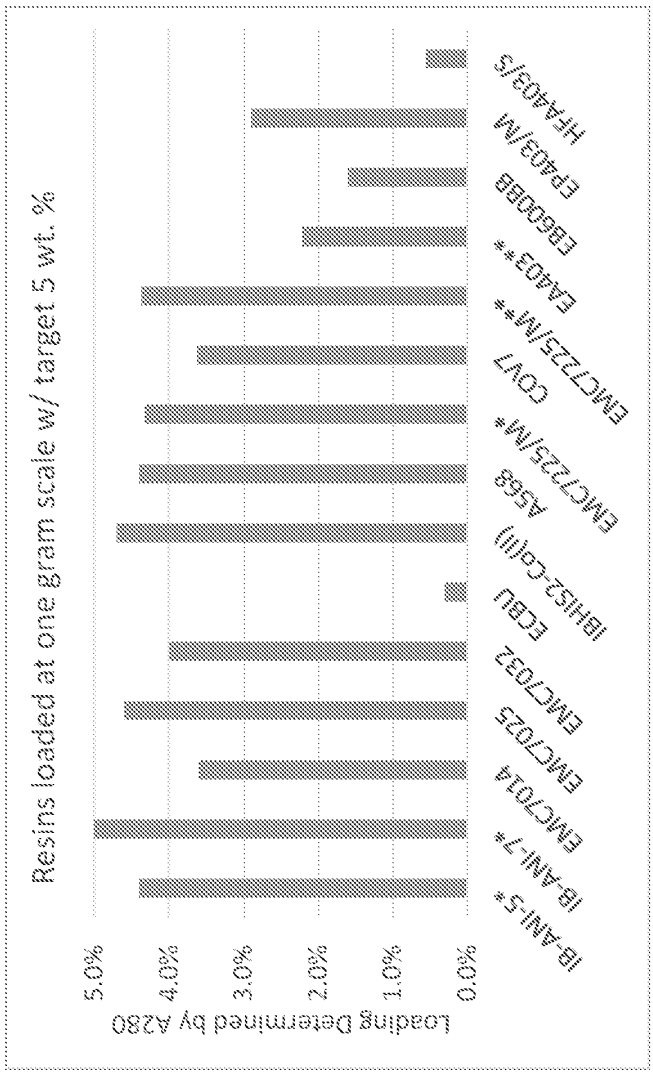
FIG. 1



*amine resin using glutaraldehyde
**amine resin uses glyoxal (or mix of glyoxal and glutaraldehyde)

FIG. 2

Fraction:	1	2	3	4	5	6	7	8	9	10	11	12
IB-ANI-5	0.0	0.0	0.0	#N/A	1.0	0.5	1.1	0.9	1.2	1.1	1.0	1.0
IB-ANI-7	0.6	1.1	1.1	1.4	0.0	0.0	1.0	0.5	1.1	1.1	0.0	1.0
EMC7014	0.0	0.0	1.0	1.1	1.0	#N/A	1.1	0.9	1.1	0.0	1.3	1.2
EMC7025	1.0	1.3	1.1	1.0	0.7	1.2	1.0	1.1	1.2	1.1	1.1	0.8
ECBU	672.4	637.4	525.0	30.3	12.0	8.7	6.6	4.5	5.4	6.2	4.4	4.1
IBHIS2	0.0	0.0	0.0	1.0	1.6	1.1	0.6	1.2	0.0	0.7	1.4	1.4
EMC7032	2.0	1.3	0.0	0.9	1.4	0.7	1.1	0.9	1.2	0.8	0.9	0.9
A568	0.9	0.8	1.0	0.0	0.9	0.0	0.0	0.7	0.8	0.9	1.1	1.1
EMC7225/M	1.9	-1.0	0.5	0.1	0.1	-0.3	-0.4	-0.8	-2.6	-0.3	-0.1	0.7
COV7	0.3	0.7	0.4	0.2	1.2	-0.1	0.3	0.6	0.4	0.4	-2.6	0.0
EMC7225/M*	-2.6	-2.6	0.9	-0.4	0.0	0.5	0.1	0.3	0.8	0.6	-2.6	0.3
EA403	-0.2	0.1	1.6	0.6	1.6	1.0	0.2	0.2	0.2	0.4	0.5	-0.1
COV7	0.6	0.0	0.8	0.8	0.7	0.0	0.0	0.8	0.8	0.7	0.8	0.7
COV7*	0.6	0.6	0.8	1.0	0.6	0.6	0.5	0.8	0.6	0.7	0.6	0.8
EP600BB	0.8	0.8	1.2	0.6	0.6	0.8	0.7	0.8	0.0	0.0	0.0	0.8
HFA403/S	0.7	1.8	0.0	0.9	1.3	1.2	0.6	0.8	0.9	0.7	0.9	0.0

FIG. 3

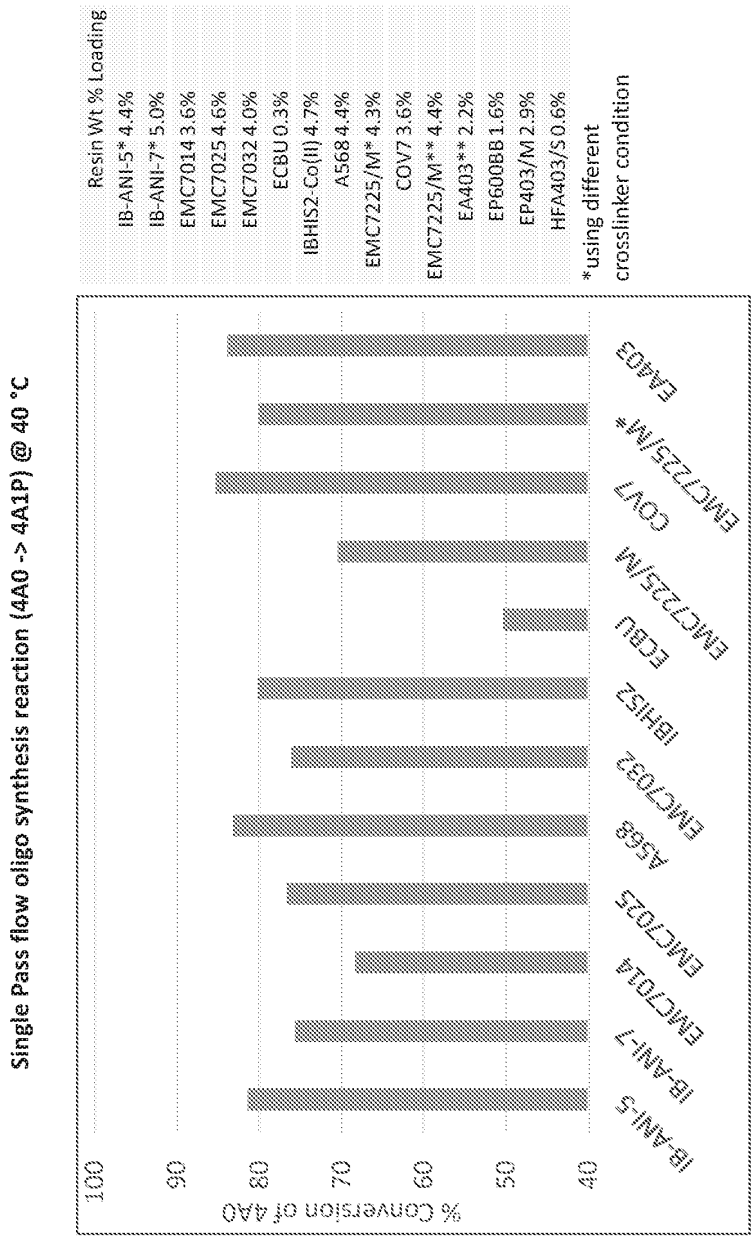

Single Pass flow oligo synthesis reaction (4A0 -> 4A1P) @ 40 °C
% Conversion of 4A0
100
90
80
70
60
50
40
IB-ANI-5
IB-ANI-7
EMC7014
EMC7025
A568
EMC7032
IBHIS2
ECBU
EMC7225/M
COV7
EMC7225/M*
EA403
Resin Wt % Loading
IB-ANI-5* 4.4%
IB-ANI-7* 5.0%
EMC7014 3.6%
EMC7025 4.6%
EMC7032 4.0%
ECBU 0.3%
IBHIS2-Co(II) 4.7%
A568 4.4%
EMC7225/M* 4.3%
COV7 3.6%
EMC7225/M** 4.4%
EA403** 2.2%
EP600BB 1.6%
EP403/M 2.9%
HFA403/S 0.6%
*using different crosslinker condition

FIG. 4

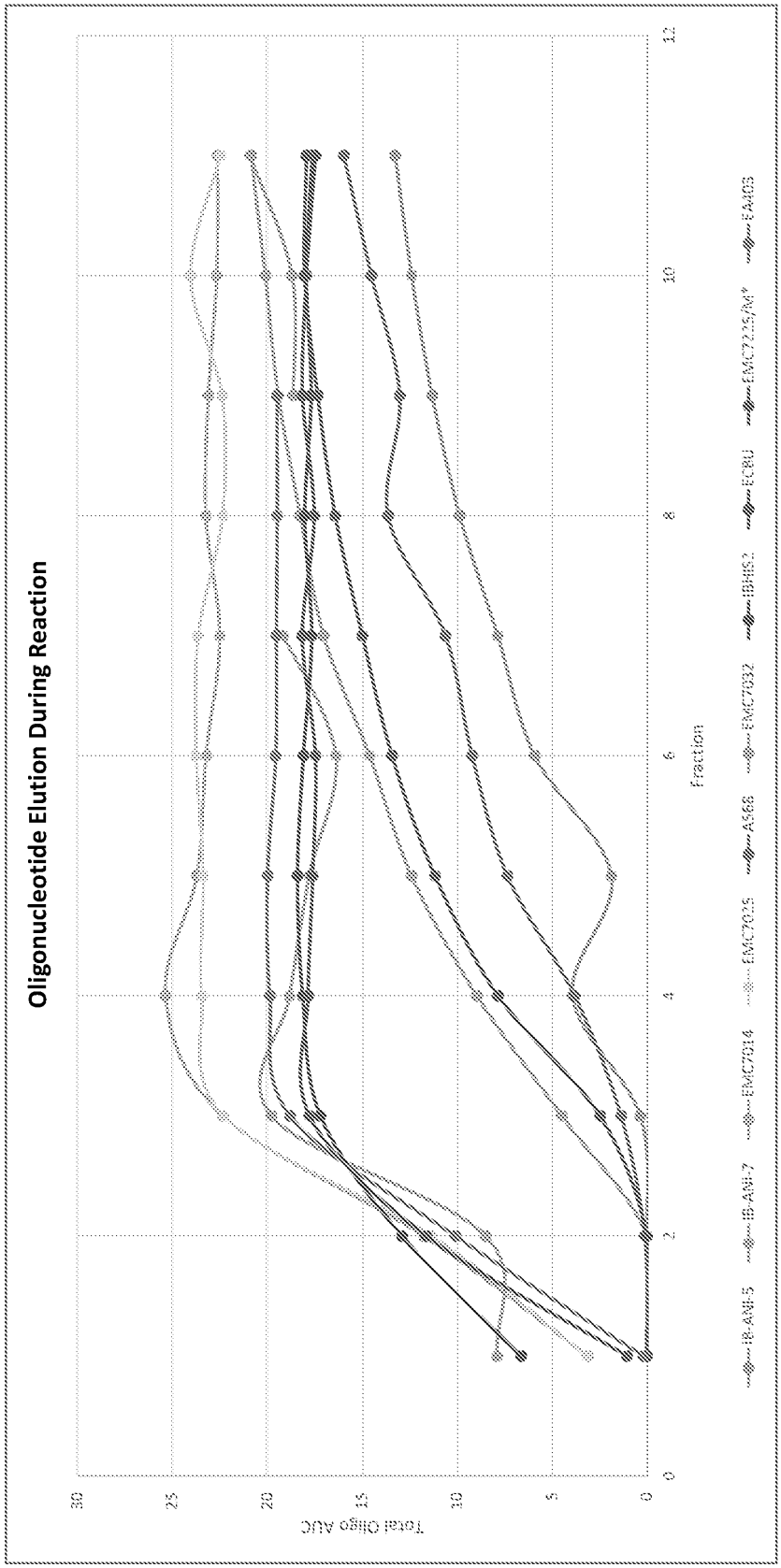

Oligonucleotide Elution During Reaction
Total Oligo AUC
Fraction
0
5
10
15
20
25
30
0
2
4
6
8
10
12
IB-AM15
IB-AM-7
EMC7014
EMC7025
A566
EMC7032
IBHIS2
ECBU
EMC7225/M*
EAM05

FIG. 5

		Resin	Mass (mg)	Vol Mock Soln (uL)	A280	% Retention
IEX	1	A568	155	517	0.294	97%
Epoxy	2	EMC7032/S	156	520	1.082	91%
	3	EMC7025/S	156	520	0.797	93%
	4	EMC7014/S	157	523	0.517	96%
	5	IB-COV-7	160	533	3.299	71%
Amine	6	EMC7225/M	146	487	0.267	98%
	7	IB-ANI-7	150	500	0.245	98%
Affinity	8	IB-HIS-2	154	513	6.998	39%
	9	-	-	500	11.562	

Tests with EA403 (using just glyoxal as crosslinker) show it retains oligo at similar levels to IB-COV-7

FIG. 6

COOH Activation Chemistry

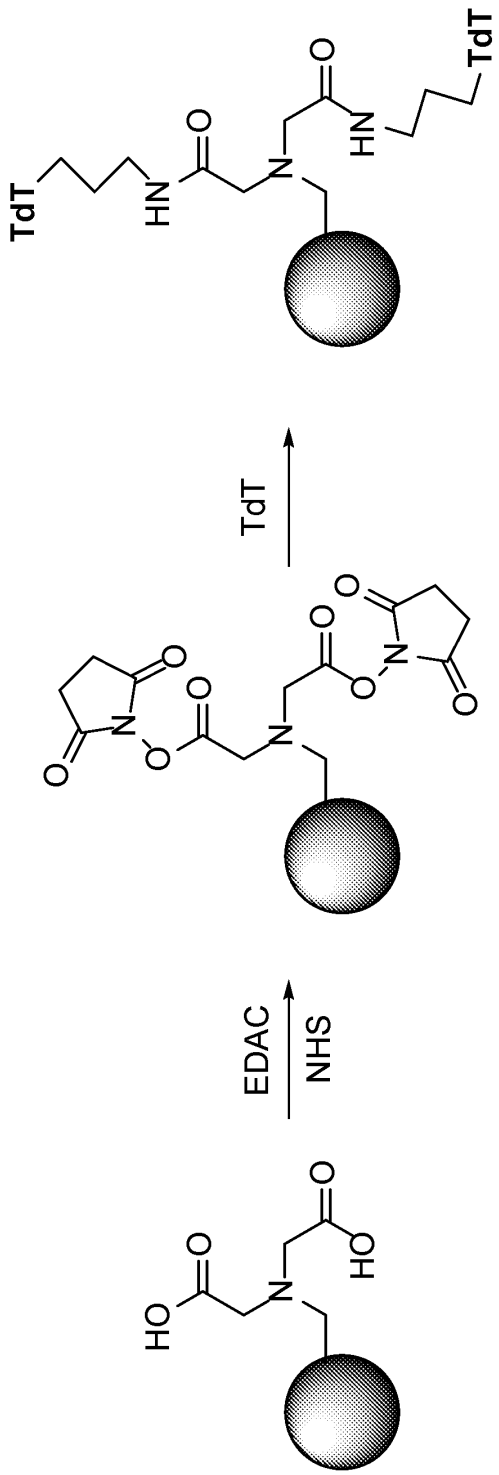


FIG. 7

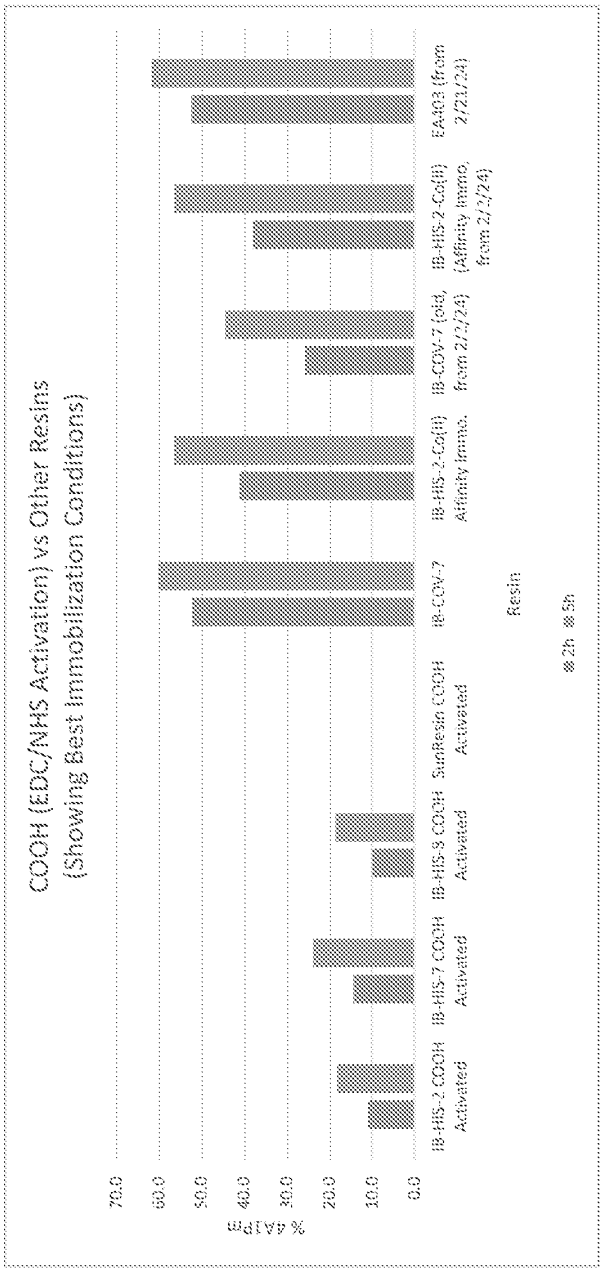

COOH (EDC/NHS Activation) vs Other Resins
(Showing Best Immobilization Conditions)
% 4A1PM
75.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
IB-HIS-2 COOH Activated
IB-HIS-7 COOH Activated
IB-HIS-8 COOH Activated
SunResin COOH Activated
IB-COV-7
IB-HIS-2-Co(II) Affinity Imma.
IB-COV-7 (old, from 2/2/24)
IB-HIS-2-Co(II) (Affinity Imma. from 2/2/24)
EA-303 (from 2/21/24)
Resin
2h
5h

FIG. 8

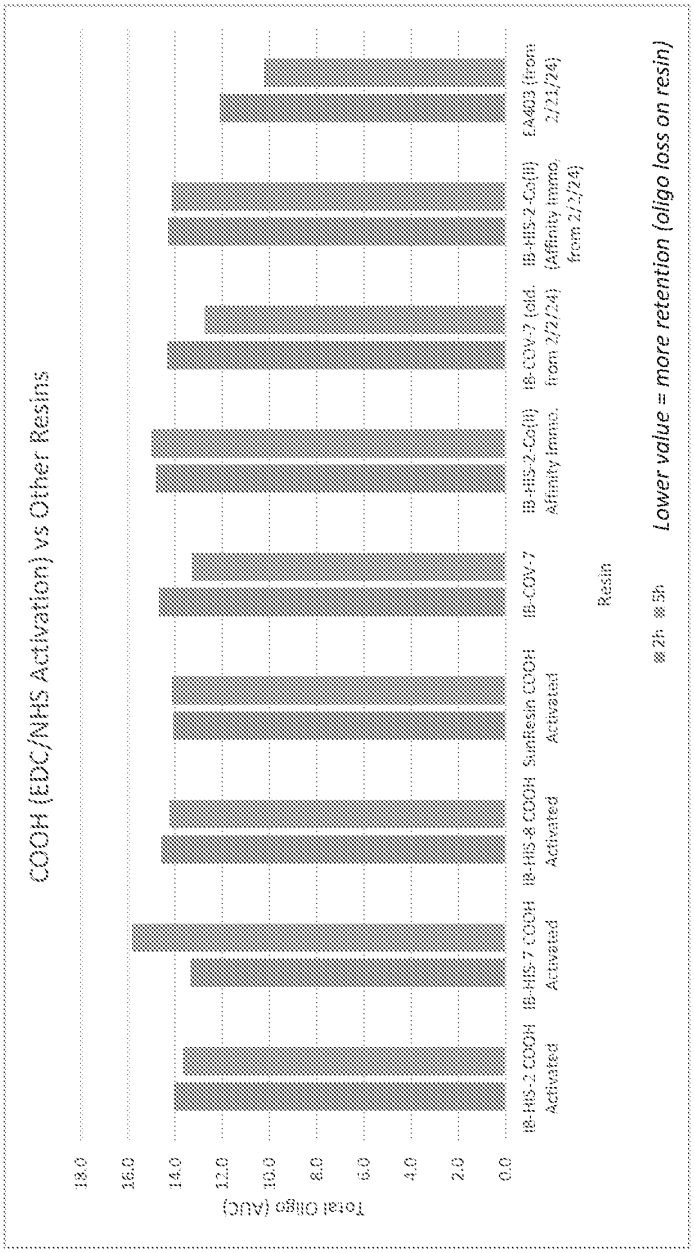

COOH (EDC/NHS Activation) vs Other Resins
Total Oligo (AUC)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
IB-HIS-2 COOH Activated
IB-HIS-7 COOH Activated
IB-HIS-8 COOH Activated
SunResin COOH Activated
IB-COV-7
IB-HIS-2-Co(II) Affinity immo.
IB-COV-7 (old, from 2/2/24)
IB-HIS-2-Co(II) (Affinity immo. from 2/2/24)
EA403 (from 2/21/24)
Resin
2h
5h
Lower value = more retention (oligo loss on resin)

FIG. 9

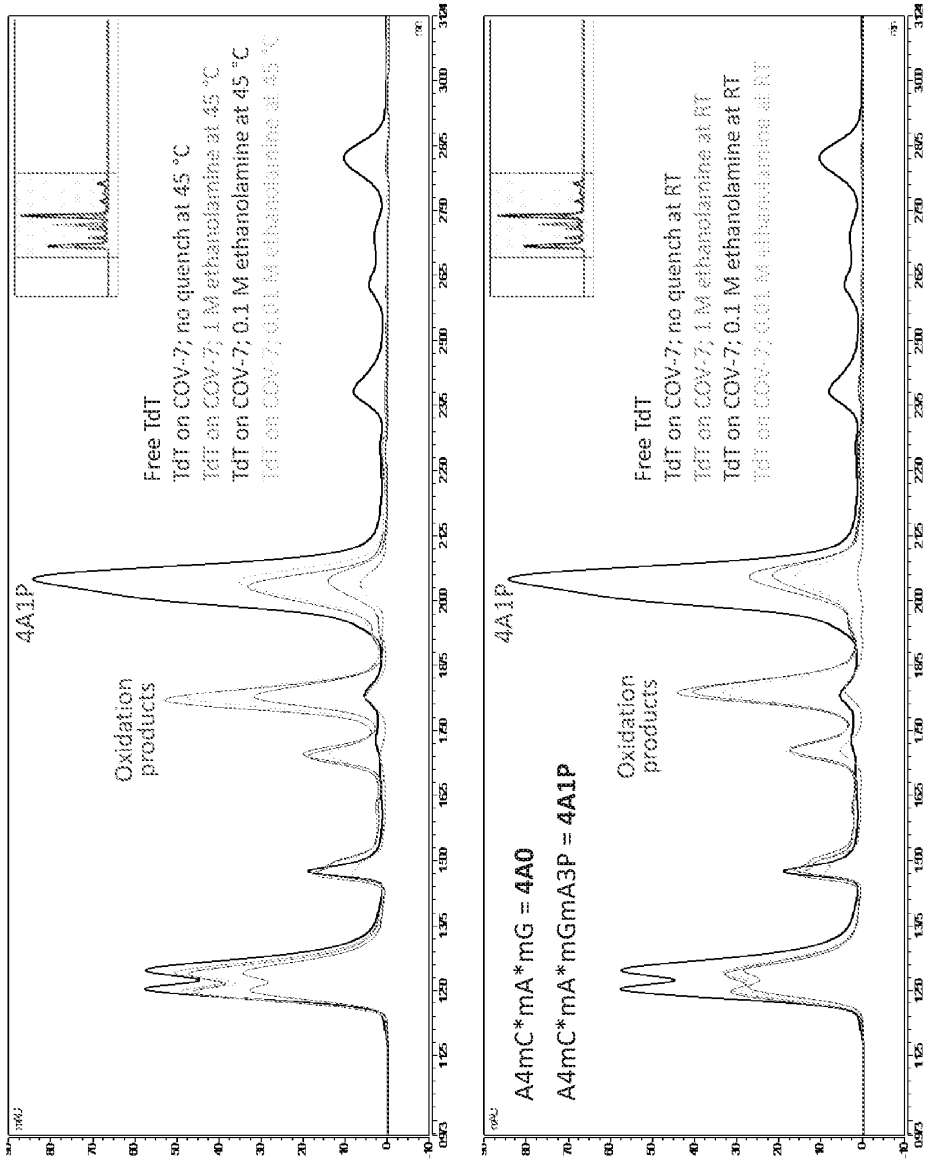

4A1P
Oxidation products
Free TdT
TdT on COV-7; no quench at 45 °C
TdT on COV-7; 1 M ethanolamine at 45 °C
TdT on COV-7; 0.1 M ethanolamine at 45 °C
TdT on COV-7; 0.01 M ethanolamine at 45 °C
4A1P
Oxidation products
A4mC*mA*mG = 4A0
A4mC*mA*mGmA3P = 4A1P
Free TdT
TdT on COV-7; no quench at RT
TdT on COV-7; 1 M ethanolamine at RT
TdT on COV-7; 0.1 M ethanolamine at RT
TdT on COV-7; 0.01 M ethanolamine at RT

FIG. 10

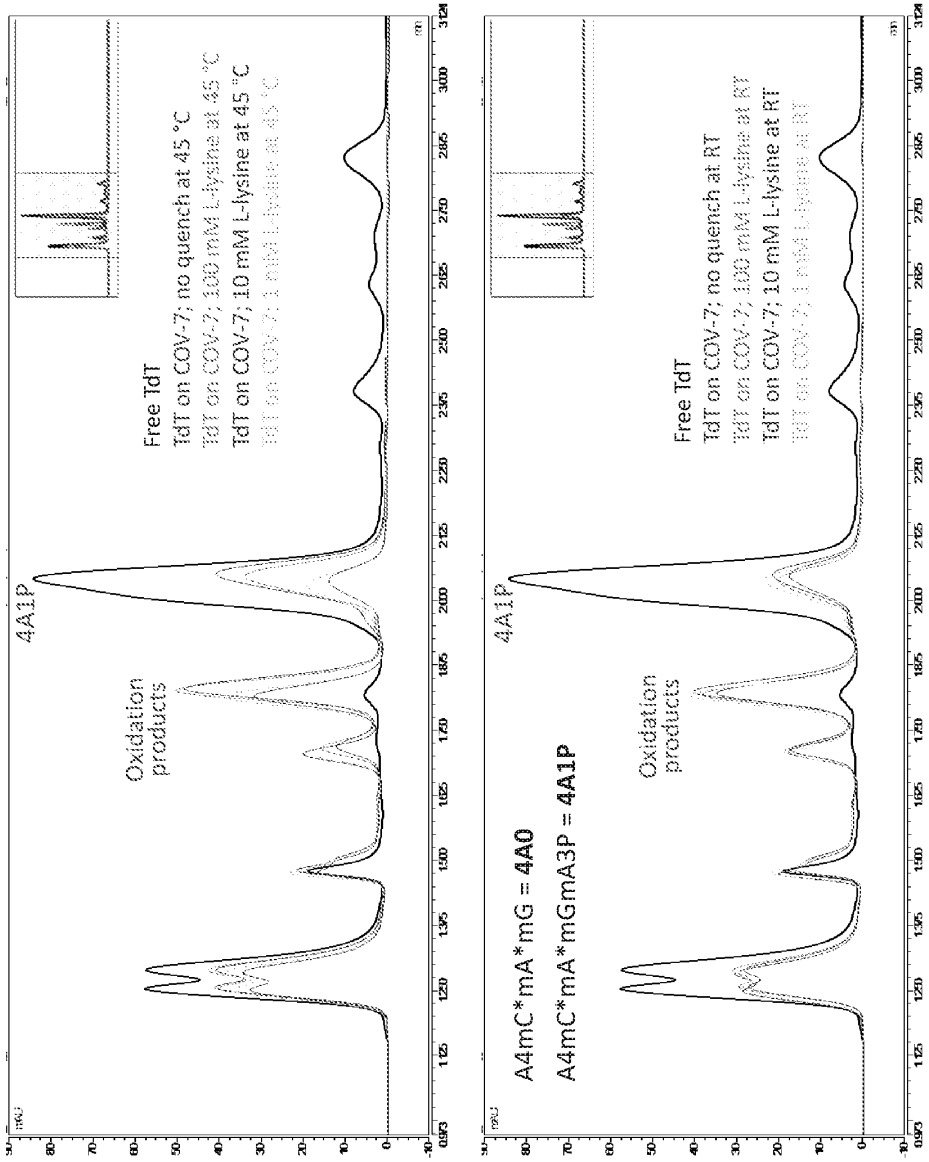

4A1P
Oxidation products
Free TdT
TdT on COV-7; no quench at 45 °C
TdT on COV-7; 100 mM L-lysine at 45 °C
TdT on COV-7; 10 mM L-lysine at 45 °C
4A1P
Oxidation products
A4mC*mA*mG = 4A0
A4mC*mA*mGmA3P = 4A1P
Free TdT
TdT on COV-7; no quench at RT
TdT on COV-7; 100 mM L-lysine at RT
TdT on COV-7; 10 mM L-lysine at RT

FIG. 11

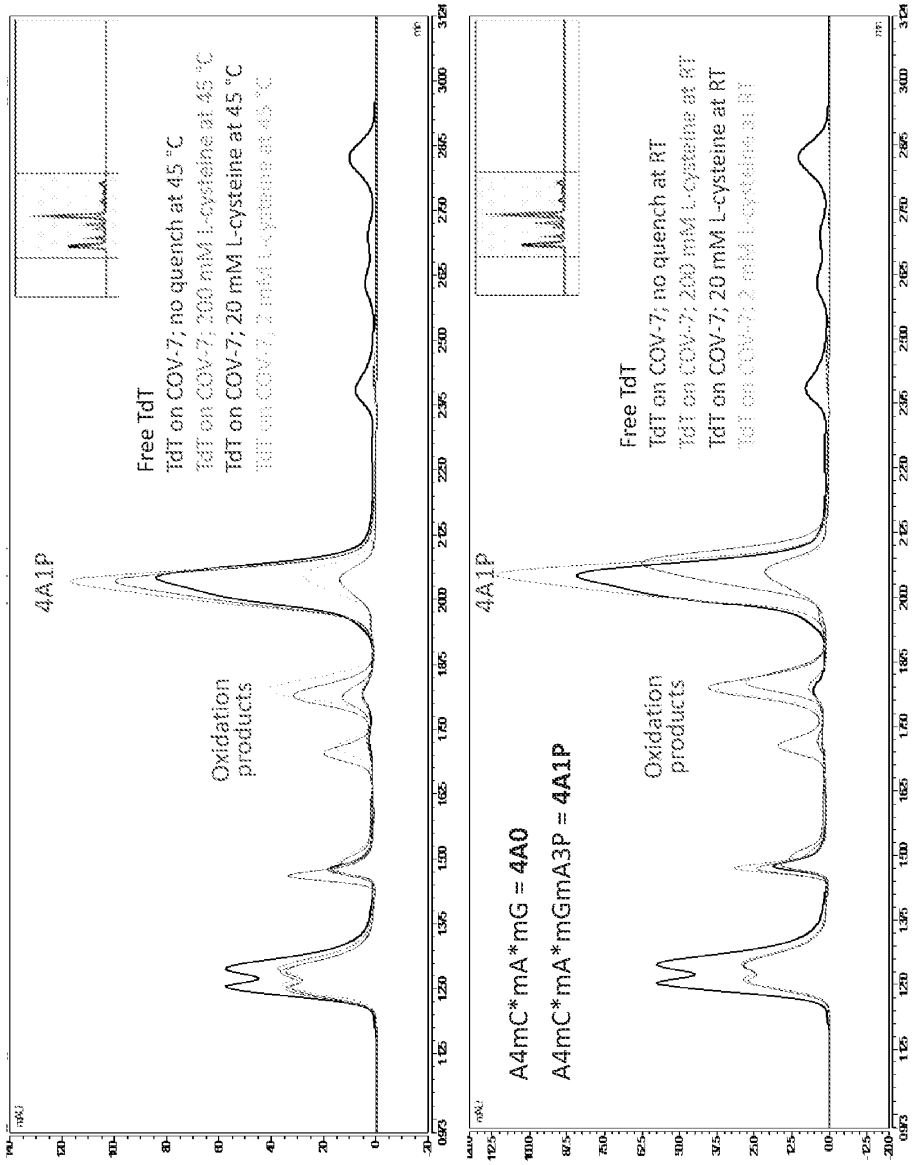

4A1P
Free TdT
TdT on COV-7; no quench at 45 °C
TdT on COV-7; 200 mM L-cysteine at 45 °C
TdT on COV-7; 20 mM L-cysteine at 45 °C
Oxidation products
4A1P
A4mC*mA*mG = 4A0
A4mC*mA*mGmA3P = 4A1P
Free TdT
TdT on COV-7; no quench at RT
TdT on COV-7; 200 mM L-cysteine at RT
TdT on COV-7; 20 mM L-cysteine at RT
Oxidation products

FIG. 12

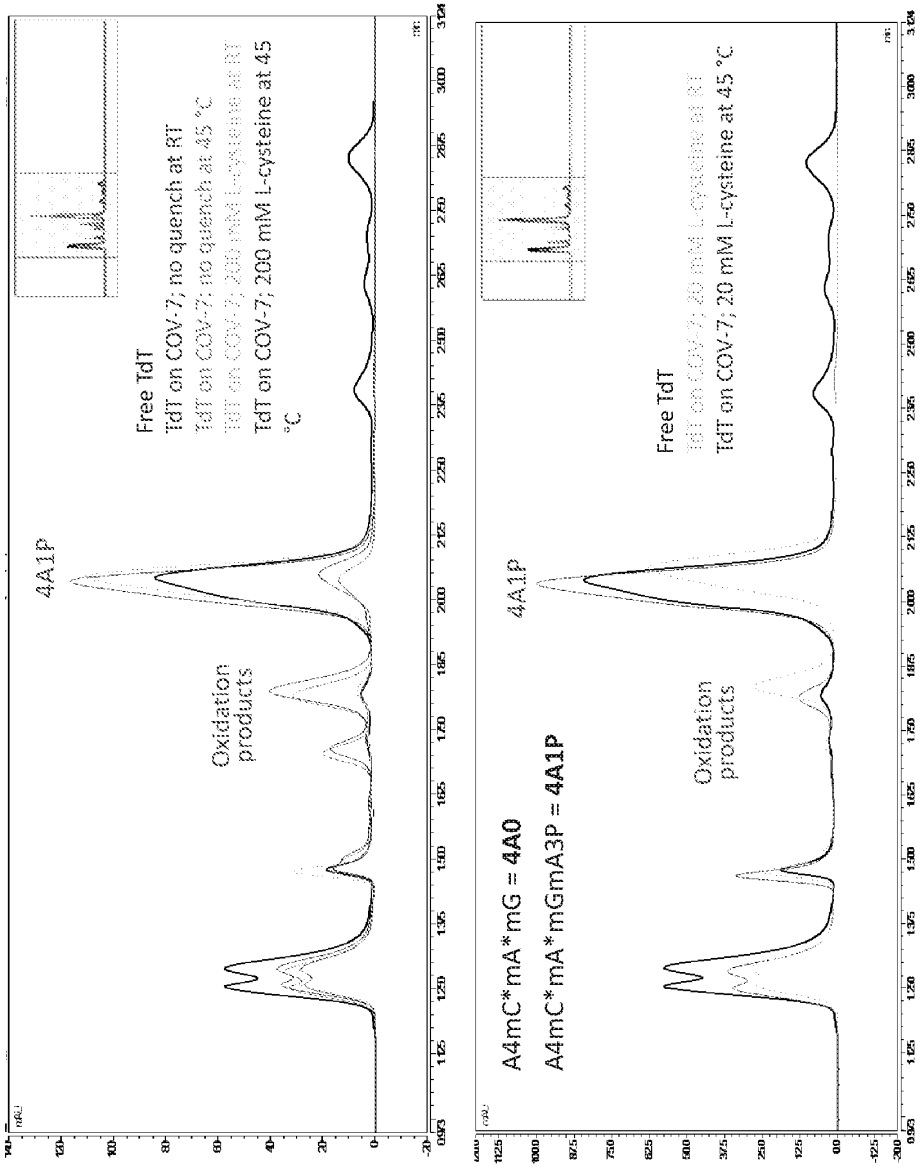

4A1P
Free TdT
TdT on COV-7; no quench at RT
TdT on COV-7; no quench at 45 °C
TdT on COV-7; 200 mM L-cysteine at RT
TdT on COV-7; 200 mM L-cysteine at 45 °C
Oxidation products
A4mC*mA*mG = 4A0
A4mC*mA*mGmA3P = 4A1P
4A1P
Free TdT
TdT on COV-7; 20 mM L-cysteine at RT
TdT on COV-7; 20 mM L-cysteine at 45 °C
Oxidation products

FIG. 13A

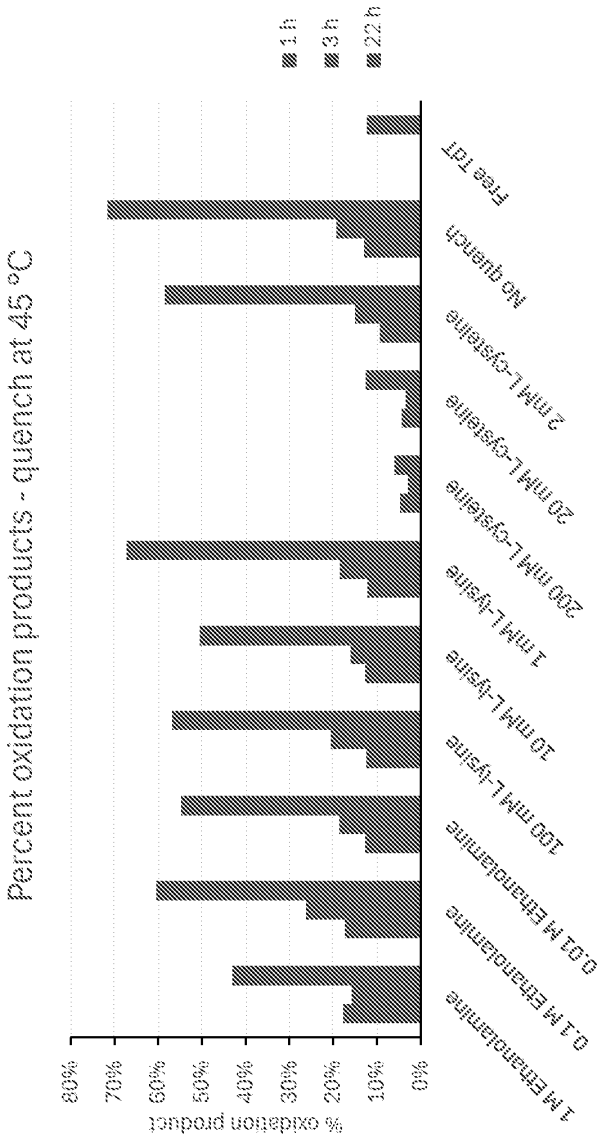

Percent oxidation products - quench at 45 °C
1 h
3 h
22 h
% oxidation product
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 M Ethanolamine
0.1 M Ethanolamine
0.01 M Ethanolamine
100 mM L-lysine
10 mM L-lysine
1 mM L-lysine
200 mM L-cysteine
20 mM L-cysteine
2 mM L-cysteine
No quench
Free tdT

FIG. 13B

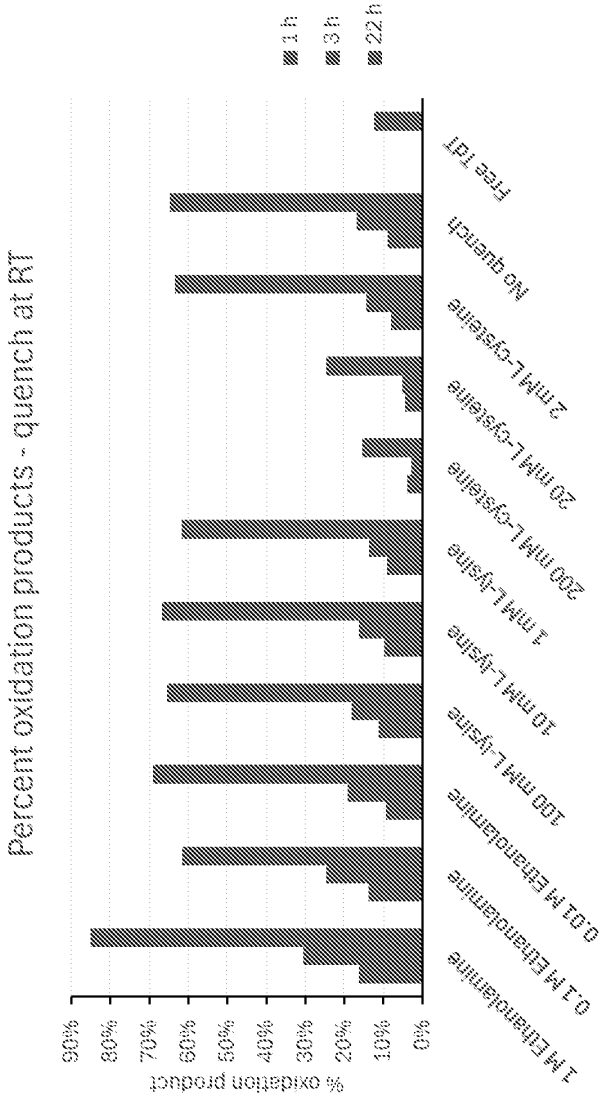
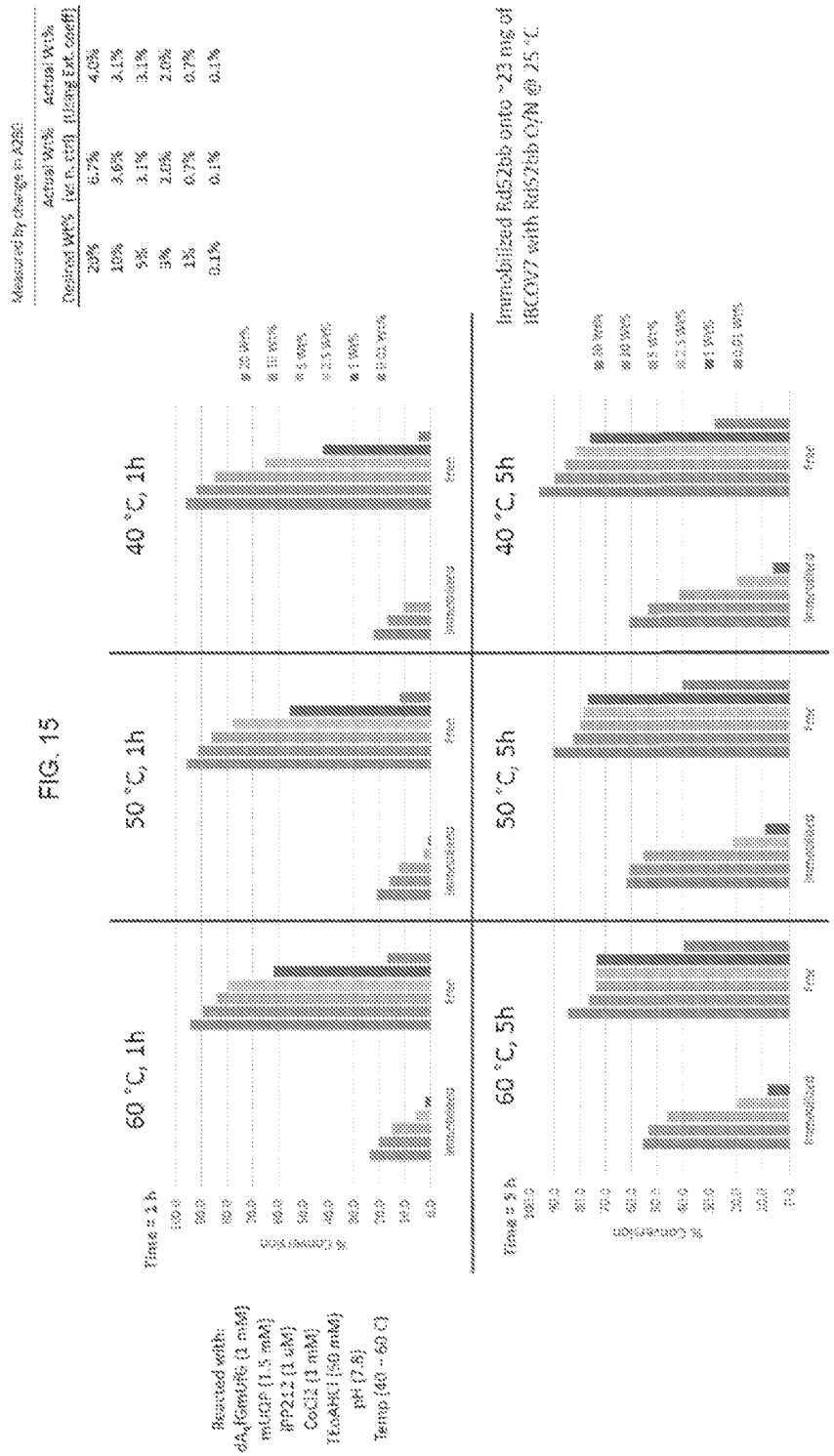

Percent oxidation products - quench at RT
% oxidation product
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 h
3 h
22 h
1M Ethanolamine
0.1 M Ethanolamine
0.01 M Ethanolamine
100 mM L-lysine
10 mM L-lysine
1 mM L-lysine
200 mM L-cysteine
20 mM L-cysteine
2 mM L-cysteine
No quench
Free Tdt

FIG. 14

Name	Activating Agent	Support	Crosslinker	Immobilization Density	Pore Size (Å)	Bead Size (µm)
IB-COV-7	Epoxy	ChiralVision / Sunresin	Glutaraldehyde + Glyoxal	97 umole/g	~500	300 - 600
EMC7225/M	Primary Amine (C2 linker)	Sunresin		880 umole/g	500 - 700	300 - 700
EA403/M	Primary Amine (C2 linker)	Resindion (Relizyme)	Glyoxal	500 umole/g	400 - 600	200 - 500

FIG. 15


Measured by change in A280
Desired Wt% | Actual Wt% (w.r.t. ctrl) | Actual Wt% (Using Ext. coeff)
20% 6.7% 4.0%
10% 3.6% 3.1%
5% 3.1% 3.1%
3% 2.0% 2.0%
1% 0.7% 0.7%
0.1% 0.1% 0.1%
Immobilized Rd52bb onto ~23 mg of IBCOV7 with Rd52bb O/N @ 25 °C
60 °C, 1h
50 °C, 1h
40 °C, 1h
60 °C, 5h
50 °C, 5h
40 °C, 5h
Time = 1 h
Time = 5 h
% Conversion
Immobilized
Free
Reacted with:
dA4/GmUfG (1 mM)
mUQP (1.5 mM)
IPP212 (1 uM)
CoCl2 (1 mM)
TEoAHCl (50 mM)
pH (7.8)
Temp (40 – 60 C)