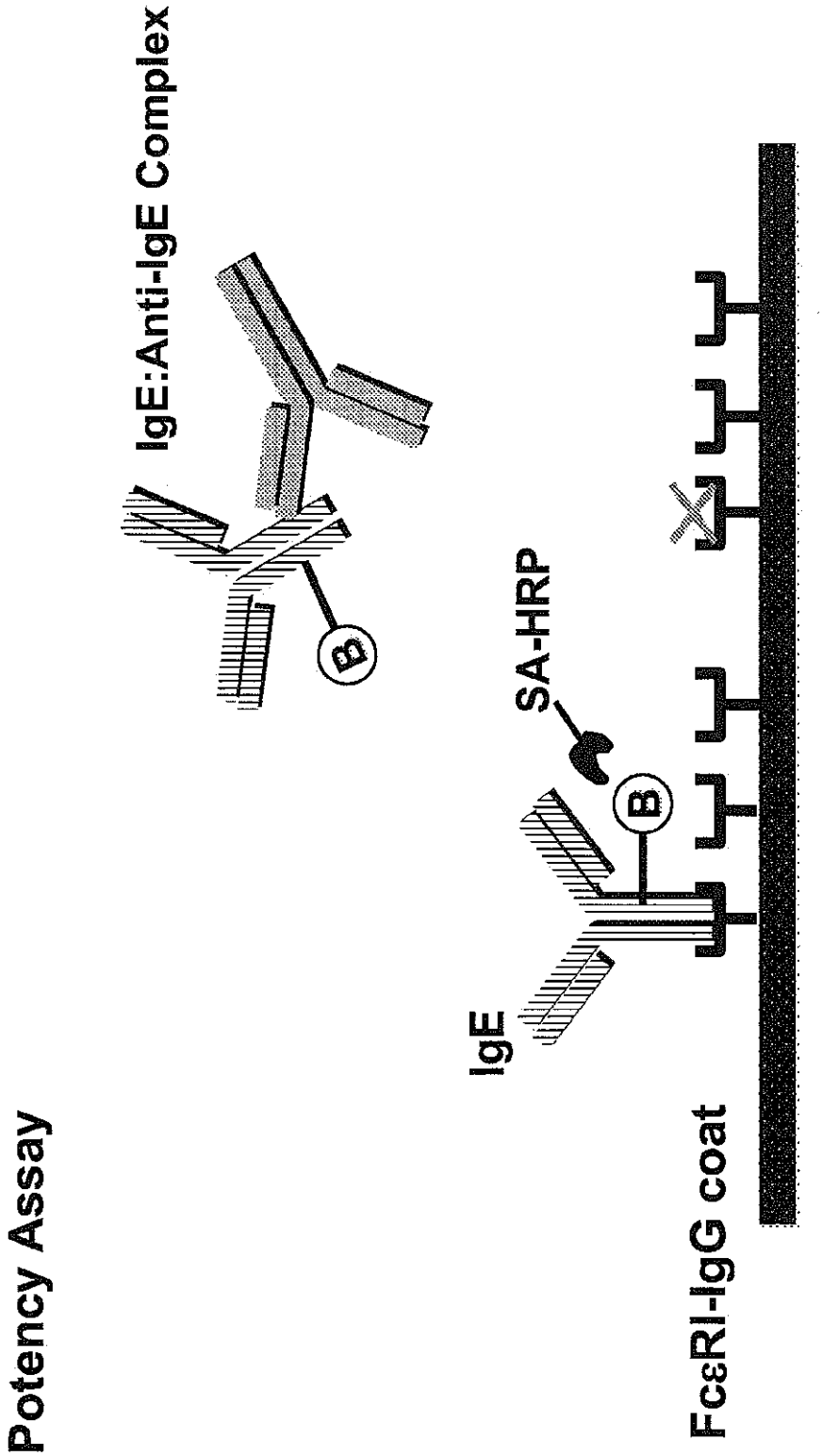

Potency Assay
IgE:Anti-IgE Complex
B
SA-HRP
B
IgE
FcεRI-IgG coat

Figure 3

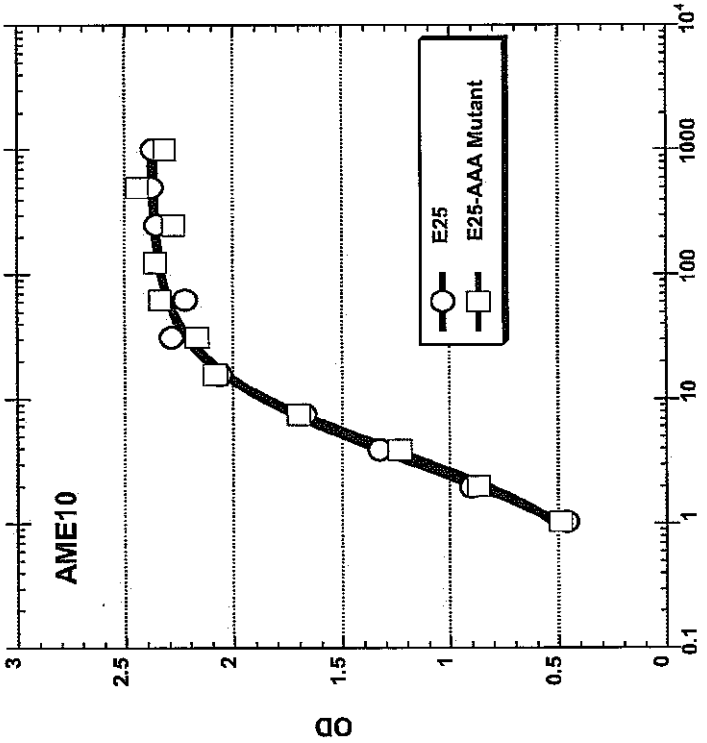


Figure 4B

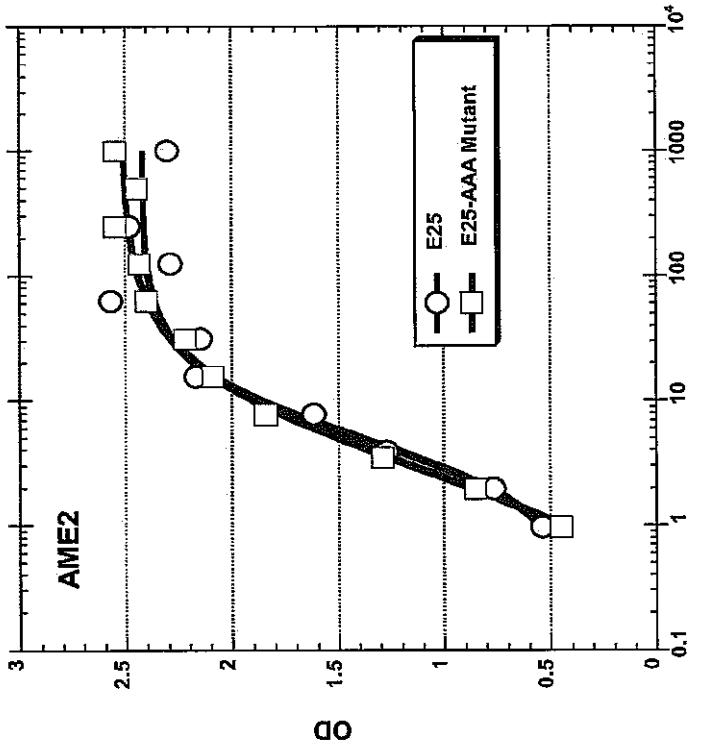


Figure 4A

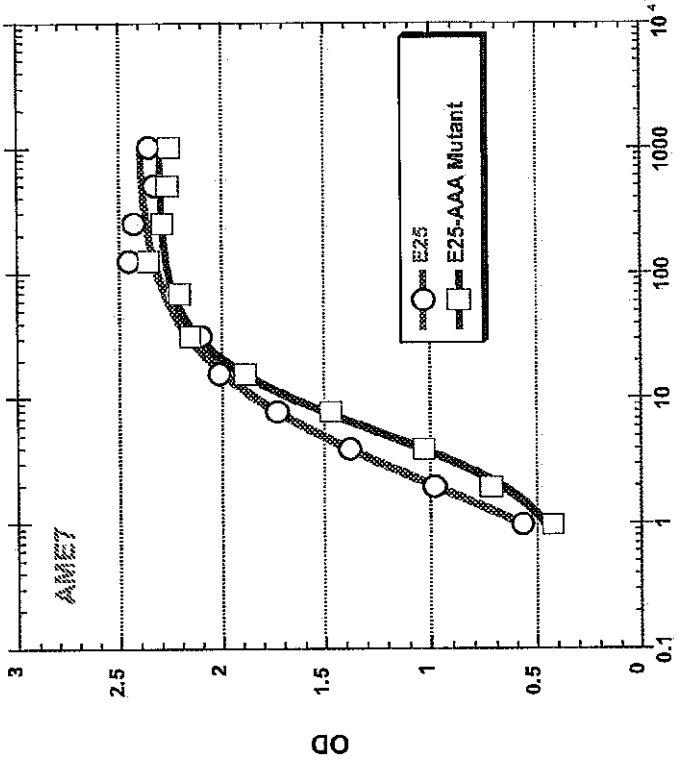


Figure 4D

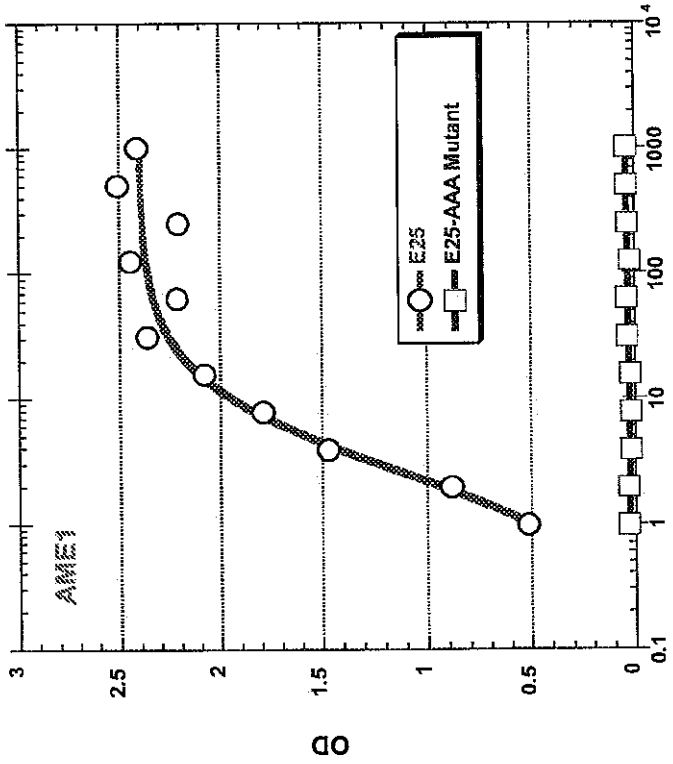


Figure 4C

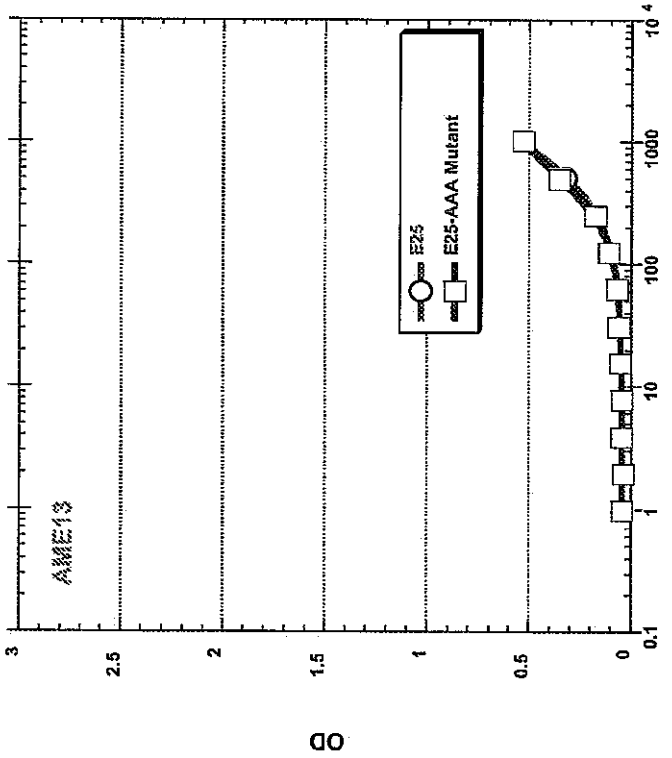


Figure 4F

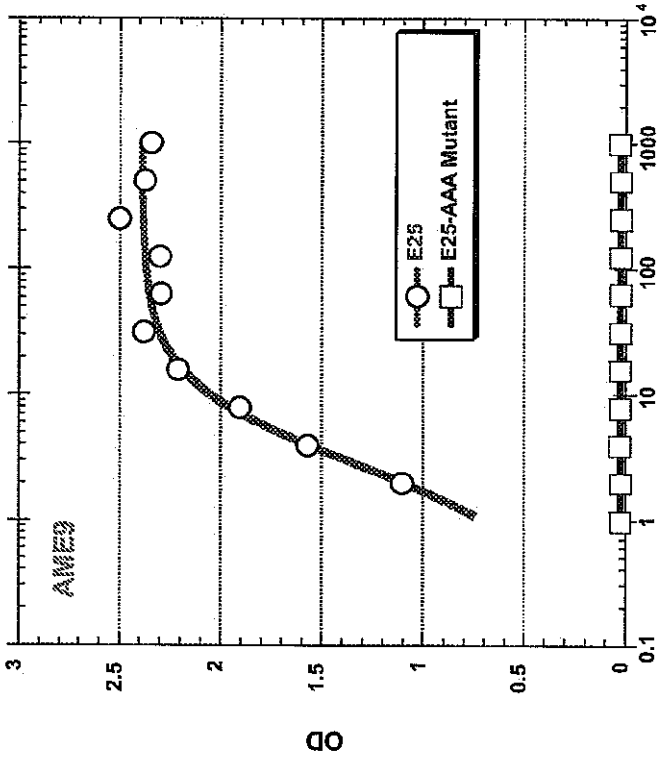


Figure 4E

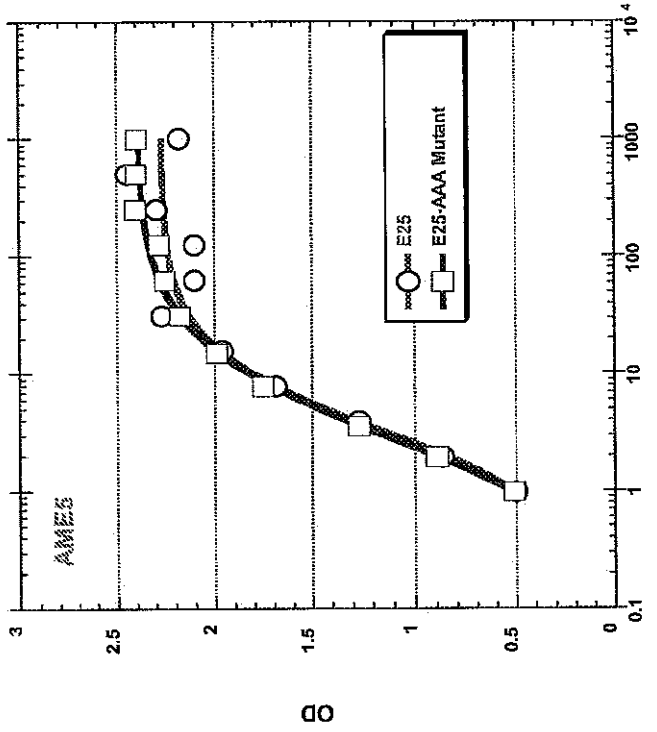


Figure 4H

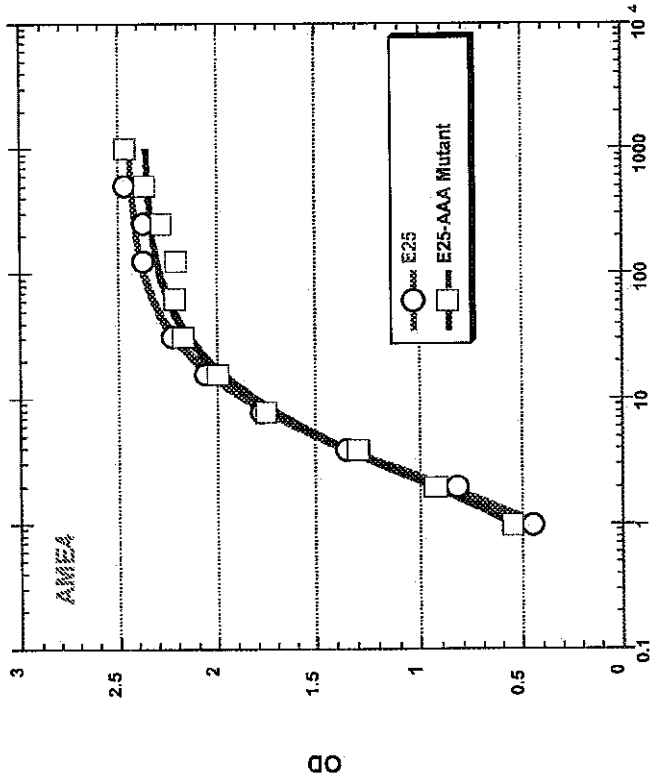


Figure 4G

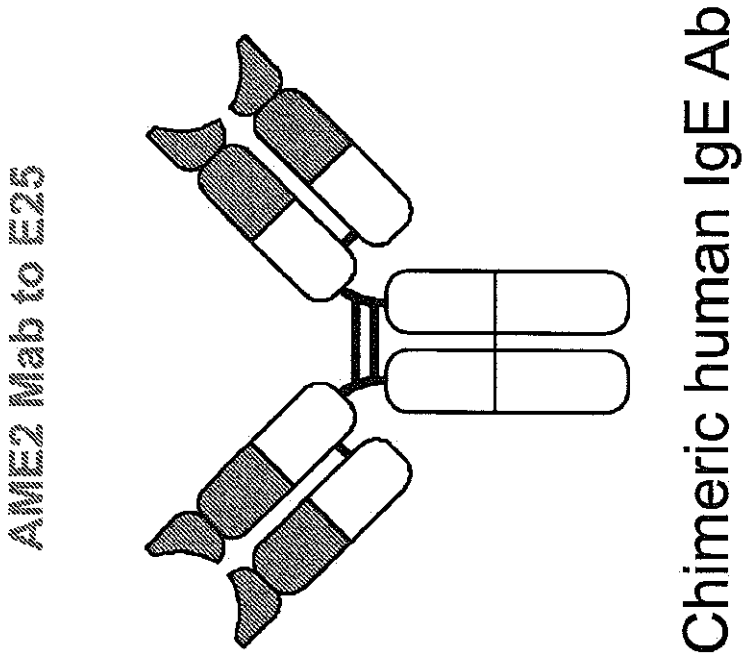


Figure 5

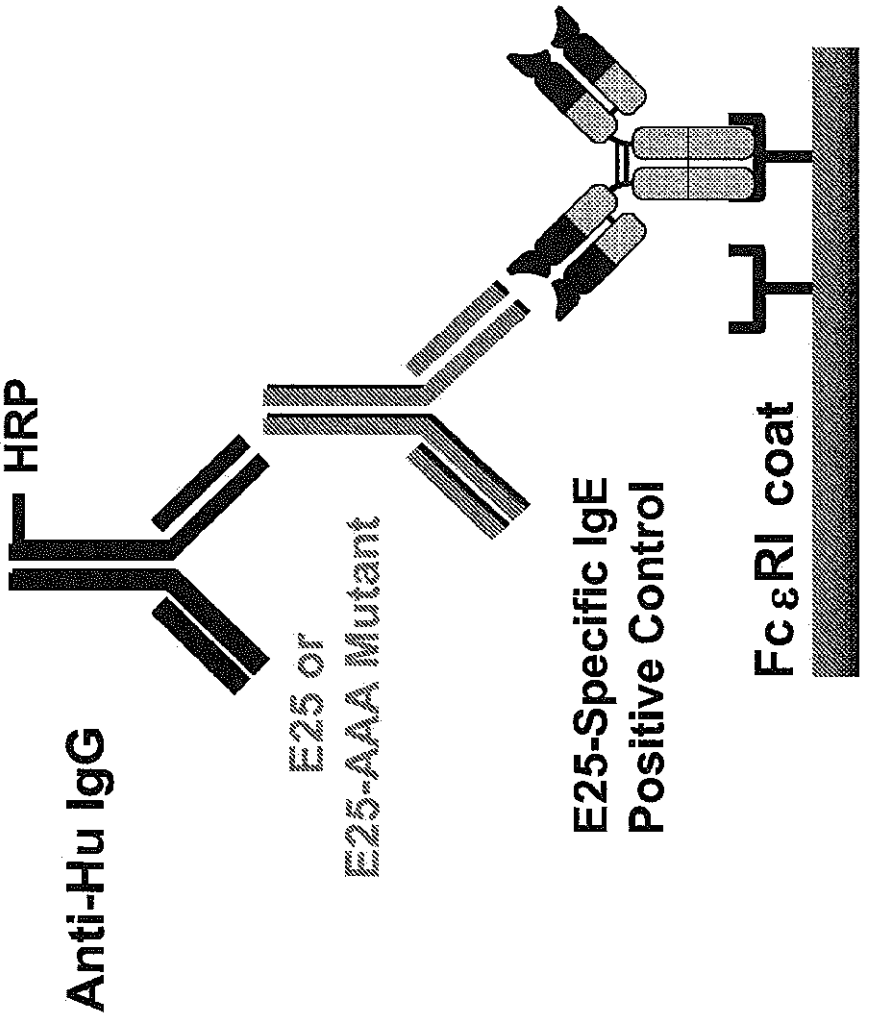

HRP
Anti-Hu IgG
E25 or
E25-AAA Mutant
E25-Specific IgE
Positive Control
FcεRI coat

Figure 6A

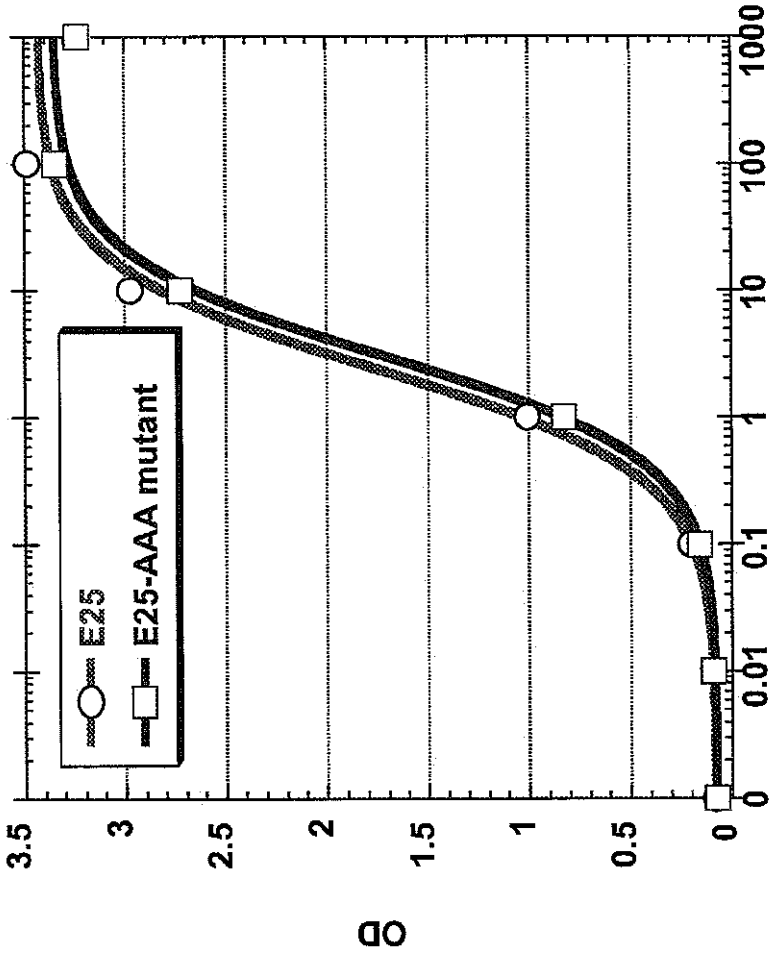

OD
3.5
3
2.5
2
1.5
1
0.5
0
0
0.01
0.1
1
10
100
1000
[E25-Specific IgE], ng/mL
E25
E25-AAA mutant

Figure 6B

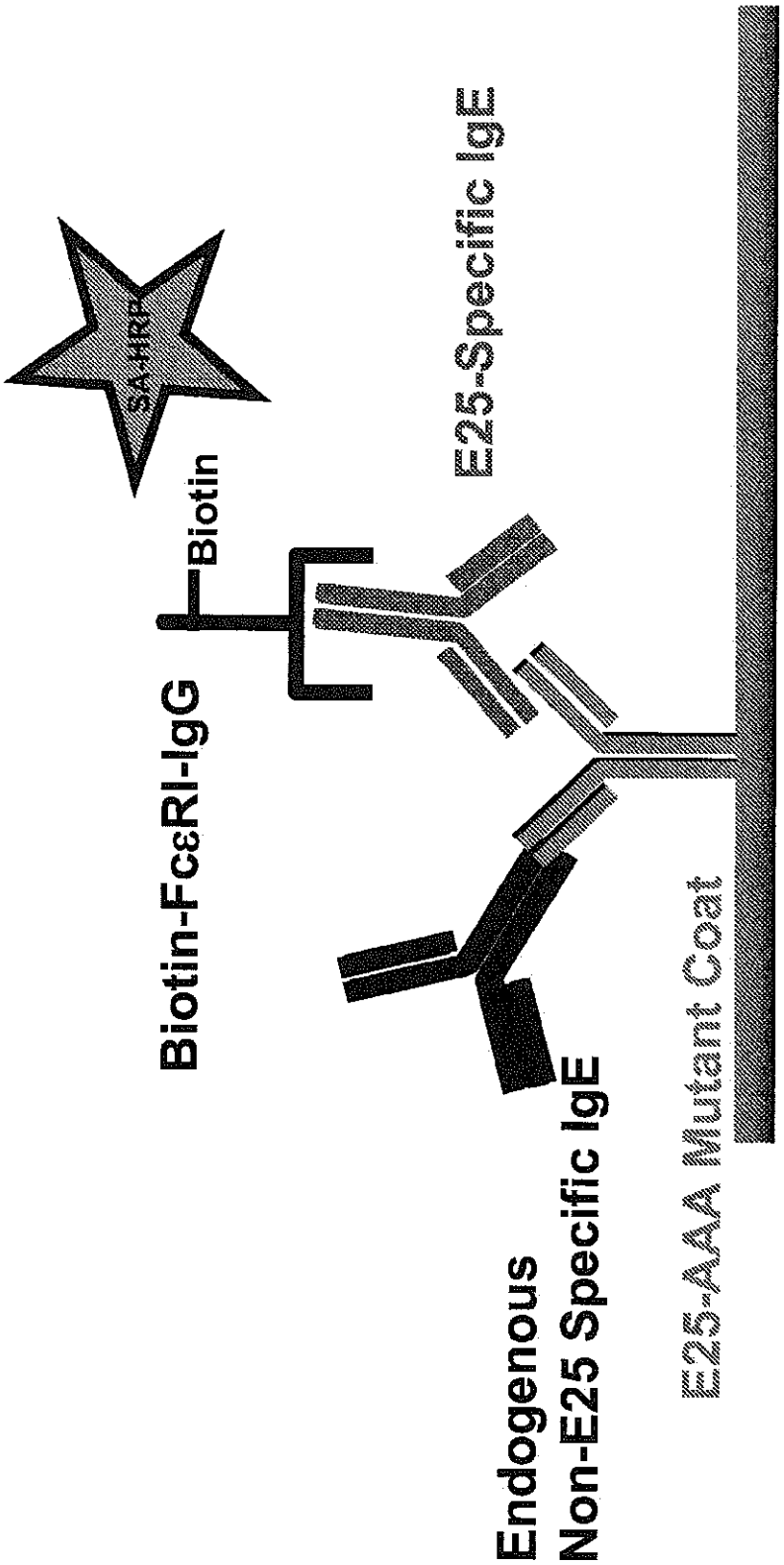

SA-HRP
Biotin
Biotin-FcεRI-IgG
E25-Specific IgE
Endogenous
Non-E25 Specific IgE
E25-AAA Mutant Coat

Figure 7

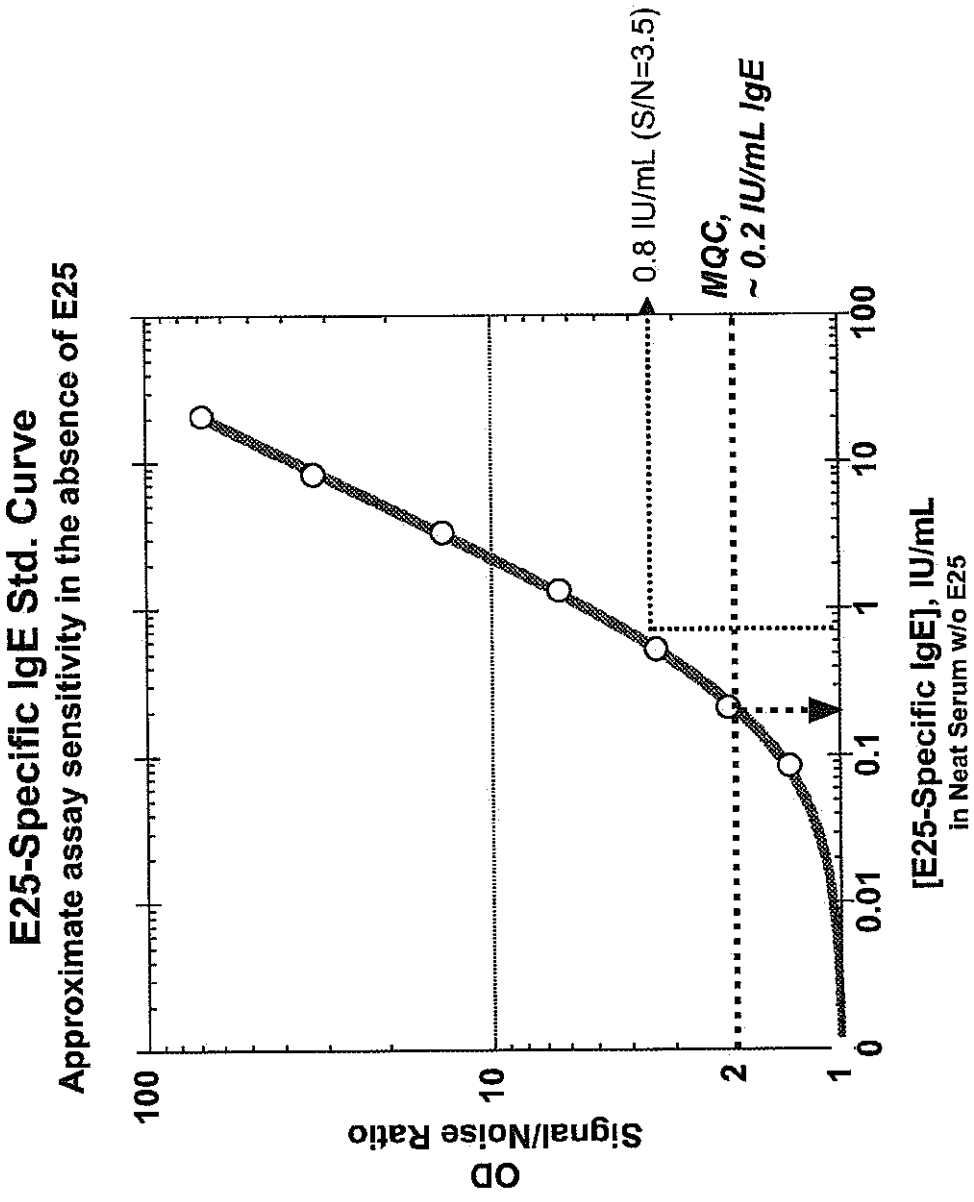

E25-Specific IgE Std. Curve
Approximate assay sensitivity in the absence of E25
OD
Signal/Noise Ratio
100
10
2
1
0.8 IU/mL (S/N=3.5)
MQC,
~ 0.2 IU/mL IgE
0
0.01
0.1
1
10
100
[E25-Specific IgE], IU/mL
in Neat Serum w/o E25

Figure 8

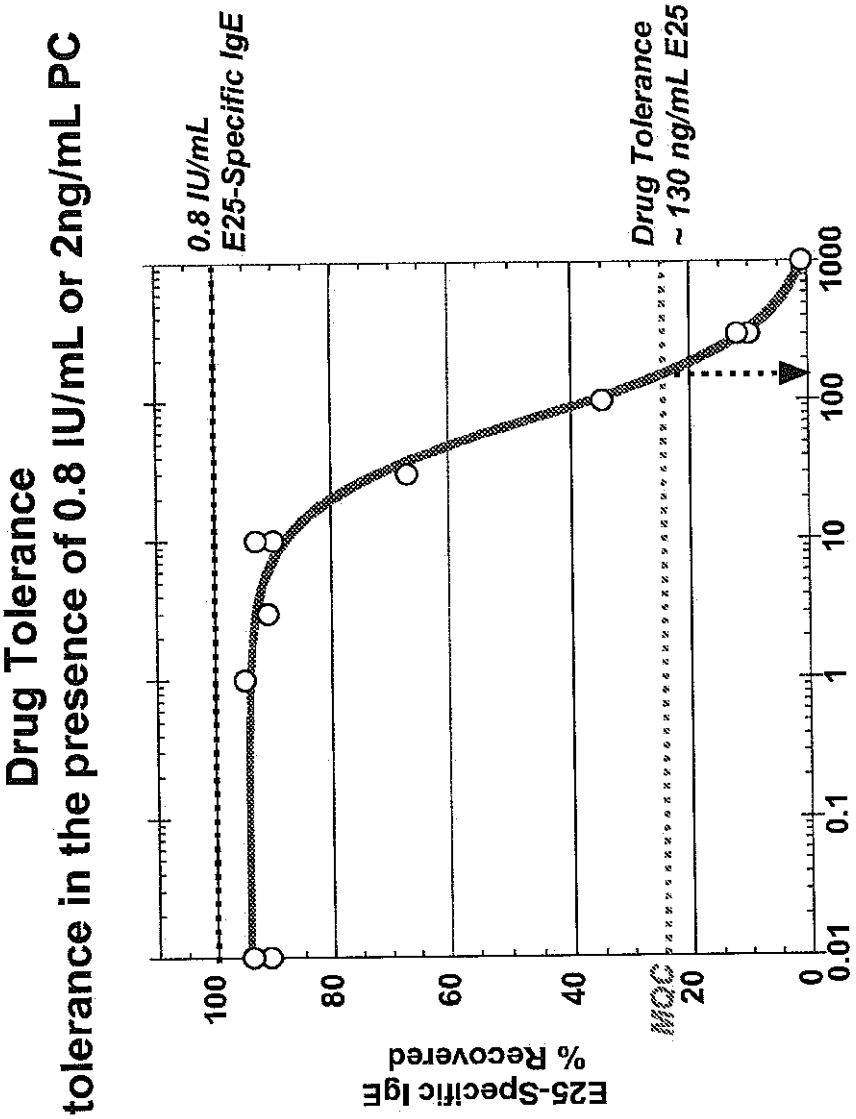

Drug Tolerance
E25 tolerance in the presence of 0.8 IU/mL or 2ng/mL PC
0.8 IU/mL
E25-Specific IgE
Drug Tolerance
~ 130 ng/mL E25
E25-Specific IgE
% Recovered
100
80
60
40
20
0
MOC
0.01
0.1
1
10
100
1000
[E25], ng/mL
in Neat Serum w/0.8 IU/mL E25-Specific IgE
Serum contains 124 IU/mL or 300ng/mL IgE

Figure 9

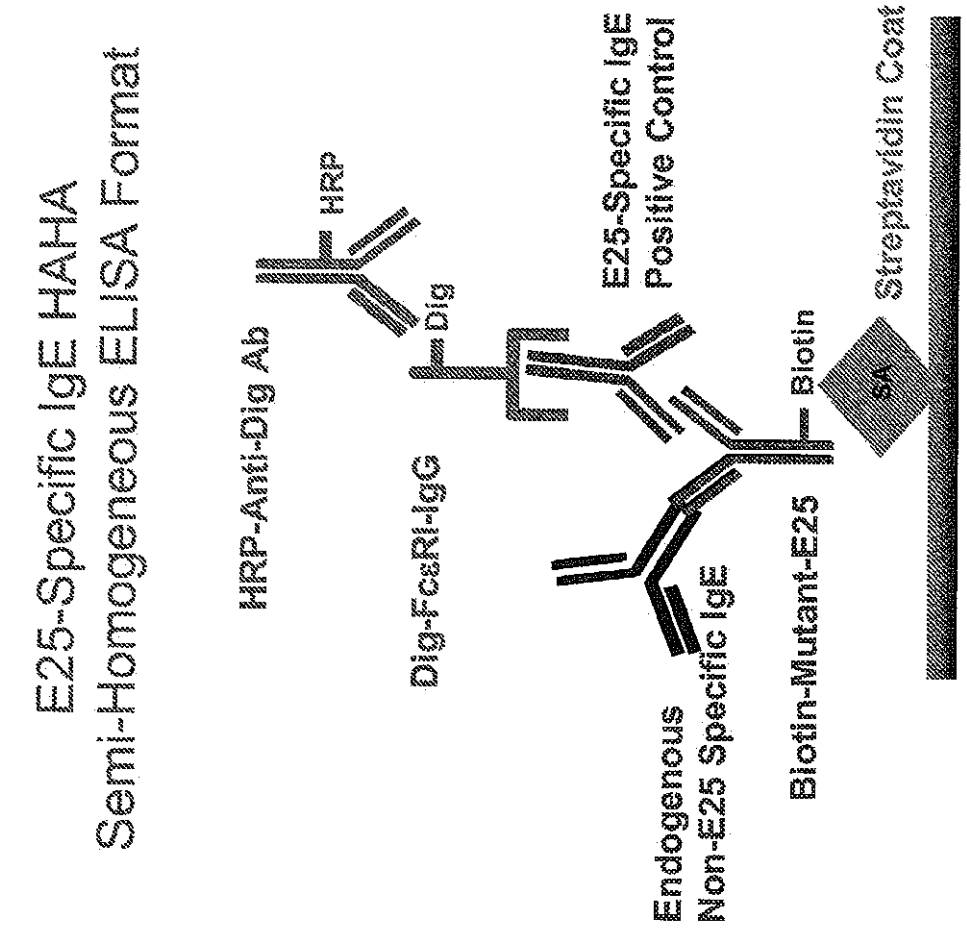

E25-Specific IgE HAHA
Semi-Homogeneous ELISA Format
HRP-Anti-Dig Ab
HRP
Dig
Dig-FcεRI-IgG
E25-Specific IgE
Positive Control
Endogenous
Non-E25 Specific IgE
Biotin-Mutant-E25
Biotin
SA
Streptavidin Coat

Figure 10

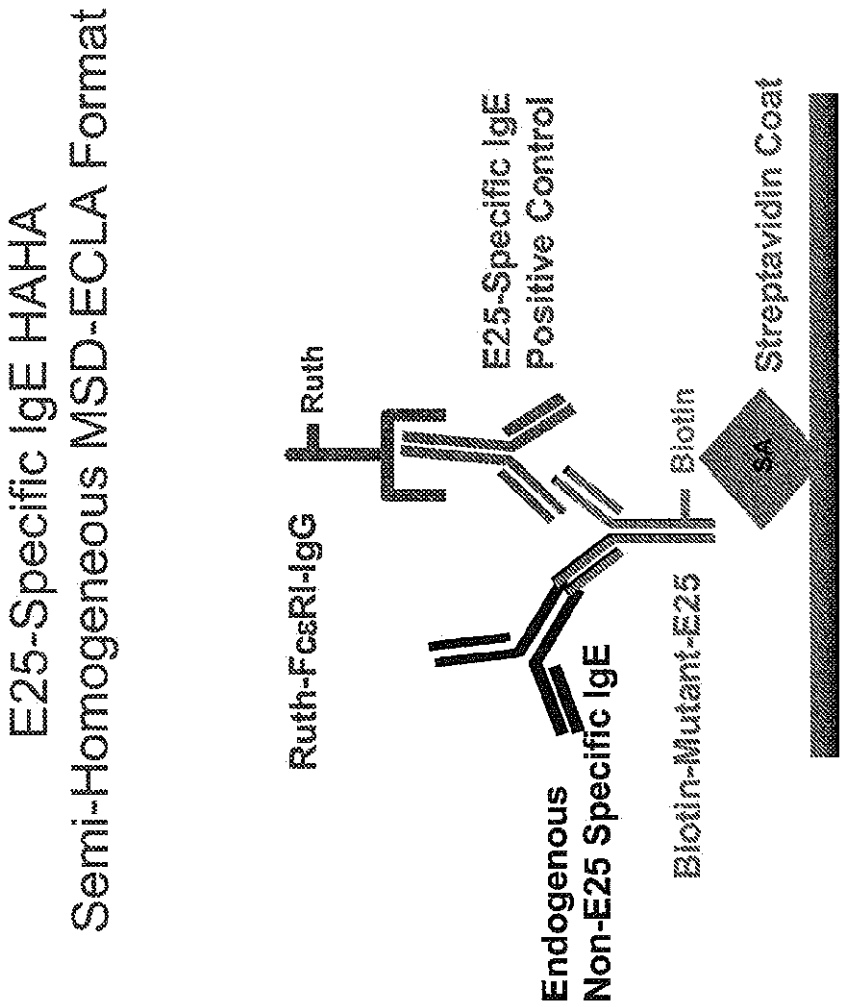

E25-Specific IgE HAHA
Semi-Homogeneous MSD-ECLA Format
Ruth-FcεRI-IgG
Ruth
E25-Specific IgE
Positive Control
Endogenous
Non-E25 Specific IgE
Biotin-Mutant-E25
Biotin
Streptavidin Coat

Figure 11

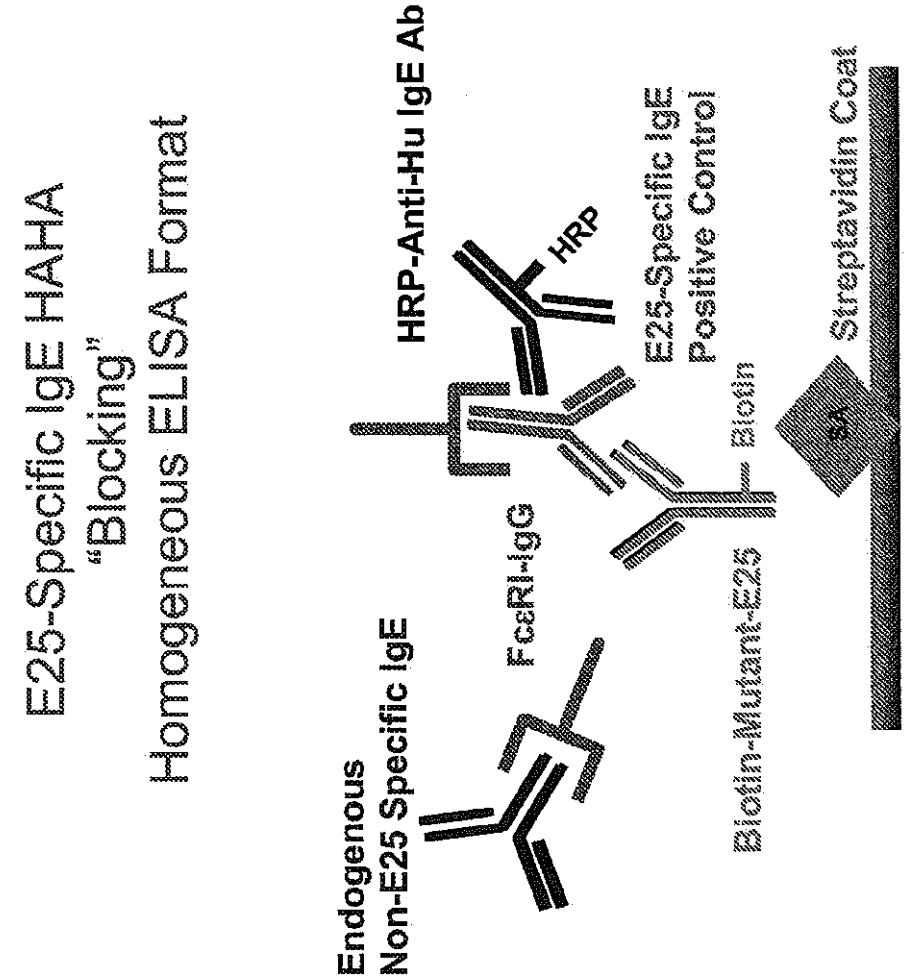

E25-Specific IgE HAHA
"Blocking"
Homogeneous ELISA Format
Endogenous
Non-E25 Specific IgE
FcεRI-IgG
HRP-Anti-Hu IgE Ab
HRP
E25-Specific IgE
Positive Control
Biotin
Biotin-Mutant-E25
SA
Streptavidin Coat

Figure 12

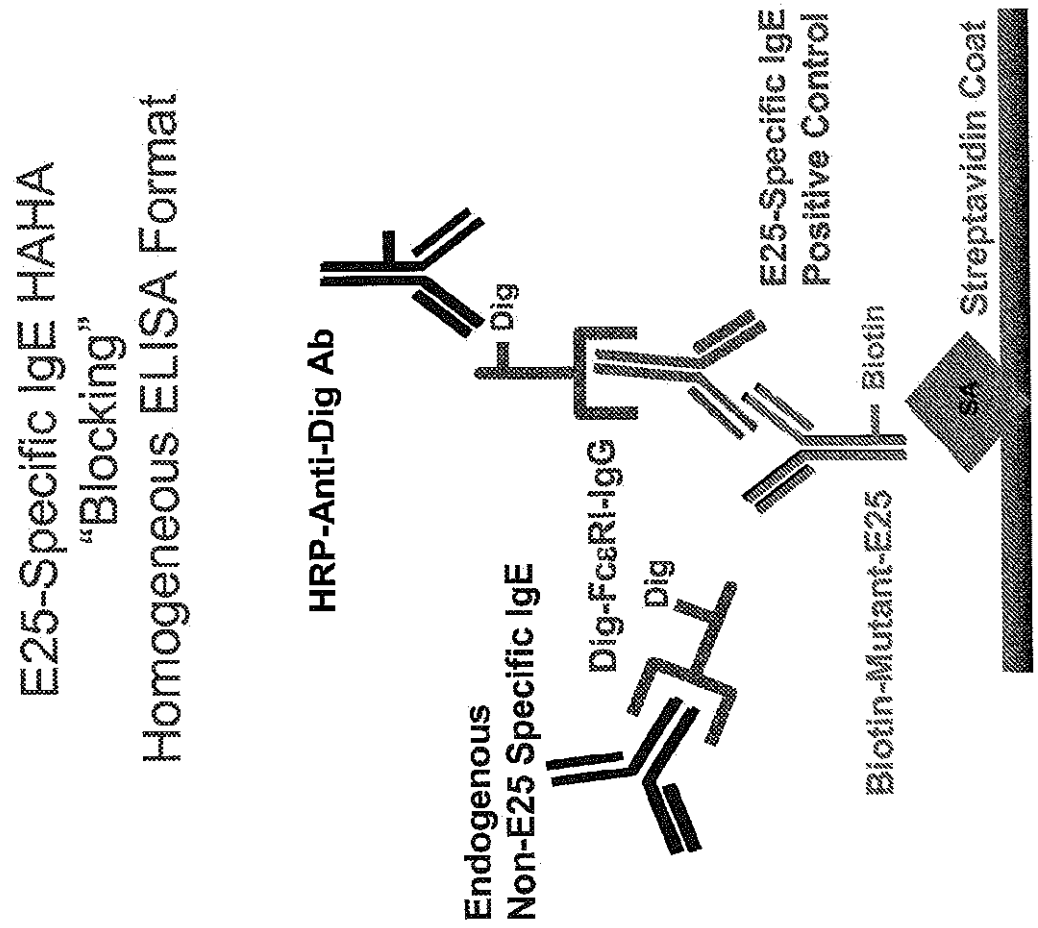

E25-Specific IgE HAHA
"Blocking"
Homogeneous ELISA Format
HRP-Anti-Dig Ab
Dig
Endogenous
Non-E25 Specific IgE
Dig-FcεRI-IgG
Dig
E25-Specific IgE
Positive Control
Biotin
Biotin-Mutant-E25
SA
Streptavidin Coat

Figure 13

E25-Specific IgE HAHA
"Blocking"
Homogeneous MSD-ECLA Format

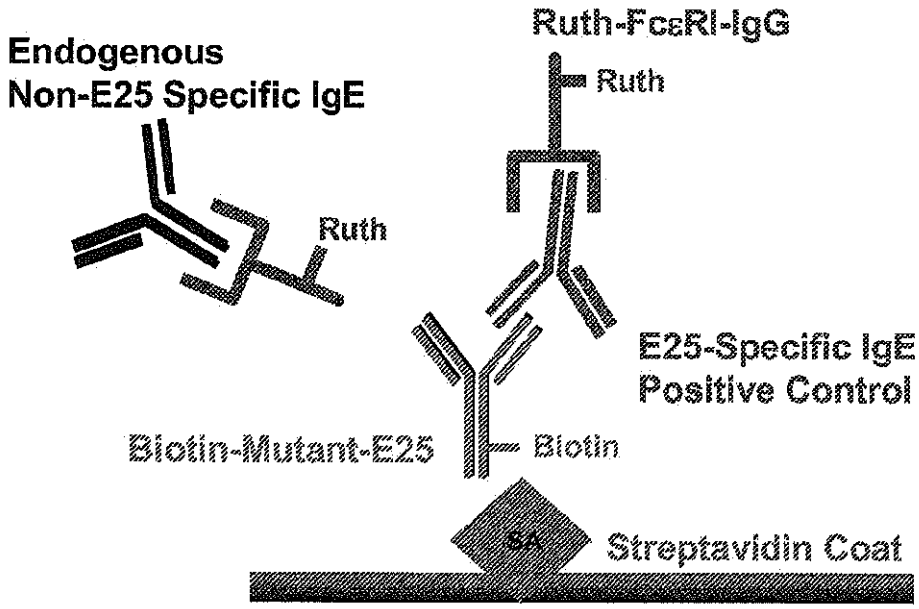


Figure 14

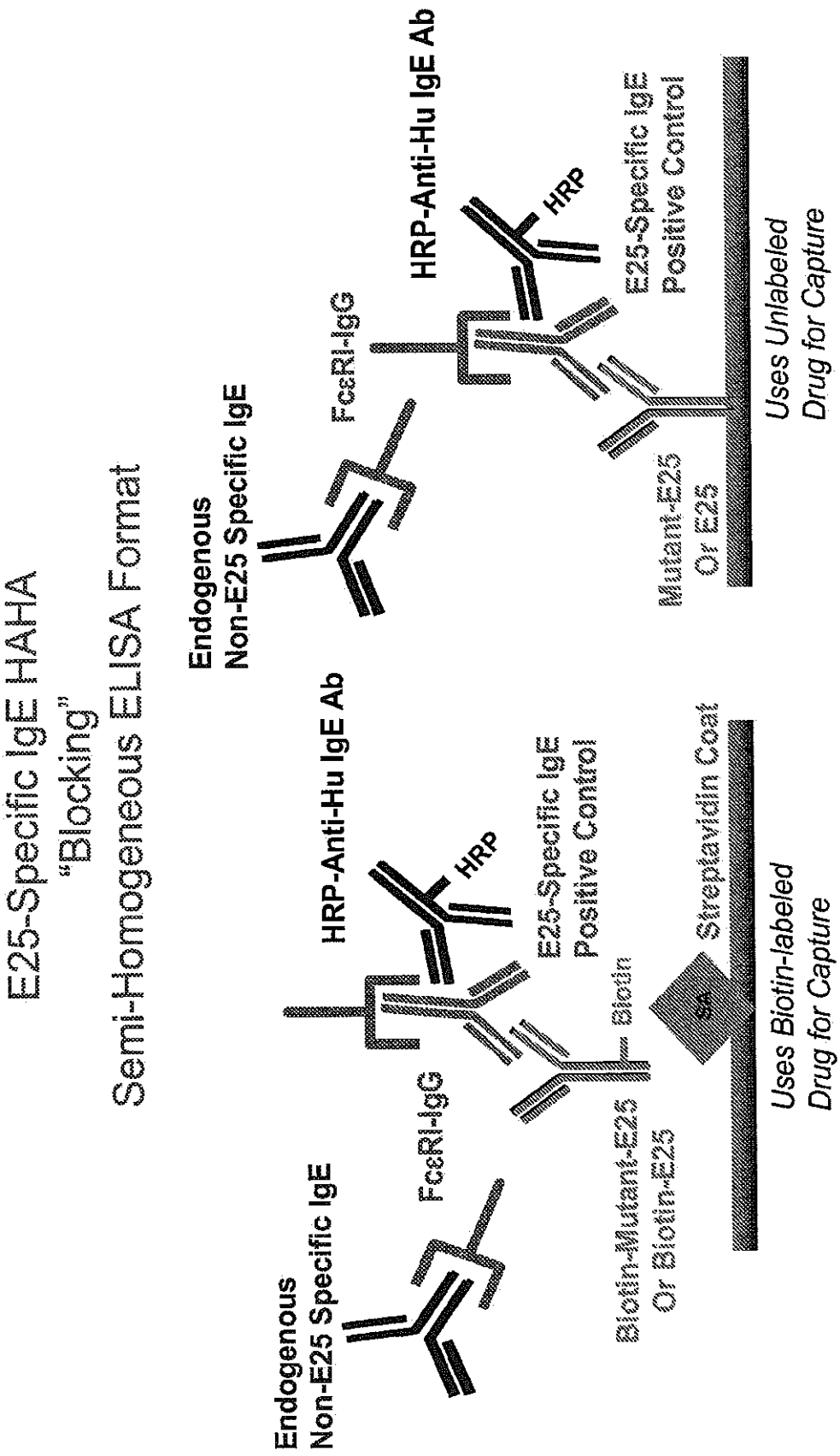

E25-Specific IgE HAHA
"Blocking"
Semi-Homogeneous ELISA Format
Endogenous
Non-E25 Specific IgE
FcεRI-IgG
HRP-Anti-Hu IgE Ab
HRP
E25-Specific IgE
Positive Control
Biotin
Biotin-Mutant-E25
Or Biotin-E25
SA
Streptavidin Coat
Uses Biotin-labeled
Drug for Capture
Endogenous
Non-E25 Specific IgE
FcεRI-IgG
HRP-Anti-Hu IgE Ab
HRP
E25-Specific IgE
Positive Control
Mutant-E25
Or E25
Uses Unlabeled
Drug for Capture

Figure 15

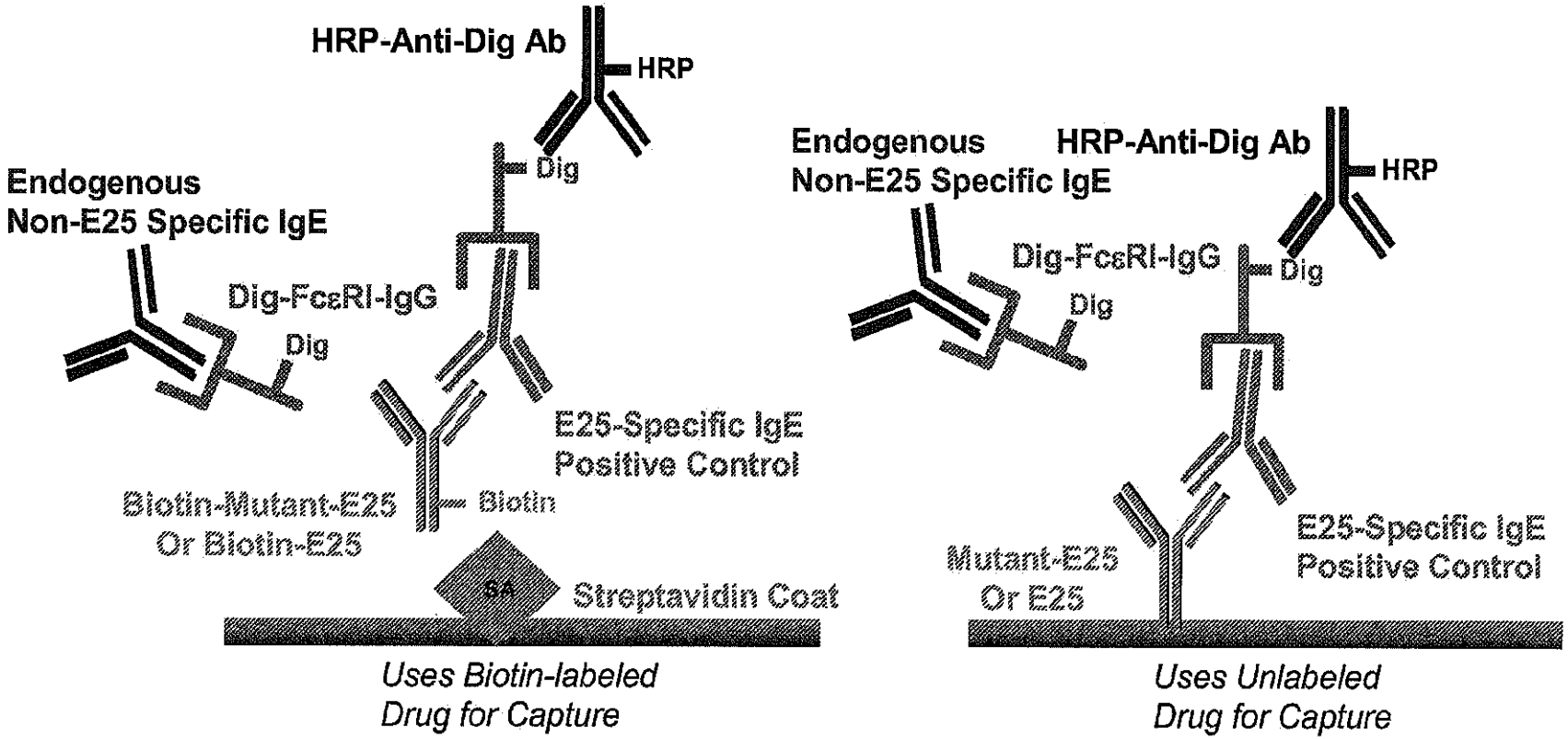

E25-Specific IgE HAHA
"Blocking"
Semi-Homogeneous ELISA Format
HRP-Anti-Dig Ab
HRP
Dig
Endogenous
Non-E25 Specific IgE
Dig-FcεRI-IgG
Dig
E25-Specific IgE
Positive Control
Biotin-Mutant-E25
Or Biotin-E25
Biotin
SA
Streptavidin Coat
Uses Biotin-labeled
Drug for Capture
Endogenous
Non-E25 Specific IgE
HRP-Anti-Dig Ab
HRP
Dig-FcεRI-IgG
Dig
Dig
E25-Specific IgE
Positive Control
Mutant-E25
Or E25
Uses Unlabeled
Drug for Capture

Figure 16

E25-Specific IgE HAHA
"Blocking"
Semi-Homogeneous MSD-ECLA Format

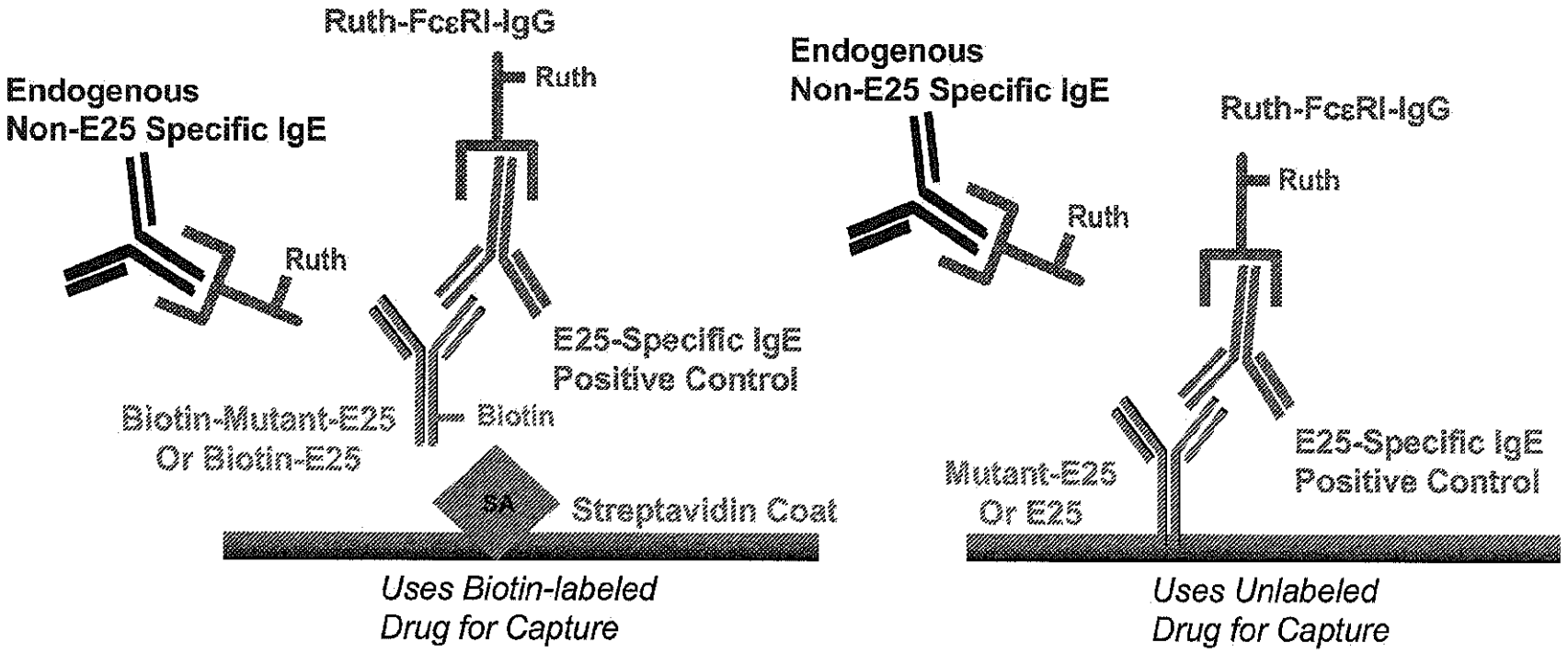


Figure 17