

	ECL					
	TX100			PEG18TDE		
	Amine	BTI	FT	D100	BTI	FT
tBDEA	21392	3288	49	15089	2359	59
MDEA	68779	8346	63	33035	5473	70
EDEA	122422	46940	105	117014	43538	83
BDEA	148197	43948	93	154964	41687	112
DBA-BS	72635	164340	12718	13287	22076	1737
BEA-BS	77341	28776	197	15385	6930	67
DEA-BS	68844	14328	86	53359	14333	100
DBA-PS	87009	120247	316	71404	59261	167
DEA-PS	60096	9516	88	61135	12407	119
DEAE	94857	54448	49	14972	9202	40
DBAE	96864	36114	108	78598	24713	124
TBA	190813	227059	632	201228	231329	636
TEA	42711	4238	79	49550	4701	125
PIPES	184192	38699	51	1798	248	46

FIG. 1A

	TX100			PEG18TDE		
	Amine	BTI/FT	S/B	Amine	BTI/FT	S/B
tBDEA		6.5	434	tBDEA	6.4	257
MDEA		8.2	1092	MDEA	6.0	474
EDEA		2.6	1170	EDEA	2.7	1410
BDEA		3.4	1594	BDEA	3.7	1379
DBA-BS		0.4	6	DBA-BS	0.6	8
BEA-BS		2.7	392	BEA-BS	2.2	228
DEA-BS		4.8	804	DEA-BS	3.7	532
DBA-PS		0.7	275	DBA-PS	1.2	428
DEA-PS		6.3	686	DEA-PS	4.9	515
DEAE		1.7	1936	DEAE	1.6	377
DBAE		2.7	900	DBAE	3.2	634
TBA		0.8	302	TBA	0.9	316
TEA		10.1	541	TEA	10.5	396
PIPES		4.8	3612	PIPES	7.3	39

FIG. 1B

Co-reactant	ECL			STDEV		
	BTI	D100	500nm FT	BTI	D100	500nm FT
1600 mM TEA	164300	120	10324	4151	4	239
1200 mM TEA	139369	83	7987	4753	3	63
800 mM TEA	98989	51	6183	4300	4	62
400 mM TEA	57782	28	4747	7692	5	676
200mM TEA	33260	20	3857	347	4	30
100 mM TEA	14509	15	2552	130	3	39
50 mM TEA	5429	14	1530	161	4	14

FIG.2B

Co-reactant	BTI/FT	S/B	BTI ECL vs PIPES+TX100
1600 mM TEA	15.9	1365	89%
1200 mM TEA	17.4	1683	76%
800 mM TEA	16.0	1954	54%
400 mM TEA	12.2	2047	31%
200mM TEA	8.6	1663	18%
100 mM TEA	5.7	967	8%
50 mM TEA	3.5	379	3%
PIPES, TX100	4.8		100%

FIG.2C

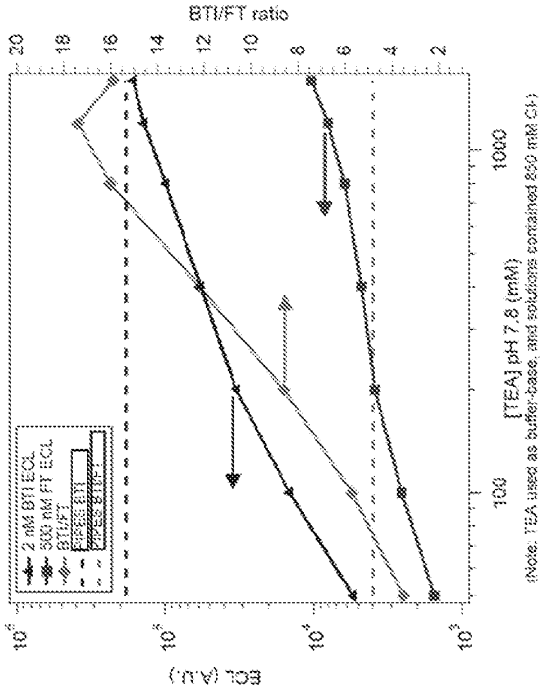


FIG.2A

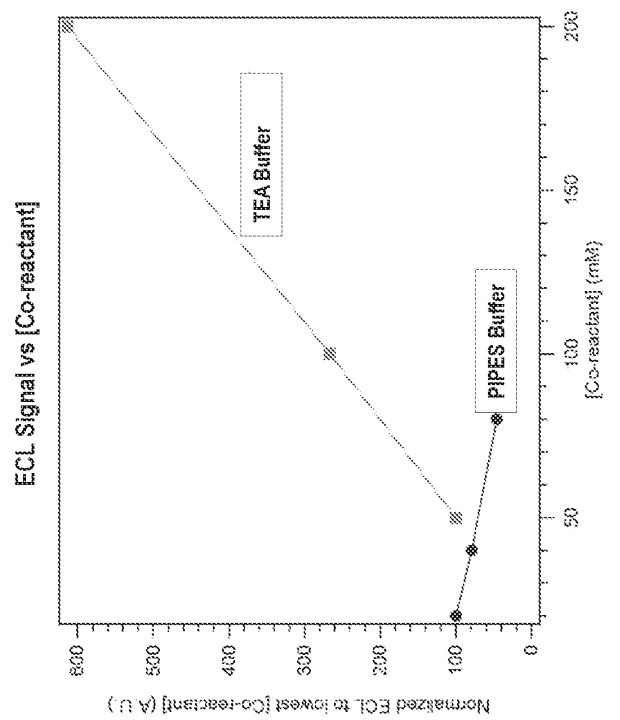


FIG. 3A

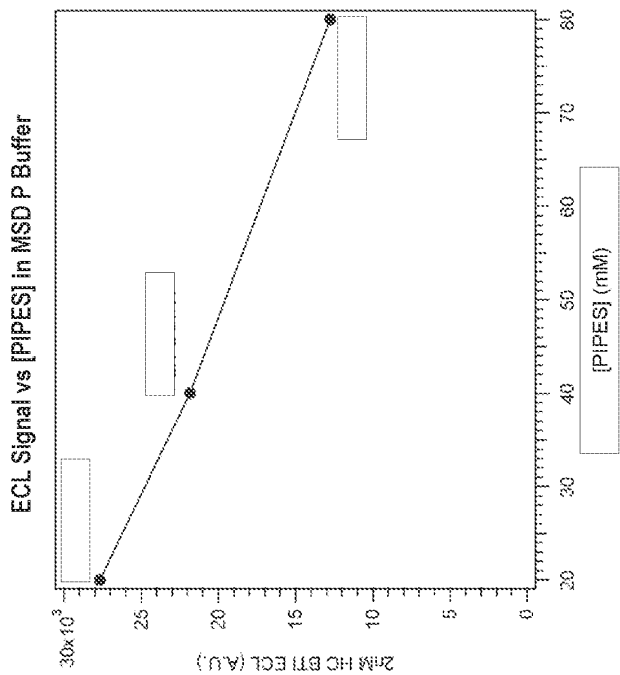


FIG. 3B

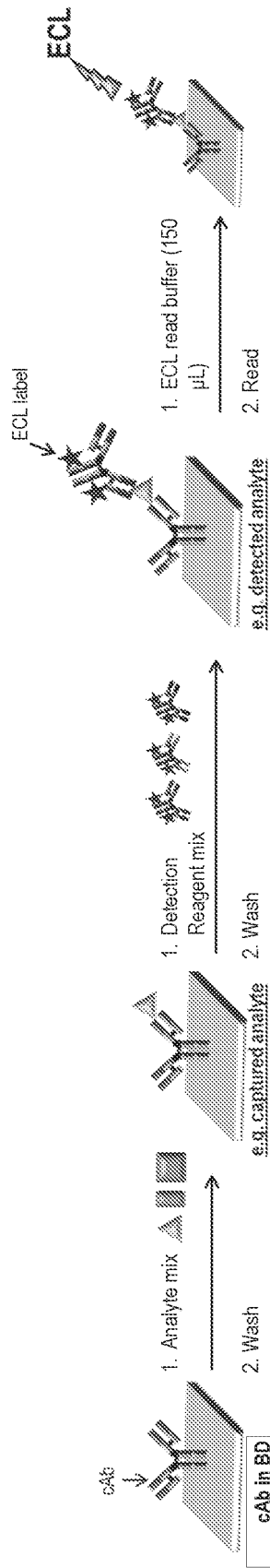


FIG. 4A

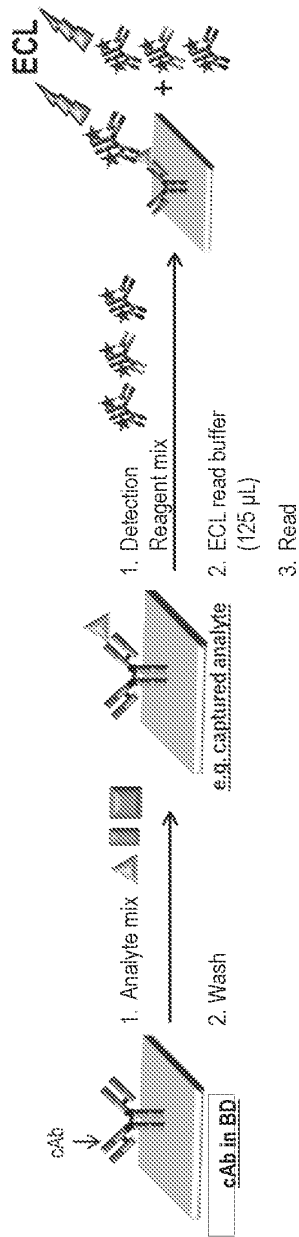


FIG. 4B

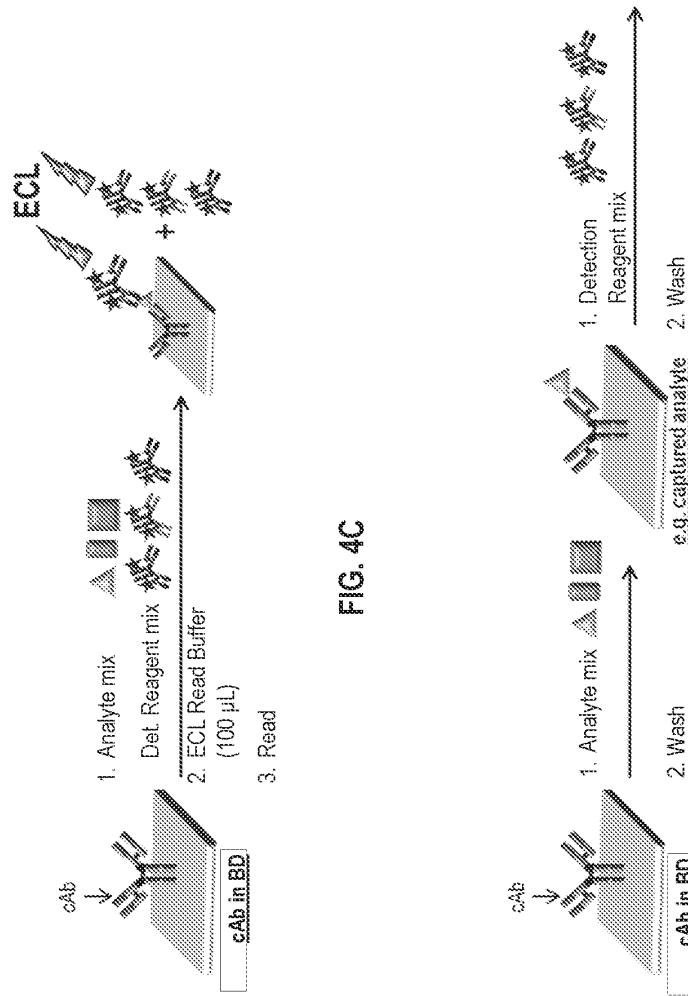


FIG. 4C

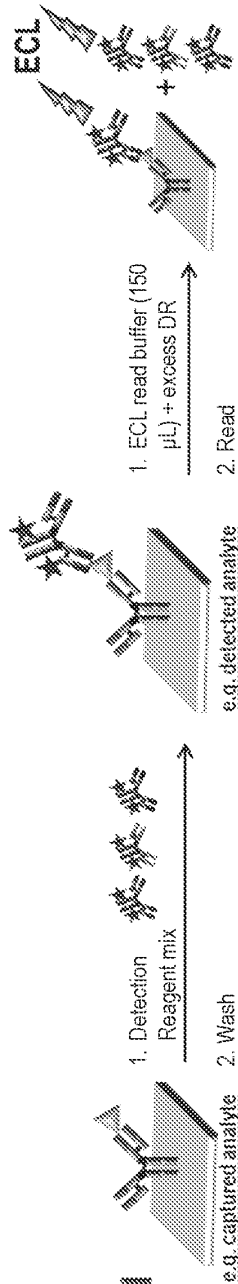


FIG. 4D

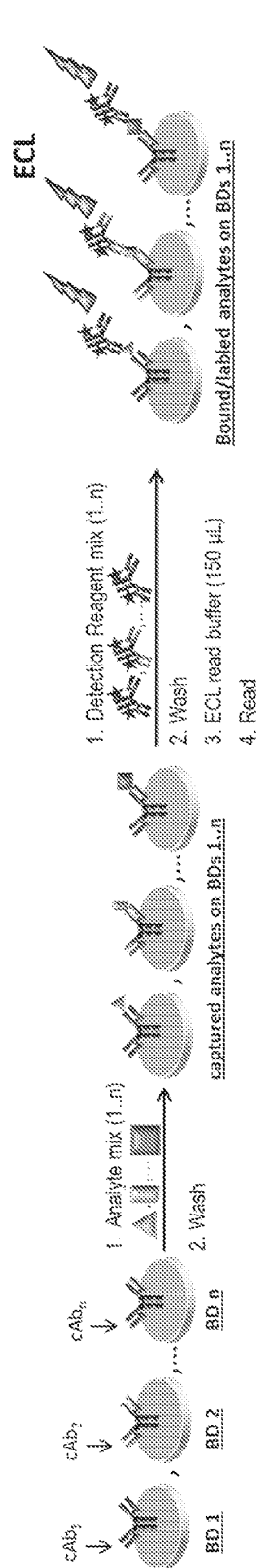


FIG. 4E

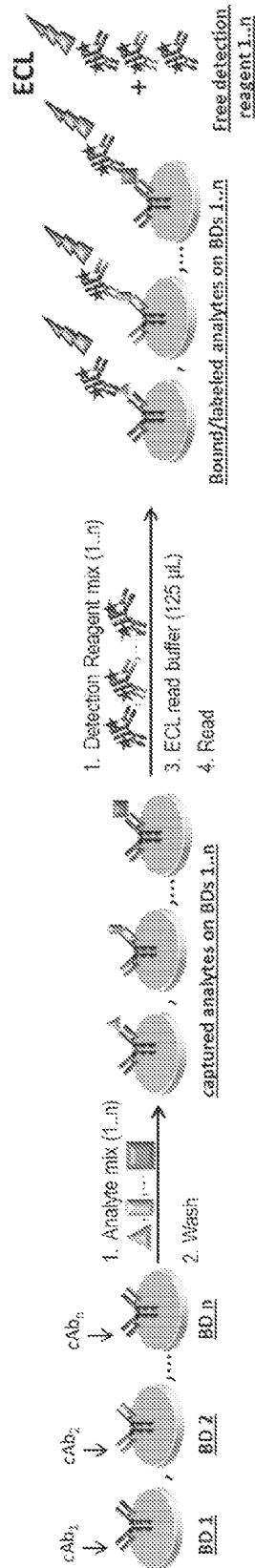


FIG. 4F

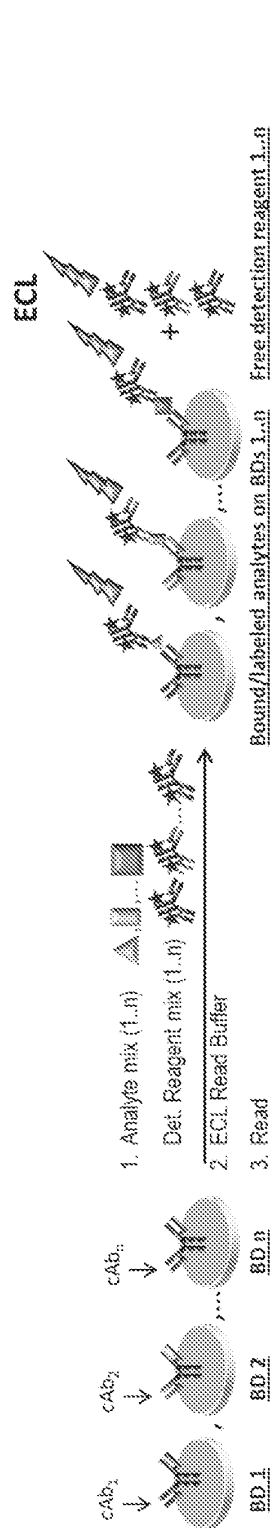


FIG. 4G

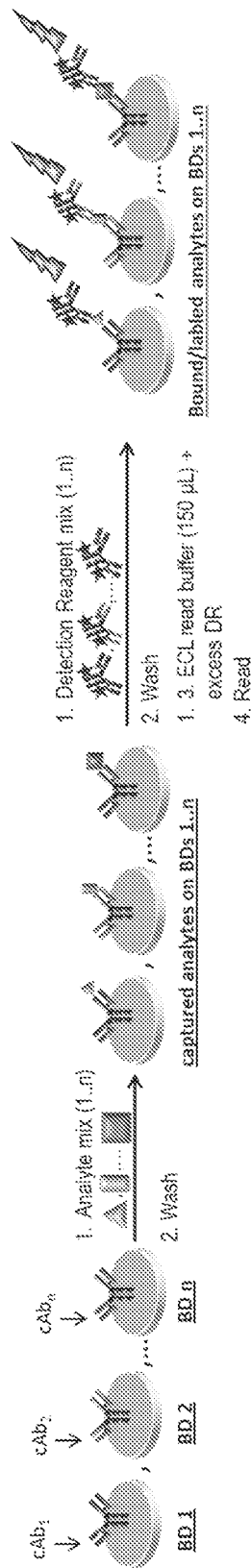


FIG. 4H

	Assay	Standard 2-Step				1 Step				Mock ECL label			
		TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA
ECL	IFN- γ	10,414	10,527	1,703	5,214	25,457	11,949	1,379	5,664	85,673	70,777	4,691	12,471
	IL-1 β	75,574	64,396	12,857	32,685	71,309	51,221	6,624	33,184	161,965	130,693	15,311	43,668
	IL-2	90,866	88,271	19,983	33,634	75,501	68,319	12,133	36,617	172,589	154,141	23,684	44,091
	IL-4	61,019	65,406	14,087	32,655	55,938	52,129	8,035	32,565	136,226	125,887	18,690	42,065
	IL-6	98,891	89,481	17,475	46,365	84,829	71,017	9,258	45,882	179,342	153,444	23,144	51,973
	IL-8	43,563	42,067	9,834	21,054	61,099	40,792	6,139	23,405	127,180	107,491	15,537	29,064
	IL-10	53,009	56,204	10,255	26,201	58,624	46,839	5,805	28,357	130,383	116,538	14,152	33,791
	IL12p70	43,580	43,700	7,624	20,426	48,426	37,091	3,974	21,504	113,482	101,841	10,743	27,729
	IL-13	30,578	42,708	8,162	19,438	50,140	40,839	4,191	22,929	110,400	109,417	12,658	28,254
	TNF- α	23,705	21,397	5,066	11,532	43,698	23,474	3,192	13,604	97,602	83,672	8,668	19,388
NSB ECL	Average	53,120	52,416	10,705	24,920	57,502	44,367	6,073	26,371	131,484	115,390	14,728	33,250
	IFN- γ	249	379	190	287	18,921	4,092	524	437	73,660	59,417	3,394	7,021
	IL-1 β	652	784	390	402	21,780	3,910	1,158	637	80,780	64,604	3,847	7,624
	IL-2	438	429	373	284	22,100	4,431	1,053	684	82,537	63,978	5,414	7,417
	IL-4	135	134	82	155	23,981	4,726	683	568	73,478	59,815	4,486	6,738
	IL-6	160	145	82	162	24,289	4,794	650	754	76,258	62,046	3,398	7,170
	IL-8	168	153	106	178	31,727	10,030	1,082	1,856	81,793	63,216	5,298	7,317
	IL-10	226	254	154	212	25,097	5,614	797	1,282	79,671	60,323	4,756	7,196
	IL12p70	242	342	159	253	25,501	6,673	602	1,511	69,129	57,119	3,403	6,606
	IL-13	151	146	132	164	29,449	9,829	861	1,993	75,767	61,766	4,343	6,891
	TNF- α	190	226	124	181	22,570	7,827	621	2,004	72,283	58,958	3,818	6,673
	Average	261	299	179	228	24,542	6,193	803	1,173	76,536	61,124	4,216	7,065

FIG.5A

	Assay	Standard 2-Step				1 Step				Mock ECL label			
		TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA
LLOD (pg/ml)	IFN- γ	0.421	0.445	2.503	0.564	34.928	27.696	8.194	1.051	29.636	16.422	46.400	8.216
	IL-1 β	0.044	0.147	0.271	0.045	0.104	0.210	8.751	0.154	1.975	1.493	1.346	0.641
	IL-2	0.076	0.069	0.458	0.187	2.776	0.095	1.588	0.314	7.368	1.297	7.480	1.247
	IL-4	0.013	0.011	0.030	0.013	1.139	0.326	0.160	0.032	0.872	0.307	0.648	0.216
	IL-6	0.030	0.028	0.081	0.036	4.822	0.012	0.862	0.161	1.330	0.809	3.389	0.473
	IL-8	0.036	0.042	0.196	0.076	5.404	3.074	0.933	1.324	3.552	0.871	2.678	0.351
	IL-10	0.022	0.030	0.067	0.065	0.888	0.039	0.847	0.178	2.309	0.571	3.362	0.147
	IL12p70	0.044	0.176	0.258	0.185	7.626	2.386	0.944	0.682	2.172	0.884	0.530	0.741
	IL-13	0.129	0.052	0.418	0.157	2.441	4.626	2.140	1.077	5.407	1.434	3.164	1.033
	TNF- α	0.036	0.049	0.160	0.074	5.286	3.379	1.039	1.285	2.647	0.117	2.257	0.467
Average		0.085	0.105	0.444	0.140	6.541	4.184	2.546	0.626	5.727	2.421	7.125	1.353

FIG.5B

Assay	Standard 2-Step					1 Step					Mock ECL label			
	TPA	BDEA	PIPES	1.2 M TEA		TPA	BDEA	PIPES	1.2 M TEA		TPA	BDEA	PIPES	1.2 M TEA
Normalized ECL to BDEA A 2-Step Assay	IFN- γ	1.00	1.01	0.16	0.50	2.44	1.15	0.13	0.54		8.23	6.80	0.45	1.20
	IL-1 β	1.00	0.85	0.17	0.43	0.94	0.68	0.09	0.44		2.14	1.73	0.20	0.58
	IL-2	1.00	0.97	0.22	0.37	0.83	0.75	0.13	0.40		1.90	1.70	0.26	0.49
	IL-4	1.00	1.07	0.23	0.54	0.92	0.85	0.13	0.53		2.23	2.06	0.31	0.69
	IL-6	1.00	0.90	0.18	0.47	0.86	0.72	0.09	0.46		1.81	1.55	0.23	0.53
	IL-8	1.00	0.97	0.23	0.48	1.40	0.94	0.14	0.54		2.92	2.47	0.36	0.67
	IL-10	1.00	1.06	0.19	0.49	1.11	0.88	0.11	0.53		2.46	2.20	0.27	0.64
	IL12p70	1.00	1.00	0.17	0.47	1.11	0.85	0.09	0.49		2.60	2.34	0.25	0.64
	IL-13	1.00	1.40	0.27	0.64	1.64	1.34	0.14	0.75		3.61	3.58	0.41	0.92
	TNF- α	1.00	0.90	0.21	0.49	1.84	0.99	0.13	0.57		4.12	3.53	0.37	0.82
Normalized NSB ECL to BDEA A 2-Step Assay	Average	1.00	1.01	0.20	0.49	1.31	0.91	0.12	0.53		3.20	2.79	0.31	0.72
	IFN- γ	1.00	1.52	0.76	1.15	75.99	16.44	2.11	1.75		295.82	238.62	13.63	28.20
	IL-1 β	1.00	1.20	0.60	0.62	33.39	5.99	1.78	0.98		123.83	99.04	5.90	11.69
	IL-2	1.00	0.98	0.85	0.65	50.42	10.11	2.40	1.56		188.30	145.96	12.35	16.92
	IL-4	1.00	0.99	0.60	1.14	177.20	34.92	5.04	4.19		542.94	441.98	33.15	49.79
	IL-6	1.00	0.91	0.51	1.01	151.49	29.90	4.05	4.70		475.62	386.98	21.19	44.72
	IL-8	1.00	0.91	0.63	1.06	189.22	59.82	6.45	11.07		487.83	377.04	31.60	43.64
	IL-10	1.00	1.12	0.68	0.94	111.05	24.84	3.53	5.67		352.53	266.91	21.04	31.84
	IL12p70	1.00	1.42	0.66	1.05	105.52	27.61	2.49	6.25		286.05	236.35	14.08	27.34
	IL-13	1.00	0.97	0.88	1.09	195.46	65.24	5.71	13.23		502.88	409.95	28.82	45.73
	TNF- α	1.00	1.19	0.65	0.95	118.58	41.12	3.26	10.53		379.77	309.76	20.06	35.06
	Average	1.00	1.12	0.68	0.97	120.83	31.60	3.68	5.99		363.56	291.26	20.18	33.49

FIG.5C

	Assay	Standard 2-Step				1 Step				Mock ECL label			
		TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA
S/B ratios	IFN- γ	42	28	9	18	1.3	2.9	2.6	13	1.2	1.2	1.4	1.8
	IL-1 β	116	82	33	81	3.3	13.1	5.7	52	2.0	2.0	4.0	5.7
	IL-2	207	206	54	119	3.4	15.4	11.5	54	2.1	2.4	4.4	5.9
	IL-4	451	489	172	211	2.3	11.0	11.8	57	1.9	2.1	4.2	6.2
	IL-6	617	616	212	287	3.5	14.8	14.2	61	2.4	2.5	6.8	7.2
	IL-8	260	275	92	118	1.9	4.1	5.7	13	1.6	1.7	2.9	4.0
	IL-10	235	222	67	123	2.3	8.3	7.3	22	1.6	1.9	3.0	4.7
	IL12p70	180	128	48	81	1.9	5.6	6.6	14	1.6	1.8	3.2	4.2
	IL-13	203	293	62	118	1.7	4.2	4.9	12	1.5	1.8	2.9	4.1
	TNF- α	125	95	41	64	1.9	3.0	5.1	7	1.4	1.4	2.3	2.9
S/N	Average	243	243	79	122	2.4	8.2	7.5	30	1.7	1.9	3.5	4.7
	IFN- γ	610	540	147	1,622	21	11	33	269	43	92	16	59
	IL-1 β	2,793	639	424	3,843	68	153	14	461	110	160	108	185
	IL-2	4,780	5,011	655	1,527	48	840	219	668	73	989	46	309
	IL-4	2,712	3,535	2,006	5,019	55	115	168	945	60	198	67	113
	IL-6	7,691	8,933	11,440	30,353	43	742	181	1,005	169	644	46	441
	IL-8	5,122	3,368	587	2,585	30	52	183	125	76	251	61	477
	IL-10	5,223	2,559	2,538	1,175	42	1,539	92	278	80	180	47	4,181
	IL12p70	2,903	685	413	641	24	59	95	209	76	164	105	162
	IL-13	2,648	11,845	598	2,225	62	48	103	372	93	239	72	376
TNF- α		3,560	2,516	440	1,078	16	24	65	58	89	694	63	168
Average		3,804	3,963	1,925	5,007	41	358	115	439	87	361	63	647

FIG.5D

		Standard 2-Step				1 Step				1 Step Non Wash			
	Assay	TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA
ECL	IFN- γ	10,414	10,527	1,703	5,214	25,457	11,949	1,379	5,664	27,487	17,362	308	6,826
	IL-1 β	75,574	64,396	12,857	32,685	71,309	51,221	6,624	33,184	32,015	22,261	391	7,956
	IL-2	90,866	88,271	19,983	33,634	75,501	68,319	12,133	36,617	74,043	99,159	958	49,102
	IL-4	61,019	65,406	14,087	32,655	55,938	52,129	8,035	32,565	28,147	41,527	397	24,144
	IL-6	98,891	89,481	17,475	46,365	84,829	71,017	9,258	45,882	43,250	56,936	491	32,738
	IL-8	43,563	42,067	9,834	21,054	61,099	40,792	6,139	23,405	19,249	13,109	165	3,501
	IL-10	53,009	56,204	10,255	26,201	58,624	46,839	5,805	28,357	26,124	39,616	203	20,592
	IL12p70	43,580	43,700	7,624	20,426	48,426	37,091	3,974	21,504	11,941	33,970	168	18,667
	IL-13	30,578	42,708	8,162	19,438	50,140	40,839	4,191	22,929	27,354	68,976	478	39,878
	TNF- α	23,705	21,397	5,066	11,532	43,698	23,474	3,192	13,604	10,919	14,293	134	4,099
	Average	53,120	52,436	10,705	24,920	57,502	44,367	6,073	26,371	30,053	40,721	369	20,750
NSB ECL	IFN- γ	249	379	190	287	18,921	4,092	524	437	16,567	6,131	153	448
	IL-1 β	652	784	390	402	21,780	3,910	1,158	637	17,366	8,586	308	1,048
	IL-2	438	429	373	284	22,100	4,431	1,053	684	15,819	6,280	153	524
	IL-4	135	134	82	155	23,981	4,726	683	568	13,204	6,505	100	544
	IL-6	160	145	82	162	24,289	4,794	650	754	14,356	6,462	99	742
	IL-8	168	153	106	178	31,727	10,030	1,082	1,856	11,134	7,194	136	821
	IL-10	226	254	154	212	25,097	5,614	797	1,282	9,988	6,341	104	709
	IL12p70	242	342	159	253	25,501	6,673	602	1,511	3,309	6,773	123	1,457
	IL-13	151	146	132	164	29,449	9,829	861	1,993	7,165	8,861	124	1,375
	TNF- α	190	226	124	181	22,570	7,827	621	2,004	8,139	8,139	102	1,558
	Average	261	299	179	228	24,542	6,193	803	1,173	11,705	7,127	140	923

FIG.6A

	Standard 2-Step					1 Step				1 Step Non Wash			
	Assay	TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA	TPA	BDEA	PIPES	1.2 M TEA
LLOD (pg/ml)	IFN- γ	0.421	0.445	2.503	0.564	34.928	27.697	8.194	1.051	6.120	8.085	9.977	0.571
	IL-1 β	0.044	0.147	0.271	0.045	0.104	0.210	8.751	0.154	1.454	0.569	30.265	2.684
	IL-2	0.076	0.069	0.458	0.187	2.776	0.095	1.588	0.314	0.770	0.044	3.061	0.723
	IL-4	0.013	0.011	0.030	0.013	1.139	0.326	0.160	0.032	3.641	0.818	1.248	0.097
	IL-6	0.030	0.028	0.081	0.036	4.822	0.012	0.862	0.161	4.341	0.883	4.573	0.272
	IL-8	0.036	0.042	0.196	0.076	5.404	3.074	0.933	1.324	50.301	15.962	125.791	0.837
	IL-10	0.022	0.030	0.067	0.065	0.888	0.039	0.847	0.178	5.897	0.799	10.920	0.281
	IL12p70	0.044	0.176	0.258	0.185	7.626	2.386	0.944	0.682	0.214	0.348	51.369	0.385
	IL-13	0.129	0.052	0.418	0.157	2.441	4.626	2.140	1.077	14.431	3.265	8.731	3.185
	TNF- α	0.036	0.049	0.160	0.074	5.286	3.379	1.039	1.285	0.532	8.384	230.860	1.124
	Average	0.085	0.105	0.444	0.140	6.541	4.184	2.546	0.626	8.770	3.916	47.679	1.016

FIG.6B

	Assay	Standard 2-Step					1 Step					1 Step Non Wash				
		TPA	BDEA	PIPES	1.2 M TEA		TPA	BDEA	PIPES	1.2 M TEA		TPA	BDEA	PIPES	1.2 M TEA	
Normalized Mid-Cal ECL to 2-Step Assay (Cal 3)	IFN- γ	1.00	1.01	0.16	0.50		2.44	1.15	0.13	0.54		2.64	1.67	0.03	0.66	
	IL-1 β	1.00	0.85	0.17	0.43		0.94	0.68	0.09	0.44		0.42	0.29	0.01	0.11	
	IL-2	1.00	0.97	0.22	0.37		0.83	0.75	0.13	0.40		0.81	1.09	0.01	0.54	
	IL-4	1.00	1.07	0.23	0.54		0.92	0.85	0.13	0.53		0.46	0.68	0.01	0.40	
	IL-6	1.00	0.90	0.18	0.47		0.86	0.72	0.09	0.46		0.44	0.58	0.00	0.33	
	IL-8	1.00	0.97	0.23	0.48		1.40	0.94	0.14	0.54		0.44	0.30	0.00	0.08	
	IL-10	1.00	1.06	0.19	0.49		1.11	0.88	0.11	0.53		0.49	0.75	0.00	0.39	
	IL12p70	1.00	1.00	0.17	0.47		1.11	0.85	0.09	0.49		0.27	0.78	0.00	0.43	
	IL-13	1.00	1.40	0.27	0.64		1.64	1.34	0.14	0.75		0.89	2.26	0.02	1.30	
	TNF- α	1.00	0.90	0.21	0.49		1.84	0.99	0.13	0.57		0.46	0.60	0.01	0.17	
	Average	1.00	1.01	0.20	0.49		1.31	0.91	0.12	0.53		0.73	0.90	0.01	0.44	
Normalized NSB ECL to 2-Step Assay (Cal 3)	IFN- γ	1.00	1.52	0.76	1.15		75.99	16.44	2.11	1.75		66.54	24.62	0.62	1.80	
	IL-1 β	1.00	1.20	0.60	0.62		33.39	5.99	1.78	0.98		26.62	13.16	0.47	1.61	
	IL-2	1.00	0.98	0.85	0.65		50.42	10.11	2.40	1.56		36.09	14.33	0.35	1.17	
	IL-4	1.00	0.99	0.60	1.14		177.20	34.92	5.04	4.19		97.56	48.07	0.74	4.02	
	IL-6	1.00	0.91	0.51	1.01		151.49	29.90	4.05	4.70		89.54	40.30	0.62	4.63	
	IL-8	1.00	0.91	0.63	1.06		189.22	59.82	6.45	11.07		66.41	42.91	0.81	4.90	
	IL-10	1.00	1.12	0.68	0.94		111.05	24.84	3.53	5.67		44.19	28.06	0.46	3.14	
	IL12p70	1.00	1.42	0.66	1.05		105.52	27.61	2.49	6.25		13.69	28.02	0.51	6.03	
	IL-13	1.00	0.97	0.88	1.09		195.46	65.24	5.71	13.23		47.56	58.81	0.83	9.12	
	TNF- α	1.00	1.19	0.65	0.95		118.58	41.12	3.26	10.53		19.42	42.76	0.54	8.18	
	Average	1.00	1.12	0.68	0.97		120.83	31.60	3.68	5.99		50.76	34.10	0.59	4.46	

FIG.6C

Compound
Acetamenophene
Ibuprofen
Naproxen
Ascorbic acid
Salicylic acid
Tolbutamide
EtOH

FIG. 7B

ID Name	Sample
P1	Matched Human Plasma #1
P2	Matched Human Plasma #2
P3	Matched Human Plasma #3
P4	Matched Human Plasma #4
S1	Matched Human Serum #1
S2	Matched Human Serum #2
S3	Matched Human Serum #3
S4	Matched Human Serum #4
FBS1	Fetal Bovine Serum #1
FBS2	Fetal Bovine Serum #2
FBS3	Fetal Bovine Serum #3
BS4	Bovine serum #1
BS5	Bovine serum #2
CS1	Chicken serum #1
CS2	Chicken serum #2
CS3	Chicken serum #3
D43	Diluent 43
D2	Diluent 2
DMEM	DMEM (1X)+GlutaMAX
	FBS
	100x Penicillin Streptomycin
D3	Diluent 3

FIG. 7A

Spike & []	Sample Matrix	ECL Generation			%CV			ECL generation vs No Matrix Control			Absolute Error		
		Free	H2O	H2O+Free	Free	H2O	H2O+Free	Free	H2O	H2O+Free	Free	H2O	H2O+Free
None	Hu Plasma 1	13356	91	N/A	N/A	N/A	N/A	3.6%	0.0%	N/A	N/A	N/A	N/A
	Hu Plasma 2	13954	93	N/A	N/A	N/A	N/A	5.5%	0.0%	N/A	N/A	N/A	N/A
	Hu Plasma 3	13700	89	N/A	N/A	N/A	N/A	1.5%	1.7%	N/A	N/A	N/A	N/A
	Hu Plasma 4	12737	90	N/A	N/A	N/A	N/A	2.8%	2.3%	N/A	N/A	N/A	N/A
	Hu Serum 1	11125	98	N/A	N/A	N/A	N/A	1.8%	1.6%	N/A	N/A	N/A	N/A
	Hu Serum 2	11952	96	N/A	N/A	N/A	N/A	0.9%	1.8%	N/A	N/A	N/A	N/A
	Hu Serum 3	12453	97	N/A	N/A	N/A	N/A	4.0%	3.3%	N/A	N/A	N/A	N/A
	Hu Serum 4	12340	97	N/A	N/A	N/A	N/A	2.6%	0.0%	N/A	N/A	N/A	N/A
	FB51	11929	89	N/A	N/A	N/A	N/A	2.6%	1.7%	N/A	N/A	N/A	N/A
	FB52	12573	87	N/A	N/A	N/A	N/A	1.3%	2.4%	N/A	N/A	N/A	N/A
	FB53	12702	87	N/A	N/A	N/A	N/A	1.2%	2.7%	N/A	N/A	N/A	N/A
	BS4	12390	97	N/A	N/A	N/A	N/A	2.6%	2.1%	N/A	N/A	N/A	N/A
	BS5	12907	93	N/A	N/A	N/A	N/A	0.3%	1.2%	N/A	N/A	N/A	N/A
	CS1	14096	82	N/A	N/A	N/A	N/A	1.1%	3.2%	N/A	N/A	N/A	N/A
	CS2	13596	84	N/A	N/A	N/A	N/A	1.5%	0.7%	N/A	N/A	N/A	N/A
	CS3	13331	82	N/A	N/A	N/A	N/A	1.6%	1.2%	N/A	N/A	N/A	N/A
	D2	12052	96	N/A	N/A	N/A	N/A	0.7%	1.0%	N/A	N/A	N/A	N/A
	D3	13682	95	N/A	N/A	N/A	N/A	1.0%	4.1%	N/A	N/A	N/A	N/A
	D43	11072	94	N/A	N/A	N/A	N/A	2.0%	2.4%	N/A	N/A	N/A	N/A
	DMEM	13237	90	N/A	N/A	N/A	N/A	1.3%	1.4%	N/A	N/A	N/A	N/A
	H2O	N/A	N/A	12396	77	N/A	N/A	N/A	N/A	8.4%	N/A	N/A	2.3%
				Average %		103%		119%					
				STDEV of %ECL		7%		7%					
				Max		114%		127%					
				Min		89%		107%					

FIG. 8A

FIG. 8B

Spike & []	Sample Matrix	ECL Generation				%CV				ECL generation vs EtOH Control				Absolute Error	
		Free	H2O	+Free	H2O	Free	H2O	+Free	H2O	Free	H2O	+Free	H2O	Free	H2O
Acetaminophene (1.03mM)	FB51	9995	90	N/A	N/A	6.1%	2.3%	N/A	N/A	10.1%	90%	N/A	N/A	9.0%	3.4%
	FB52	10682	94	N/A	N/A	4.7%	2.8%	N/A	N/A	11.0%	95%	N/A	N/A	8.0%	3.8%
	BS4	11304	94	N/A	N/A	4.7%	2.8%	N/A	N/A	11.4%	93%	N/A	N/A	9.1%	3.4%
	BS5	12163	92	N/A	N/A	3.4%	2.1%	N/A	N/A	11.5%	91%	N/A	N/A	7.1%	3.7%
	FB51	12331	92	N/A	N/A	4.6%	2.9%	N/A	N/A	12.5%	92%	N/A	N/A	8.2%	3.4%
Ibuprofen (1.06mM)	FB52	12549	94	N/A	N/A	1.3%	2.3%	N/A	N/A	12.9%	95%	N/A	N/A	8.2%	2.9%
	BS4	11892	92	N/A	N/A	3.0%	1.1%	N/A	N/A	12.0%	91%	N/A	N/A	10.4%	3.9%
	BS5	9886	91	N/A	N/A	5.3%	1.1%	N/A	N/A	9.4%	90%	N/A	N/A	6.2%	2.8%
	FB51	10171	96	N/A	N/A	1.9%	1.6%	N/A	N/A	10.3%	97%	N/A	N/A	6.9%	3.2%
	FB52	10989	102	N/A	N/A	4.7%	1.0%	N/A	N/A	11.3%	103%	N/A	N/A	8.3%	3.2%
Naproxen Na+ (2.17mM)	BS4	11999	93	N/A	N/A	3.2%	2.2%	N/A	N/A	12.1%	92%	N/A	N/A	9.5%	3.4%
	BS5	12090	93	N/A	N/A	8.2%	1.1%	N/A	N/A	11.5%	92%	N/A	N/A	10.4%	2.9%
	FB51	12408	96	N/A	N/A	4.0%	1.0%	N/A	N/A	12.5%	96%	N/A	N/A	9.5%	3.0%
	FB52	11783	92	N/A	N/A	4.5%	2.3%	N/A	N/A	12.1%	93%	N/A	N/A	8.7%	3.4%
	BS4	8746	96	N/A	N/A	0.9%	2.8%	N/A	N/A	8.5%	95%	N/A	N/A	6.4%	3.8%
Sodium ascorbate (0.34mM)	BS5	9200	97	N/A	N/A	1.1%	1.8%	N/A	N/A	8.7%	93%	N/A	N/A	6.4%	3.8%
	FB51	9793	106	N/A	N/A	1.3%	1.1%	N/A	N/A	9.9%	106%	N/A	N/A	6.5%	3.3%
	FB52	10220	99	N/A	N/A	2.2%	3.8%	N/A	N/A	10.5%	100%	N/A	N/A	6.3%	4.8%
	BS4	10174	97	N/A	N/A	3.1%	2.1%	N/A	N/A	10.3%	96%	N/A	N/A	8.0%	3.5%
	BS5	10580	103	N/A	N/A	3.7%	1.5%	N/A	N/A	10.0%	102%	N/A	N/A	5.4%	3.3%
Salicylic acid (4.34mM)	FB51	11025	97	N/A	N/A	1.5%	2.2%	N/A	N/A	9.7%	98%	N/A	N/A	8.2%	4.2%
	FB52	9400	97	N/A	N/A	6.4%	3.1%	N/A	N/A	9.9%	97%	N/A	N/A	9.9%	3.3%
	BS4	9858	98	N/A	N/A	6.9%	1.8%	N/A	N/A	9.4%	104%	N/A	N/A	3.8%	4.3%
	BS5	9951	105	N/A	N/A	0.4%	2.9%	N/A	N/A	10.0%	100%	N/A	N/A	9.1%	3.0%
	FB51	9900	100	N/A	N/A	6.5%	0.6%	N/A	N/A	10.0%	100%	N/A	N/A	10.2%	4.6%
Tolbutamine (2.4mM)	FB52	9719	99	N/A	N/A	5.6%	1.5%	N/A	N/A	10.0%	100%	N/A	N/A	5.7%	3.1%
	BS4	9913	101	N/A	N/A	7.2%	3.6%	N/A	N/A	N/A	100%	N/A	N/A	N/A	N/A
	BS5	10555	101	N/A	N/A	4.0%	1.1%	N/A	N/A	N/A	100%	N/A	N/A	N/A	N/A
	H2O	N/A	N/A	11916	79	N/A	N/A	N/A	N/A	N/A	N/A	100%	N/A	N/A	N/A
	H2O	N/A	N/A	11581	77	N/A	N/A	N/A	N/A	N/A	N/A	97%	98%	N/A	N/A
EtOH (5 %v/v)															
H2O															
		Average %		Free		107%		96%							
		STDEV of %ECL		17%		4%									
		Max		129%		106%									
		Min		87%		90%									

FIG. 9B

FIG. 9A

Read Buffer vol.	Spike & []	Sample Matrix	ECL Generation		%CV
			D3+STAG	H2O+STAG	
150 ul	None	Hu Plasma 1	235	N/A	5.9%
		Hu Plasma 2	231	N/A	11.0%
		Hu Plasma 3	212	N/A	5.4%
		Hu Plasma 4	234	N/A	9.3%
		Hu Serum 1	237	N/A	2.8%
		Hu Serum 2	246	N/A	2.2%
		Hu Serum 3	241	N/A	5.8%
		Hu Serum 4	262	N/A	8.4%
		FBS1	208	N/A	5.0%
		FBS2	207	N/A	5.3%
		FBS3	216	N/A	2.8%
		BS4	280	N/A	4.1%
		BS5	259	N/A	6.7%
		CS1	190	N/A	6.1%
		CS2	193	N/A	2.1%
		CS3	183	N/A	4.3%
		D2	222	N/A	7.4%
		D3	229	N/A	8.4%
		D43	205	N/A	7.2%
		DMEM	166	N/A	6.8%
		H2O	N/A	167	N/A

Read Buffer vol.	Spike & []	Sample Matrix	ECL generation vs No Matrix Control		Absolute Error	
			D3+STAG	H2O+STAG	D3+STAG	H2O+STAG
150 ul	None	Hu Plasma 1	141%	N/A	21.4%	N/A
		Hu Plasma 2	139%	N/A	24.6%	N/A
		Hu Plasma 3	127%	N/A	19.0%	N/A
		Hu Plasma 4	140%	N/A	23.5%	N/A
		Hu Serum 1	142%	N/A	20.2%	N/A
		Hu Serum 2	147%	N/A	20.8%	N/A
		Hu Serum 3	145%	N/A	21.9%	N/A
		Hu Serum 4	157%	N/A	25.7%	N/A
		FBS1	125%	N/A	18.5%	N/A
		FBS2	124%	N/A	18.6%	N/A
		FBS3	130%	N/A	18.5%	N/A
		BS4	168%	N/A	24.4%	N/A
		BS5	155%	N/A	24.1%	N/A
		CS1	114%	N/A	17.3%	N/A
		CS2	116%	N/A	16.4%	N/A
		CS3	110%	N/A	16.0%	N/A
		D2	133%	N/A	21.0%	N/A
		D3	137%	N/A	22.4%	N/A
		D43	123%	N/A	19.3%	N/A
		DMEM	100%	N/A	15.5%	N/A
		H2O	N/A	100%	N/A	19.8%

D3+STAG	
Average %	135%
STDEV of %ECL	15%
Max	168%
Min	110%

FIG. 10B

FIG. 10A

Spike & []	Sample Matrix	ECL generation vs EtOH Control			Absolute Error		
		D3+STA G	H2O +STAG	H2O	D3+STA G	H2O +STAG	H2O
Acetamenophene (1.03mM)	FBS1	80%	N/A	N/A	6%	N/A	N/A
	FBS2	79%	N/A	N/A	11%	N/A	N/A
	BS4	91%	N/A	N/A	6%	N/A	N/A
	BS5	86%	N/A	N/A	8%	N/A	N/A
Ibuprofen (1.06mM)	FBS1	66%	N/A	N/A	5%	N/A	N/A
	FBS2	78%	N/A	N/A	6%	N/A	N/A
	BS4	71%	N/A	N/A	7%	N/A	N/A
	BS5	89%	N/A	N/A	3%	N/A	N/A
Naproxen Na+ (2.17mM)	FBS1	74%	N/A	N/A	3%	N/A	N/A
	FBS2	93%	N/A	N/A	4%	N/A	N/A
	BS4	89%	N/A	N/A	5%	N/A	N/A
	BS5	83%	N/A	N/A	5%	N/A	N/A
Sodium ascorbate (0.34mM)	FBS1	79%	N/A	N/A	3%	N/A	N/A
	FBS2	83%	N/A	N/A	6%	N/A	N/A
	BS4	119%	N/A	N/A	7%	N/A	N/A
	BS5	103%	N/A	N/A	8%	N/A	N/A
Salicylic acid (4.34mM)	FBS1	102%	N/A	N/A	4%	N/A	N/A
	FBS2	108%	N/A	N/A	5%	N/A	N/A
	BS4	97%	N/A	N/A	5%	N/A	N/A
	BS5	107%	N/A	N/A	5%	N/A	N/A
Tolbutamine (2.4mM)	FBS1	91%	N/A	N/A	2%	N/A	N/A
	FBS2	114%	N/A	N/A	15%	N/A	N/A
	BS4	101%	N/A	N/A	6%	N/A	N/A
	BS5	119%	N/A	N/A	6%	N/A	N/A
EtOH (5 %v/v)	FBS1	100%	N/A	N/A	3%	N/A	N/A
	FBS2	100%	N/A	N/A	6%	N/A	N/A
	BS4	100%	N/A	N/A	7%	N/A	N/A
	BS5	100%	N/A	N/A	3%	N/A	N/A
None	H2O	N/A	100%	N/A	N/A	17%	15%
	H2O	N/A	104%	N/A	N/A	N/A	N/A

D3+STAG	
Average %	92%
STDEV of %ECL	14%
Max	119%
Min	66%

FIG. 11B

Spike & []	Sample Matrix	ECL Generation			%CV		
		D3+STA G	H2O +STAG	H2O	D3+STA G	H2O +STAG	H2O
Acetamenophene (1.03mM)	FBS1	384	N/A	N/A	7%	N/A	N/A
	FBS2	336	N/A	N/A	13%	N/A	N/A
	BS4	389	N/A	N/A	4%	N/A	N/A
	BS5	359	N/A	N/A	9%	N/A	N/A
Ibuprofen (1.06mM)	FBS1	315	N/A	N/A	8%	N/A	N/A
	FBS2	332	N/A	N/A	6%	N/A	N/A
	BS4	305	N/A	N/A	8%	N/A	N/A
	BS5	369	N/A	N/A	3%	N/A	N/A
Naproxen Na+ (2.17mM)	FBS1	352	N/A	N/A	3%	N/A	N/A
	FBS2	395	N/A	N/A	2%	N/A	N/A
	BS4	383	N/A	N/A	2%	N/A	N/A
	BS5	347	N/A	N/A	5%	N/A	N/A
Sodium ascorbate (0.34mM)	FBS1	377	N/A	N/A	3%	N/A	N/A
	FBS2	350	N/A	N/A	5%	N/A	N/A
	BS4	510	N/A	N/A	4%	N/A	N/A
	BS5	428	N/A	N/A	7%	N/A	N/A
Salicylic acid (4.34mM)	FBS1	487	N/A	N/A	3%	N/A	N/A
	FBS2	458	N/A	N/A	2%	N/A	N/A
	BS4	414	N/A	N/A	1%	N/A	N/A
	BS5	446	N/A	N/A	4%	N/A	N/A
Tolbutamine (2.4mM)	FBS1	435	N/A	N/A	1%	N/A	N/A
	FBS2	483	N/A	N/A	12%	N/A	N/A
	BS4	432	N/A	N/A	4%	N/A	N/A
	BS5	494	N/A	N/A	4%	N/A	N/A
EtOH (5 %v/v)	FBS1	478	N/A	N/A	2%	N/A	N/A
	FBS2	424	N/A	N/A	4%	N/A	N/A
	BS4	429	N/A	N/A	5%	N/A	N/A
	BS5	416	N/A	N/A	2%	N/A	N/A
None	H2O	N/A	232	N/A	N/A	12%	8%
	H2O	N/A	241	N/A	N/A	N/A	N/A

FIG. 11A

BTI ECL Values

		Coreactant #1														Coreactant #2			
		TPA	TBA	TEA	BDEA	PDEA	EDEA	MDEA	tBDEA	DBEA	DEAE	DBA-BS	DBA-PS	BEA-BS	DEA-BS	DEA-PS			
TPA (T1x)		5954	17812	7900	21427	22696	18234	11536	7624	13360	13120	17764	11284	12395	14383	13253			
TBA		0.9	13306	14427	24384	24825	22050	15503	12388	14681	15400	25130	15735	16906	17740	16876			
TEA		0.9	0.9	3203	20008	18165	11827	3826	3466	15475	5380	5623	8213	6451	7932	8078			
BDEA (RBB)		1.0	0.8	1.1	15543	19965	16641	15485	15763	15454	15205	19549	15232	15561	17594	18087			
PDEA		1.1	0.9	1.0	0.7	14212	16311	14426	15831	16523	15159	20397	15878	15728	16494	16596			
EDEA		1.4	1.1	1.1	0.7	0.8	7221	8136	9067	12705	8324	12096	9571	8247	8916	9217			
MDEA		1.4	1.0	0.7	0.9	0.9	0.9	2092	2264	11164	3949	5143	6349	5061	5861	5598			
DBDEA		1.0	0.8	0.7	0.9	1.0	1.0	0.6	1450	11793	3155	3376	5546	3805	5185	5304			
DBEA		0.8	0.6	1.2	0.6	0.7	0.7	0.9	1.0	10137	11048	14611	11294	10901	11841	12444			
DEAE		1.5	1.0	0.9	0.8	0.9	0.8	0.8	0.8	0.9	2725	5605	4996	3990	4210	4648			
DBA-BS		2.1	1.6	1.0	1.1	1.2	1.2	1.1	0.8	1.2	1.1	2553	2553	6559	7090	6910			
DBA-PS		1.3	1.0	1.3	0.8	0.9	0.9	1.2	1.2	0.9	0.9	1.4	3060	5872	6821	7794			
BEA-BS		1.4	1.0	1.0	0.8	0.9	0.8	1.0	0.9	0.8	0.7	1.2	1.0	3002	5181	5862			
DEA-BS		1.6	1.1	1.2	0.9	1.0	0.9	1.1	1.1	0.9	0.7	1.2	1.1	0.8	3146	7346			
DEA-PS		1.4	1.0	1.2	1.0	0.9	0.9	1.0	1.1	0.9	0.8	1.2	1.2	0.9	1.1	3351			

Mixed co-reactant ECL

ECL Ratios = $\frac{\text{Mixed co-reactant ECL}}{\sum \text{control ECL buffers}}$

FIG. 12

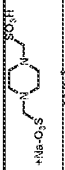
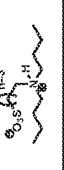
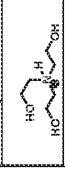
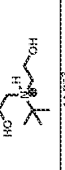
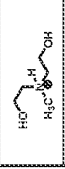
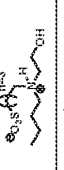
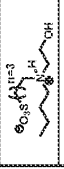
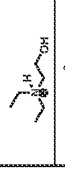
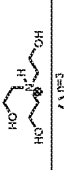
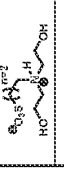
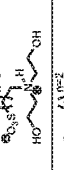
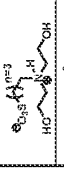
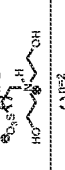
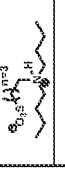
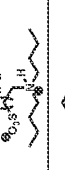
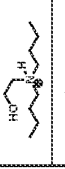
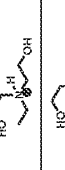
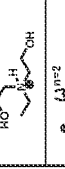
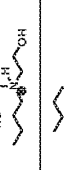
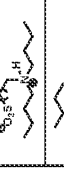
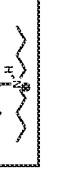
BTI ECL values			FT ECL values		
Amine	TX100	PEG18TDE	Amine	TX100	PEG18TDE
	184192	1798		38699	248
	72635	13287		3288	2359
	94857	14972		4238	4701
	21392	15089		8346	5473
	77341	15385		28776	6930
	68779	33035		54448	9202
	42711	49550		9516	12407
	68844	53359		14328	14333
	60096	61135		164340	22076
	87009	71404		36114	24713
	96864	78598		43948	41687
	122422	117014		46940	43538
	148197	154964		120247	59261
	190813	201228		227059	231329

FIG. 13A

	ECL ratio (PEG18TDE vs TX100)			Amine	pKa
	BTI	FT			
Amine	TX100 vs PEG18	TX100 vs PEG18			
TEA	0.9	0.9		TEA	7.73
TBA	0.9	1.0		TBA	10.89
BDEA	1.0	1.1		BDEA	8.90
DEA-PS	1.0	0.8		DEA-PS	?
EDEA	1.0	1.1		EDEA	8.80
DBA-PS	1.2	2.0		DBA-PS	?
DBAE	1.2	1.5		DBAE	9.83
DEA-BS	1.3	1.0		DEA-BS	?
tBDEA	1.4	1.4		tBDEA	9.03
MDEA	2.1	1.5		MDEA	8.54
BEA-BS	5.0	4.2		BEA-BS	?
DBA-BS	5.5	7.4		DBA-BS	?
DEAE	6.3	5.9		DEAE	9.73
PIPES	102.4	156.3		PIPES	6.76

FIG. 13B

FIG. 14

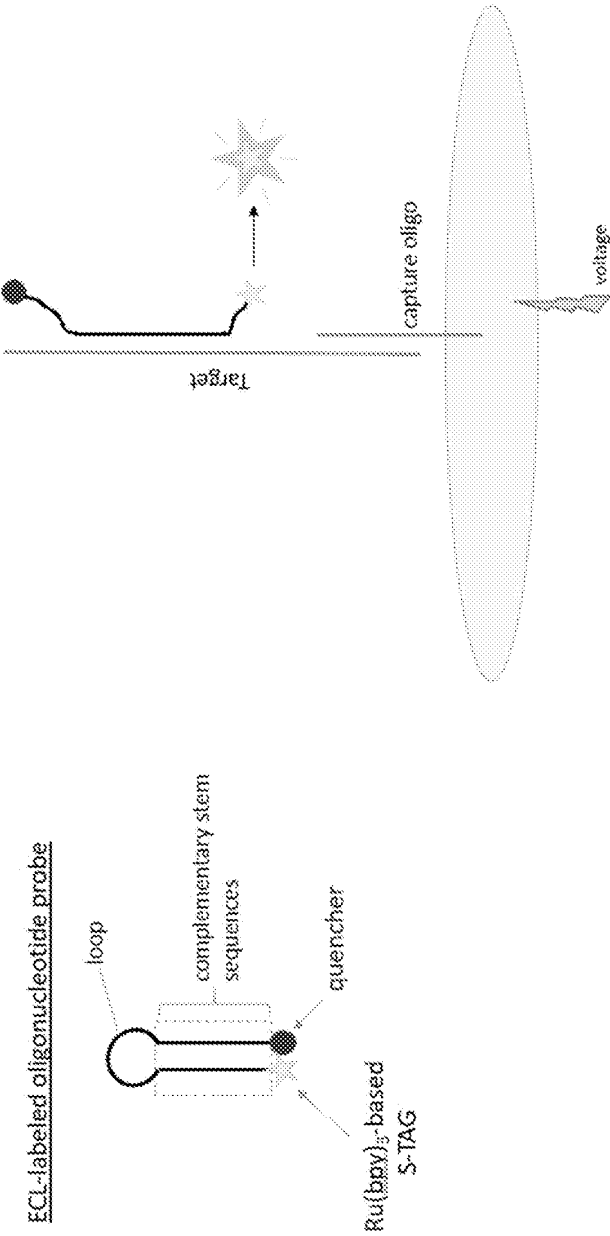


FIG. 15

Target 4.2 = 60-mer containing the sequence complementary to the loop and the top nucleotide of the stem.

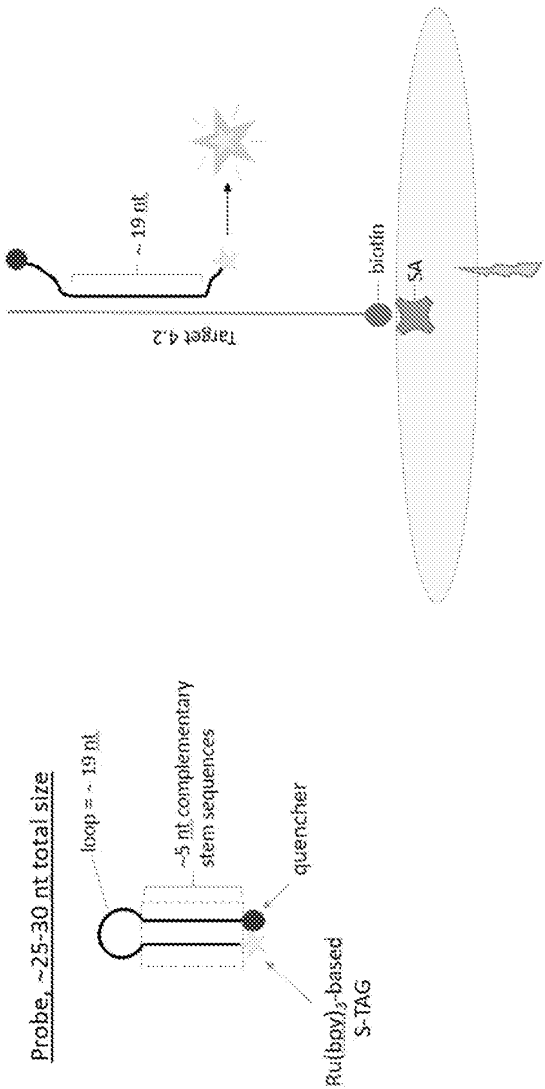


FIG. 16

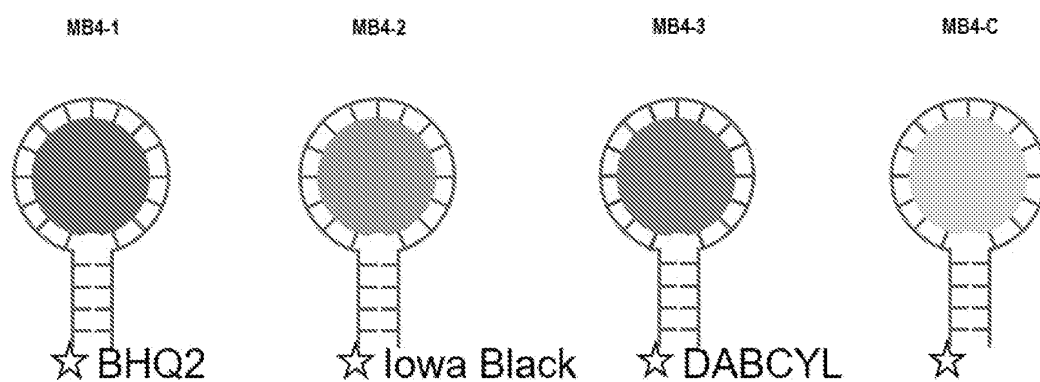


FIG. 17A

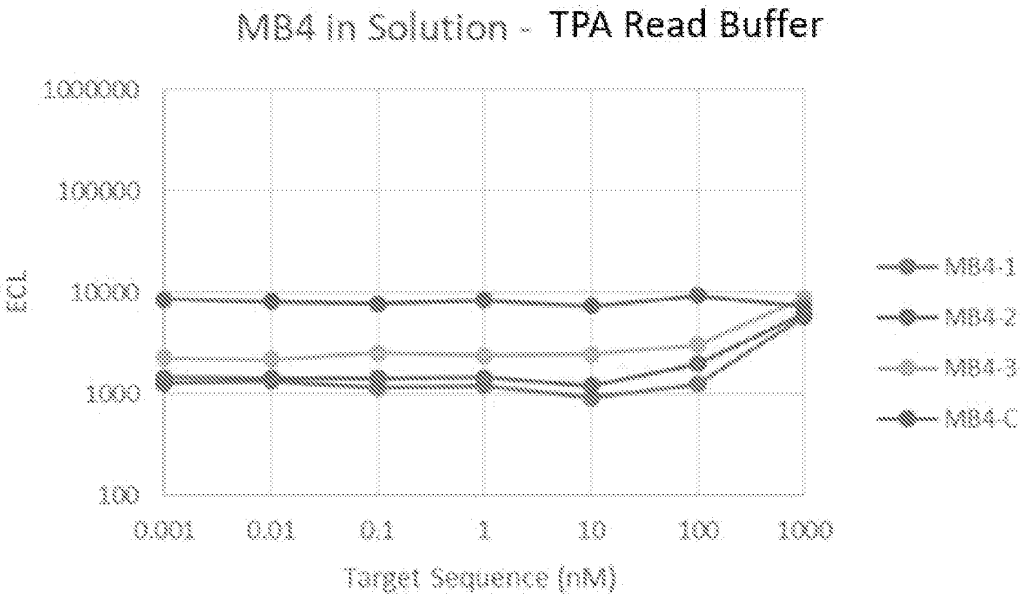


FIG. 17B

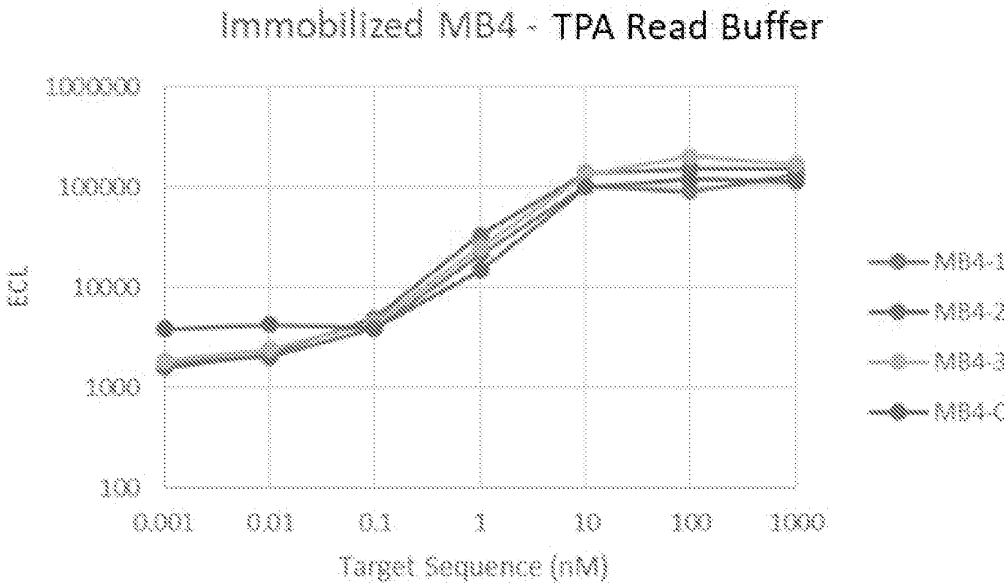


FIG. 17C

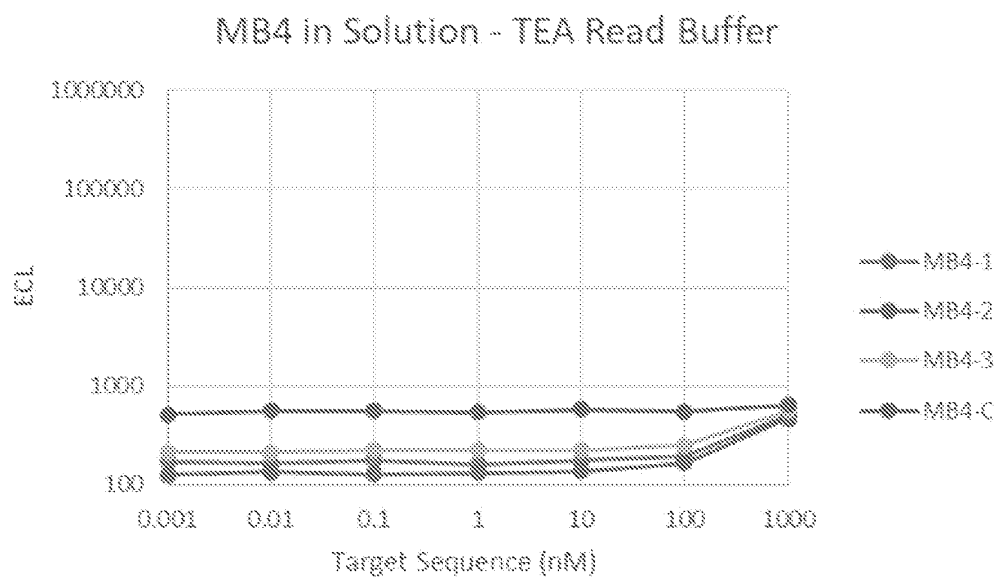
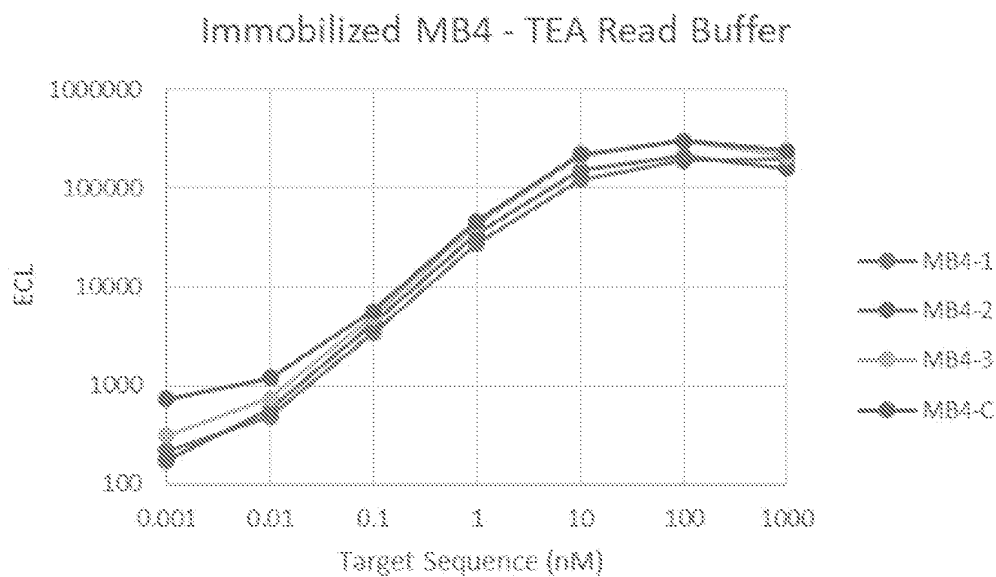


FIG. 17D



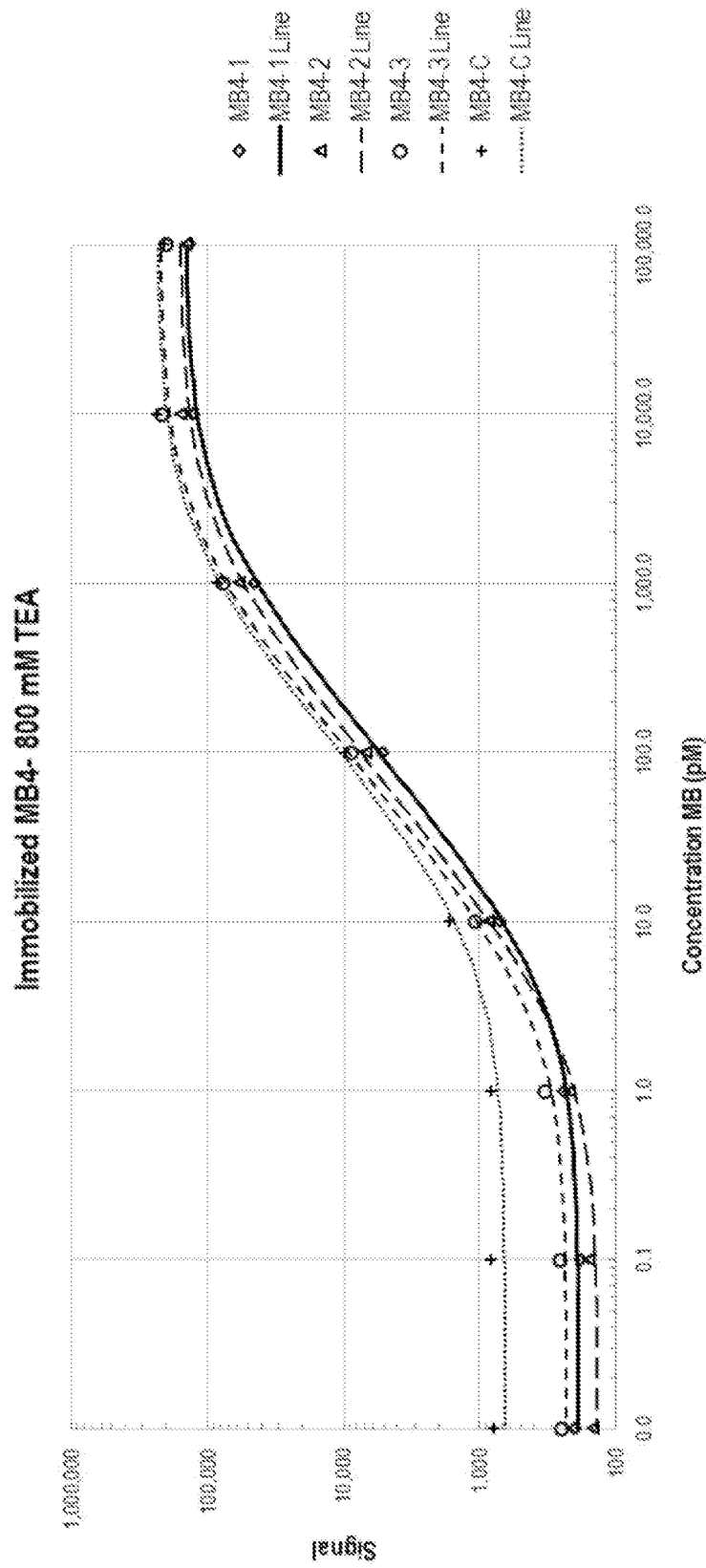


FIG. 18A

	MB4-1		MB4-2		MB4-3		MB4-C	
	Conc.	Signal	Conc.	Signal	Conc.	Signal	Conc.	Signal
Cal 01	10000.00	135243.33	100000.00	141710.67	100000.00	200662.00	100000.00	207271.00
Cal 02	10000.00	127018.33	10000.00	156471.00	10000.00	214997.33	10000.00	234353.33
Cal 03	1000.00	44957.00	1000.00	58497.33	1000.00	75642.00	1000.00	84469.33
Cal 04	100.00	5161.00	100.00	6897.33	100.00	8657.00	100.00	9811.67
Cal 05	10.00	721.33	10.00	866.00	10.00	1069.33	10.00	1664.67
Cal 06	1.00	242.00	1.00	223.00	1.00	332.33	1.00	828.33
Cal 07	0.10	184.33	0.10	158.00	0.10	252.33	0.10	822.33
Cal 08	0.01	201.00	0.01	148.00	0.01	247.00	0.01	794.33
Cal 09	0.00	177.17	0.00	115.61	0.00	199.33	0.00	498.17

Hill slope	1.04	Hill slope	1.03	Hill slope	1.06	Hill slope	1.06
LLOD	1.72503	LLOD	#NUM!	LLOD	0.56806	LLOD	1.28360
CV	20.49	CV	4.93	CV	14.25	CV	20.79
SD	36.31	SD	5.70	SD	28.40	SD	103.58

FIG. 18B

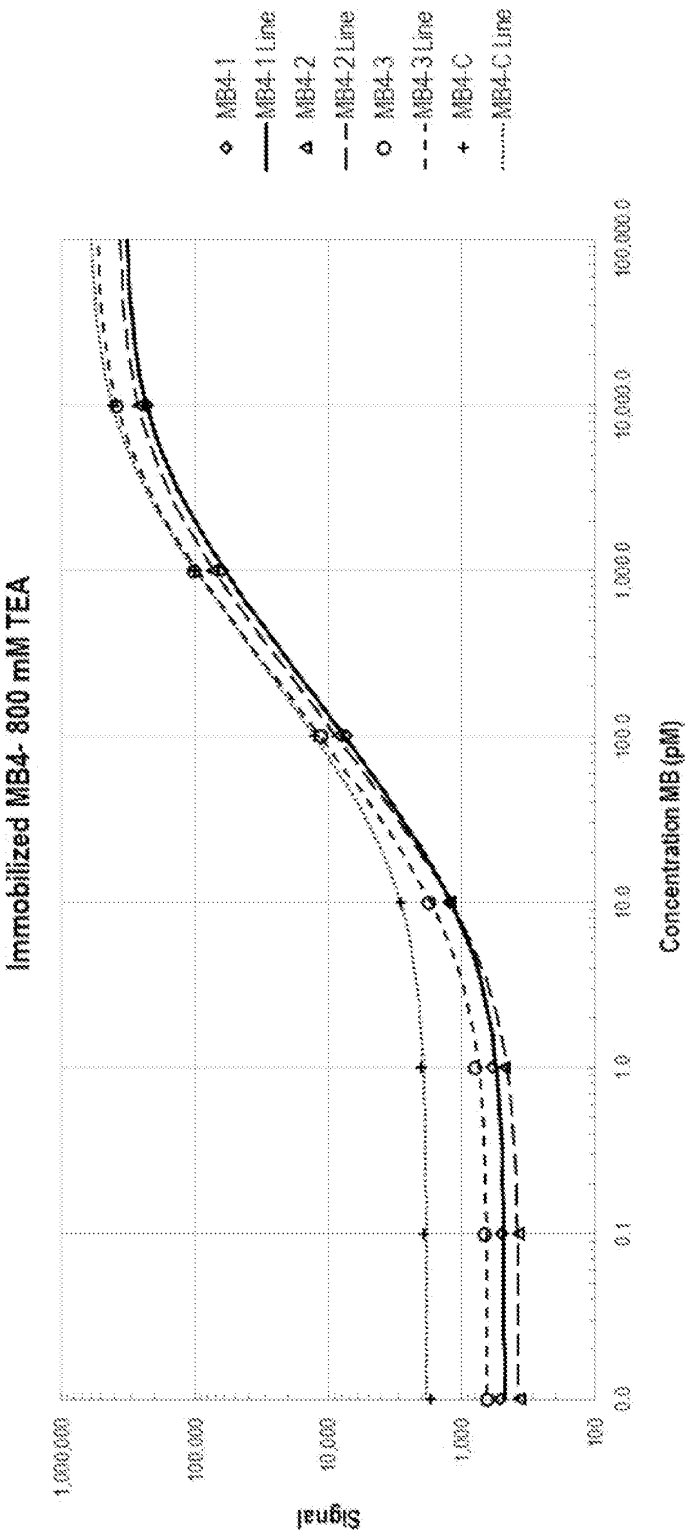


FIG. 19A

	MB4-1		MB4-2		MB4-3		MB4-C	
	Conc.	Signal	Conc.	Signal	Conc.	Signal	Conc.	Signal
Ca 01	10000.00	228432.00	10000.00	265299.50	10000.00	381015.50	10000.00	416550.50
Ca 02	1000.00	62443.00	1000.00	74122.00	1000.00	100080.00	1000.00	100392.00
Ca 03	100.00	7287.00	100.00	8452.50	100.00	11138.50	100.00	12614.50
Ca 04	10.00	1226.50	10.00	1221.50	10.00	1735.50	10.00	2903.00
Ca 05	1.00	597.50	1.00	479.00	1.00	795.00	1.00	2007.50
Ca 06	0.10	500.00	0.10	383.00	0.10	667.00	0.10	1931.50
Ca 07	0.01	510.50	0.01	366.50	0.01	635.00	0.01	1721.50
Ca 08	0.00	425.00	0.00	376.50	0.00	624.00	0.00	1863.00

Hill slope	1.00	Hill slope	1.01	Hill slope	1.02	Hill slope	1.02
LLOD	1.35851	LLOD	0.16970	LLOD	#NUM!	LLOD	0.26038
CV	14.59	CV	1.46	CV	0.32	CV	0.14
SD	62.00	SD	5.50	SD	2.00	SD	2.70

FIG. 19B

FIG. 20

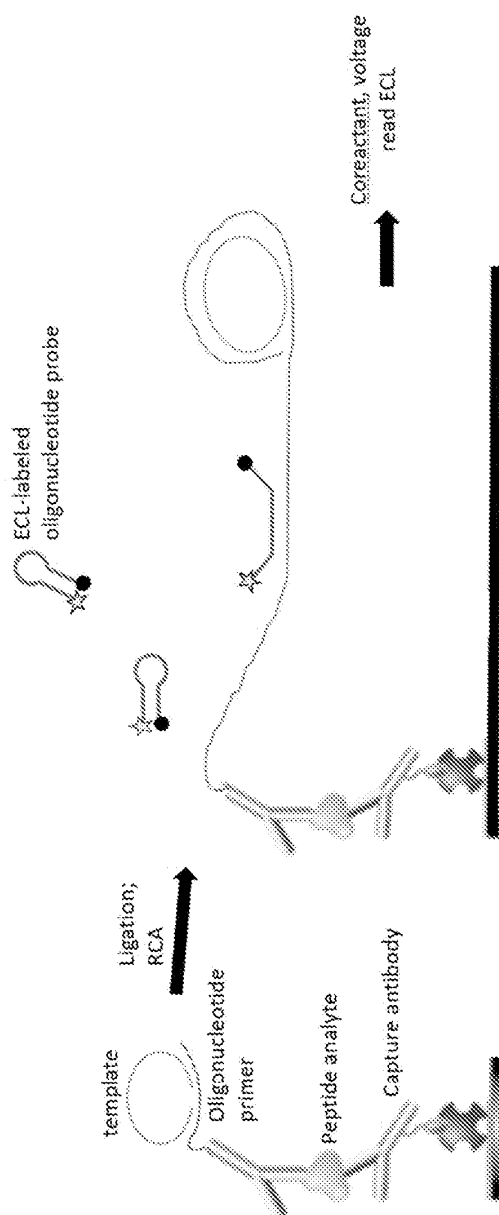


FIG. 21

