

1. OBJECTIVE

The purpose of this study was to determine the aqueous solubility of the test compounds by laser nephelometry.

2. PERSONNEL

- 2.1 Deborah Culver performed the assay(s).
- 2.2 Aiming Sun provided the test compounds.
- 2.3 Mike Natchus, Mark Lockwood, and Deborah Culver evaluated the results.

3. REFERENCES

- 3.1 Drug Aqueous Solubility Screening Assay Protocol
- 3.2 BMG LABTECH NEPHELOstar Nephelometer Standard Operating Procedure (SOP)
- 3.3 Grubb's Test for Detecting Outliers see: Grubbs, F.E. and Beck, G., *Technometrics* **1972**, *14*, 847-854.
- 3.4 Aqueous Solubility Range see: Green, C., McKee, S., and Saunders, K., Department of Drug Metabolism, Pfizer Global Research and Development, Kent CT13 9NJ, UK, (poster presentation, BMG LABTECH).

4. APPENDIX

- 4.1 Compound Stock Concentration Worksheet (Appendix I)
- 4.2 Graphs and Tables (Appendix II)

5. GENERAL METHOD

- 5.1 The test compounds and phenytoin, internal control, were dissolved in 100% DMSO to obtain a stock concentration of 30 mg/mL (Appendix I). The 30 mg/mL stock solution was serially diluted (concentration profile: 30, 20, 15, 10, 7.5, 5, 2.5, 1.25, 0.63, 0.31, and 0.15 mg/mL) in test tubes with 100% DMSO. These concentrations (plus vehicle) were transferred to row A of a 96 well microplate (Costar black clear bottom microplates). Each well in the remaining rows of the plate then received 27 μ L of phosphate buffered saline, pH 7.4 (PBS). Next, 3 μ L of each dilution in row one was transferred to each of the remaining 7 wells in its column (i.e., rows B through H); the plate was then vortexed in the nephelometer to mix drug and PBS. Next, 270 μ L of PBS was added to each well in rows B through H, followed by vortexing in the nephelometer again, to achieve a final volume of 300 μ L, a final DMSO concentration of 1%, and final drug concentrations of 300, 200, 150, 100, 75, 50, 25, 12, 6, 3 & 1.5 μ g/mL. The microplates were then incubated for 90 minutes at ambient temperature and then analyzed using the nephelometer. The segmental regression curve fit of two linear regression lines and the intersection point of the two

lines was used to determine solubility. (See reference assay protocol and nephelometer SOP for additional details)

6. PROTOCOL DEVIATIONS

6.1 NA

7. RESULTS

7.1 Summary

Table 1.

			Solubility by Nephelometer	
Compound	Structure	MW (g/mol)	$\mu\text{g/mL}$	μM
Triptolide Lot N ^o : NA		360.40	>100	>275
GB67A Lot N ^o : NA		208.25	>100	>480
GB67B Lot N ^o : NA		208.25	>100	>480
GB594 Lot N ^o : NA		242.27	>100	>400

**Aqueous Solubility Screening of Triptolide
Compounds**

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GB615 Lot N ^o : NA		224.25	>100	>440
GB625 Lot N ^o : NA		226.27	>100	>440
GB630 Lot N ^o : NA		226.27	>100	>440
GB768B Lot N ^o : NA		166.17	>100	>600
GB780 Lot N ^o : NA		210.23	>100	>475
GB807B Lot N ^o : NA		234.29	83	354

7.2 Stock Concentration Worksheet, See Appendix I

7.3 Graphs, See Appendix II.

7.4 Raw Data, See Appendix II.

7.5 All raw data generated by the assay is stored on the dedicated computer connected to the nephelometer. This data was also exported to an Excel file and used to generate the graphs contained in this report.

8. CONCLUSION

8.1 Visually, it appeared the standard curve was completely dissolved/soluble in 100% DMSO. GB67A immediately turned yellow upon the addition of DMSO. Over a period of 30 minutes, the yellow color diminished to colorless. Once diluted to a final DMSO concentration of 1%, it was visually apparent the higher concentrations, 100-300 µg/mL, for GB807B, immediately turned turbid with precipitation. There was no visual precipitation with the other compounds. The gain was high for these compounds, indicating high solubility.

Compounds contained in the literature have been classified in one of three categories based on their reported aqueous solubility: soluble (>100 µg/mL), partially soluble (15 – 100 µg/mL) and poorly soluble (<15 µg/mL), (Green, *et. al.*, Pfizer). The aqueous solubilities of the compounds in this study were determined by laser nephelometry and were classified in a similar manner in the following table.

Poorly Soluble (<15 µg/mL)	Partially Soluble (15 – 100 µg/mL)	Soluble (>100 µg/mL)
	GB807B	Triptolide GB67A GB67B GB594 GB615 GB625 GB630 GB768B GB780

APPENDIX I

Compound Stock Concentration Worksheet

**Aqueous Solubility Screening of Triptolide
Compounds**

Project Code:

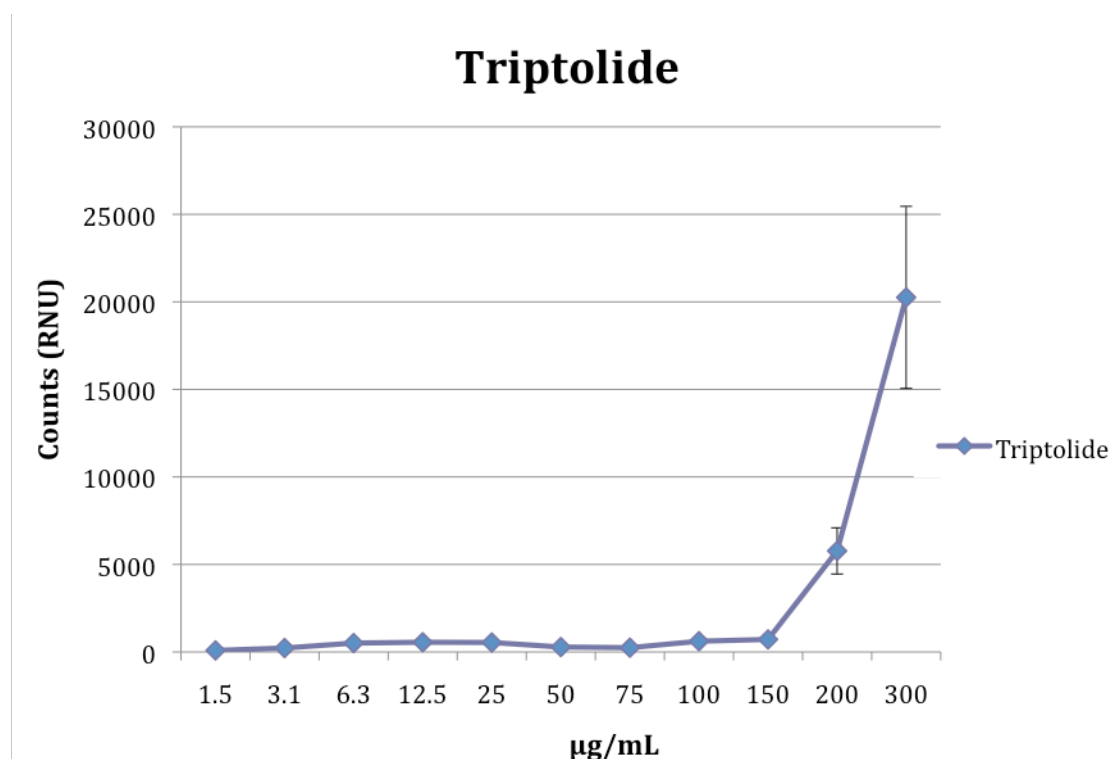
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COMPOUND STOCK CONCENTRATION WORKSHEET

Compound	Lot No.	Mol. Wt. (g/mol)	Amount (mg)	DMSO (μ L) for 30 mg/mL soln.	Comments
Triptolide	N/A	360.40	16	530	Sonicated
GB67A	N/A	208.25	15	500	Turned yellow when DMSO added; turned colorless after 30 minutes
GB67B	N/A	208.25	17	570	N/A
GB594	N/A	242.27	11	370	N/A
GB615	N/A	224.25	15	500	N/A
GB625	N/A	226.27	11	370	N/A
GB630	N/A	226.27	12	400	N/A
GB768B	N/A	166.17	10	330	N/A
GB780	N/A	210.23	11	370	N/A
GB807B	N/A	234.29	12	400	N/A

APPENDIX II

Graphs and Data Tables



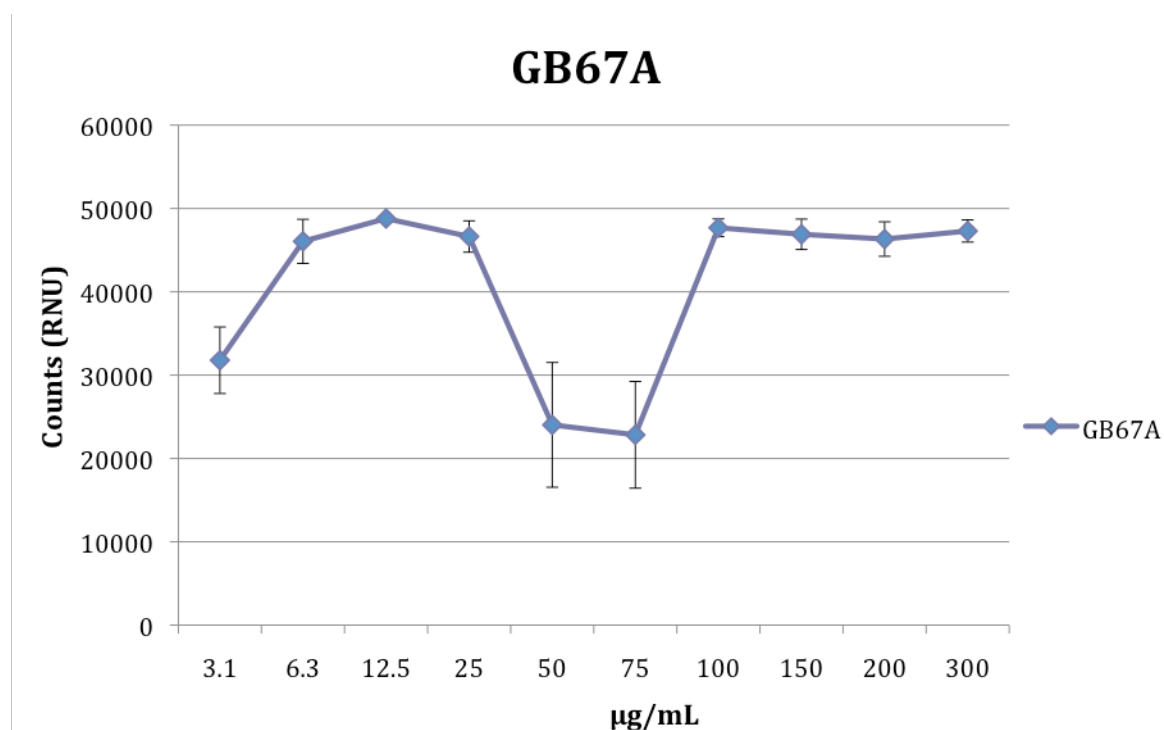
*Truncated graph (minus highest concentrations)

Well N ^o :	Triptolide (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	325	781	2956	853	(5016)	777	807	941	1017	1037	745	444
2	490	37311	(21455)	877	996	750	1023	1214	1047	959	732	565
3	538	10507	5584	1236	950	632	702	1016	1081	998	741	570
4	365	37080	4426	1330	1030	539	449	976	937	857	(2380)	462
5	487	22927	9045	1192	1263	(1069)	823	868	996	974	587	537
6	357	12067	3959	1868	969	727	706	778	797	762	621	409
7	407	24087	11214	622	1017	581	421	(428)	(591)	(537)	465	594
Avg	424	20680	8377	1140	1606	725	704	889	924	875	896	512
STDEV	75	12738	5998	378	1396	163	197	226	161	163	613	67
Avg	424	20680	6197	1140	1038	668	704	966	979	931	649	512
**Blank Corrected Avg		20256	5773	716	613	244	280	541	555	507	224	87
STDEV	81	13759	3234	408	114	98	213	148	102	102	112	72
SE	31	5200	1320	154	47	40	80	60	41	42	46	27

Raw data

() Denote outliers

**Denote raw data corrected for outliers by Grubb's T-statistic for detecting outliers.



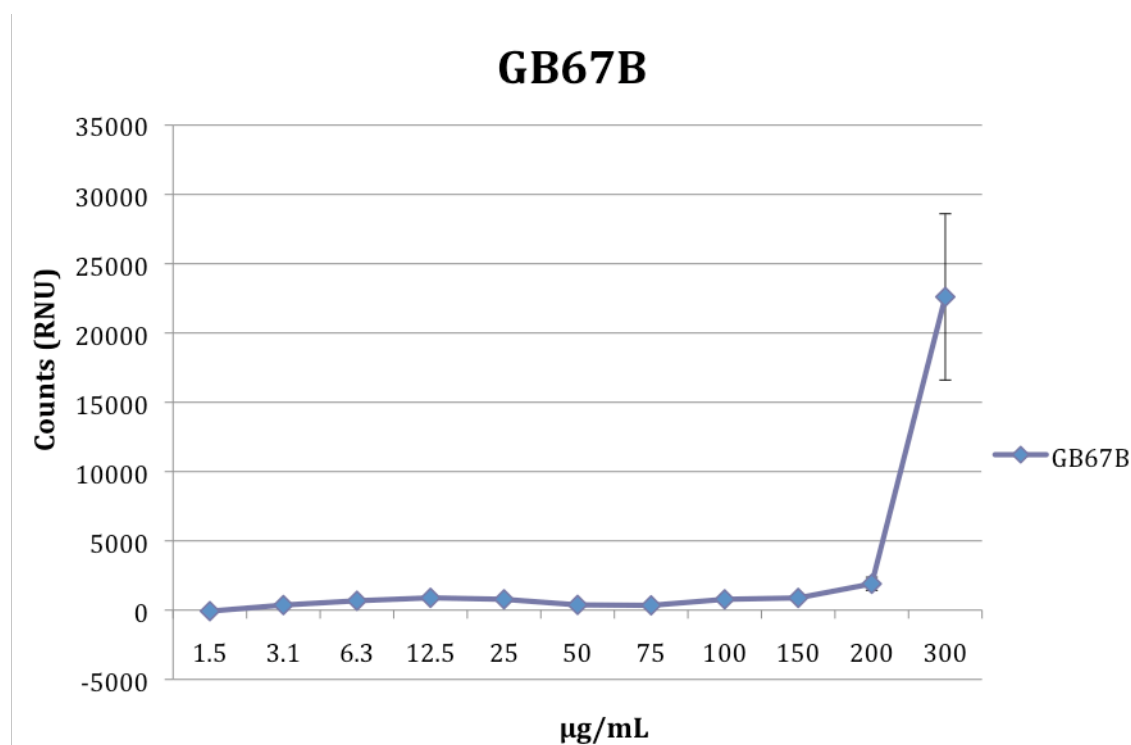
*Truncated graph (minus highest concentrations)

Raw data

Well N°:	GB67A (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	57678	(82002)	87034	88766	93635	80910	85931	99982	99982	99637	83395	45978
2	44243	99982	97206	97732	97351	84405	99982	99982	99982	99982	83441	59583
3	78866	92062	98624	99788	99982	61091	75718	99982	99982	99982	86169	50802
4	34012	99982	99982	99982	99982	58314	48162	97405	99978	98960	73460	53139
5	60163	99982	99982	99982	99982	98589	87883	99016	99366	98908	70873	66578
6	35774	96602	99982	99982	99982	80616	77922	88266	98275	83686	98197	48758
7	44888	99982	(61527)	(58879)	(61455)	51537	48236	(48233)	(60760)	(69647)	(46190)	32840
Avg	50803	95799	92048	92159	93196	73637	74833	90409	94046	92972	77389	51097
STDEV	14679	6267	13177	14101	13143	15679	18342	17653	13602	10981	15185	9863
Avg	50803	98099	97135	97705	98486	73637	74833	97439	99594	96859	82589	51097
**Blank Corrected Avg		47295	46332	46902	47682	22834	24030	46635	48791	46056	31786	293
STDEV	15855	3252	5071	4468	2599	16935	19812	4605	691	6471	9782	10653
SE	5993	1328	2070	1824	1061	6401	7488	1880	282	2642	3993	4026

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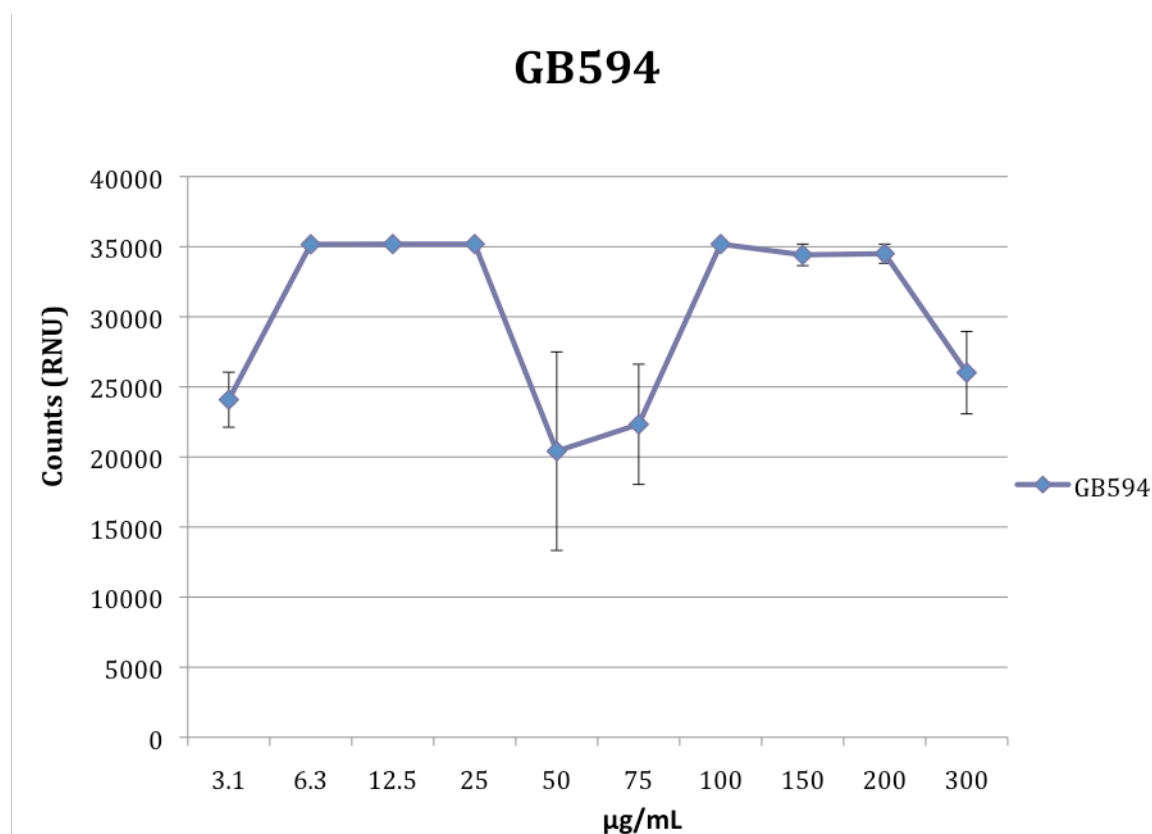
*Truncated graph (minus highest concentrations)

Raw Data

Well N ^o :	GB67B (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	820	(99982)	(11398)	1219	1165	948	1010	1427	1478	1352	1090	536
2	471	45397	1403	1404	1359	1120	1466	1700	1669	1622	1095	713
3	649	16342	3988	1519	1350	796	969	1560	1674	1554	1043	650
4	466	11348	3479	1506	1451	762	652	1276	1347	1287	886	611
5	724	35827	3233	1876	1757	1415	1306	1356	1461	1411	975	515
6	481	7708	1571	1553	1378	1008	1089	1140	1476	1086	881	467
7	779	22789	1505	1494	(685)	772	563	(632)	(719)	822	(553)	389
Avg	627	34199	3797	1510	1306	974	1008	1299	1403	1305	932	554
STDEV	142	29589	3255	182	302	219	300	321	300	256	175	103
Avg	627	23235	2530	1510	1410	974	1008	1410	1518	1305	995	554
**Blank Corrected Avg		22608	1903	883	783	347	381	783	890	678	368	-73
STDEV	154	14696	1163	197	195	236	324	200	129	276	97	111
SE	58	5999	475	74	79	89	123	82	53	104	39	42

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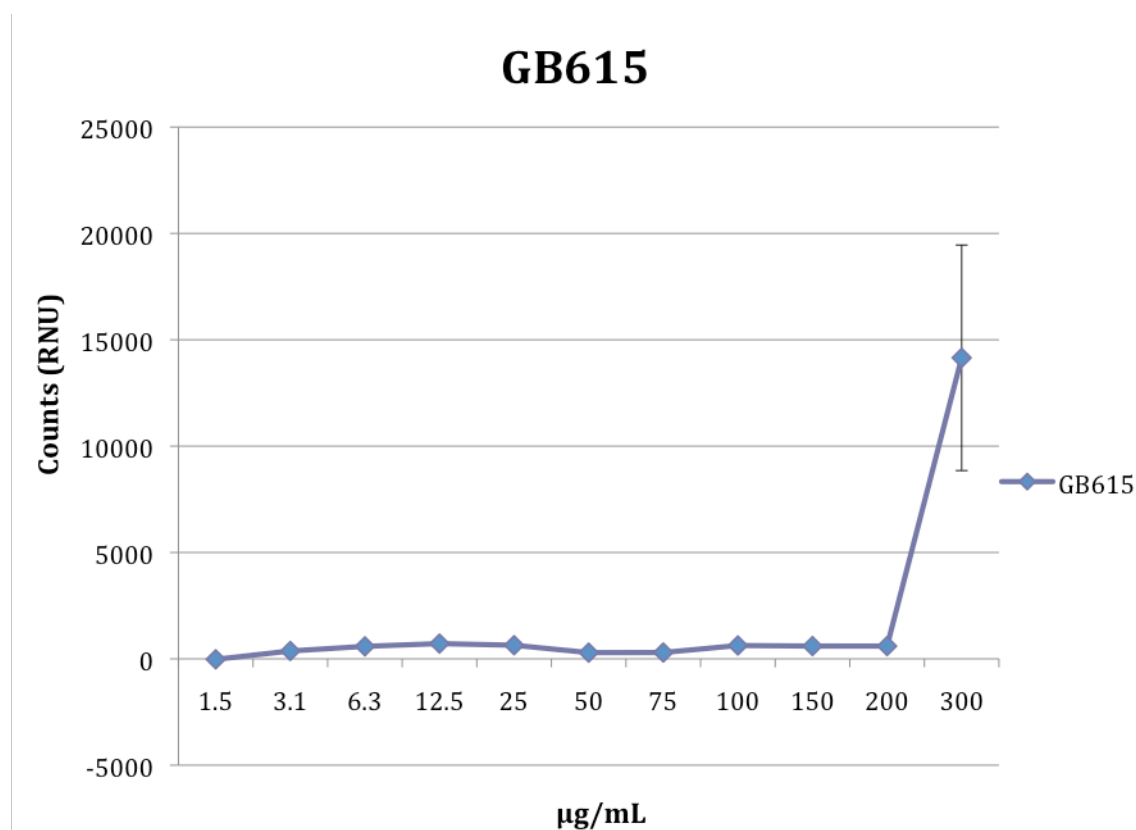
*Truncated graph (minus highest concentrations)

Raw Data

Well N ^o :	GB594 (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	88564	81921	95866	95367	99974	88522	97037	99982	99982	99982	88720	47437
2	47411	84330	99982	99982	99982	99359	99982	99982	99982	99982	94751	69672
3	99982	96650	99982	99982	99982	79944	89868	99982	99982	99982	94629	69416
4	45916	96615	99982	99982	99982	73610	62933	99982	99982	99952	85232	72168
5	69637	99015	99982	99982	99982	99982	99972	99982	99982	99982	86456	59814
6	43869	95516	99982	99982	99982	94329	92423	99982	99982	99910	83496	46902
7	58192	81613	(72956)	(71917)	(66141)	74119	54244	(57861)	(76777)	(76380)	(60324)	38474
Avg	64796	90809	95533	95313	95146	87124	85208	93965	96667	96596	84801	57698
STDEV	20587	7200	9326	9683	11841	10503	17341	14739	8120	8253	10788	12455
Avg	64796	90809	99296	99213	99981	87124	85208	99982	99982	99965	88881	57698
**Blank Corrected Avg		26013	34500	34417	35185	22328	20413	35186	35186	35169	24085	-7098
STDEV	22236	7777	1680	1884	3	11345	18730	0	0	29	4811	13453
SE	8404	2939	686	769	1	4288	7079	0	0	12	1964	5085

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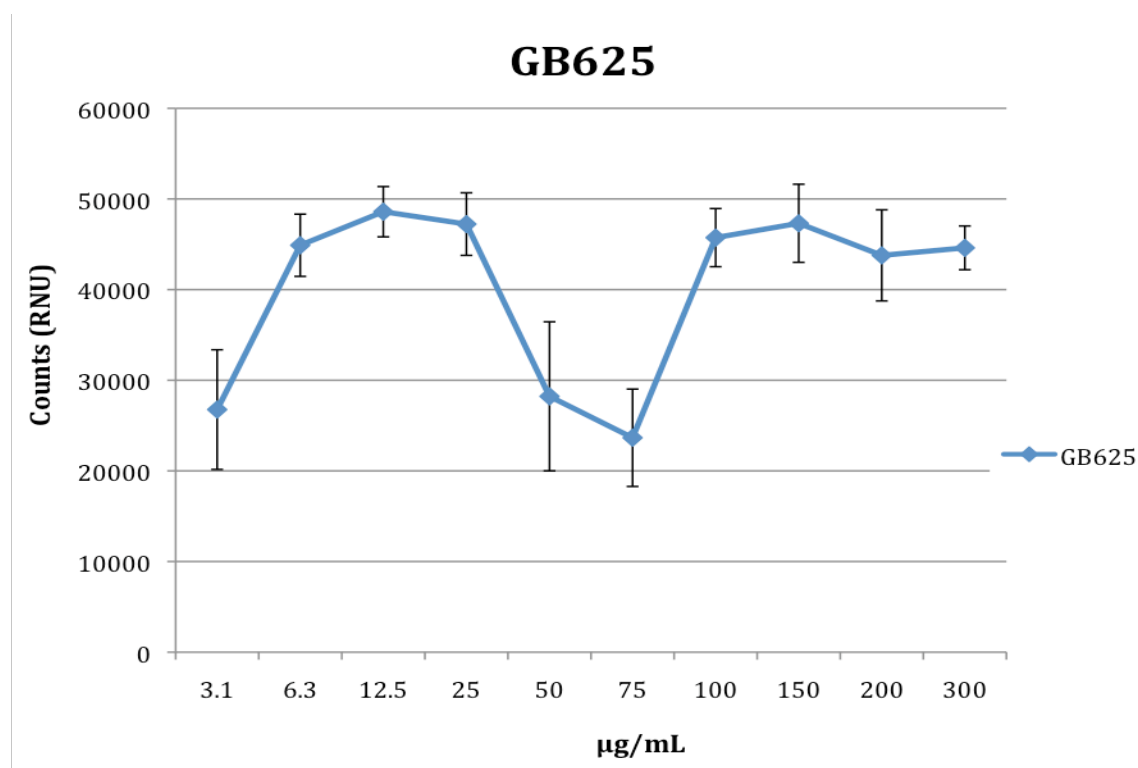
*Truncated graph (minus highest concentrations)

Raw Data

Well N ^o :	GB615 (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	706	21841	2976	1053	1207	961	1023	1306	1462	1420	1250	537
2	537	71097	1358	1090	1179	1024	1246	1541	1469	1288	961	749
3	872	13750	1254	1321	1222	763	872	1308	1454	1444	1680	525
4	431	6799	1318	1295	1308	692	571	1183	1224	1162	848	626
5	840	37421	1332	1570	1585	1298	1081	1142	1300	1279	801	604
6	418	4284	1250	1441	1271	983	985	1048	1082	994	820	509
7	501	4507	779	742	921	662	571	554	662	824	527	1530
Avg	615	22814	1467	1216	1242	912	907	1155	1236	1202	984	726
STDEV	176	22560	643	256	182	208	237	285	270	209	347	337
Avg	615	14767	1215	1216	1242	912	907	1255	1332	1202	984	592
**Blank Corrected Avg		14152	600	601	627	297	292	640	717	587	369	-23
STDEV	190	12984	218	277	196	224	256	172	159	226	375	90
SE	72	5301	89	105	74	85	97	70	65	86	142	37

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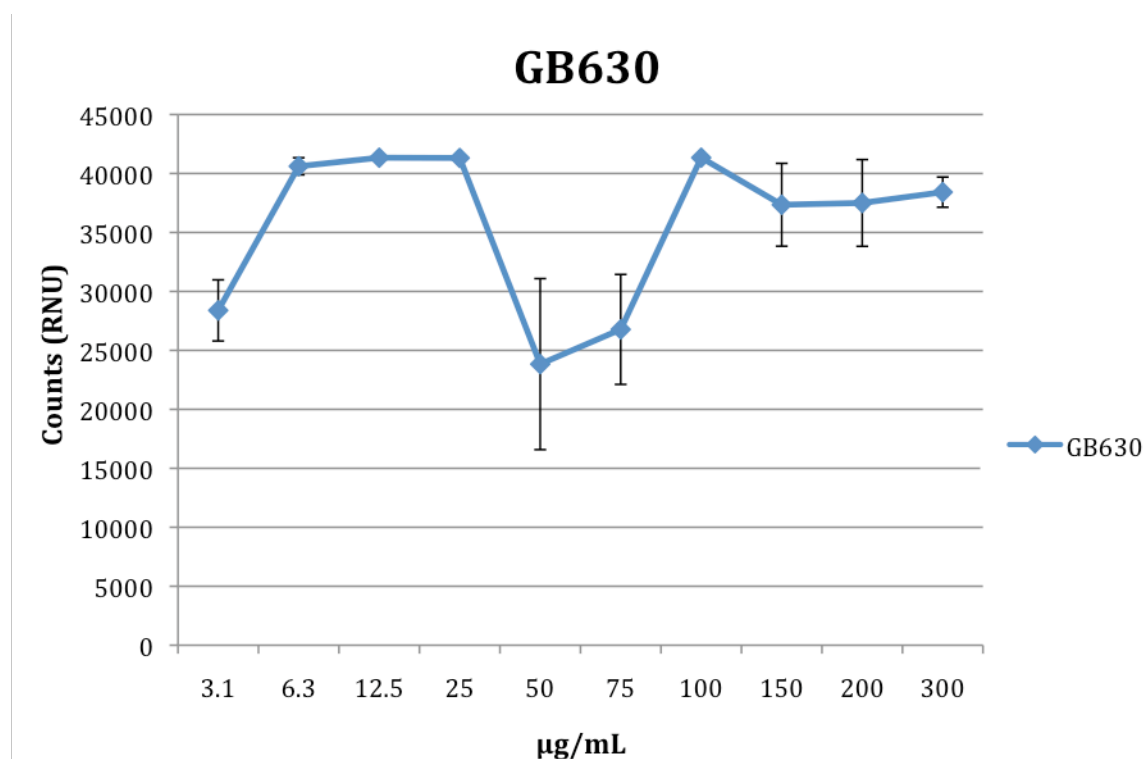
*Truncated graph (minus highest concentrations)

Raw Data

Well N ^o :	GB625 (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	42131	77332	76698	74029	76551	60790	67385	87263	89292	83102	64059	34310
2	44107	92348	78942	79941	85063	73522	89755	99982	99591	92208	68869	45619
3	60385	91165	93789	88418	84348	57011	68346	99982	96683	98887	95447	44731
4	34358	83543	94086	96010	90812	57809	50271	82689	87199	83214	85694	(68027)
5	(99982)	93145	97655	99982	99982	91856	79111	83754	90669	89650	63261	44168
6	31741	89368	97083	98005	90144	70344	99982	82187	80628	74795	63160	41698
7	39771	79919	62754	(51309)	(52653)	48837	37300	(45412)	(53457)	(54918)	41426	41137
Avg	50354	86689	85858	83956	82793	65738	70307	83038	85360	82396	68845	45670
STDEV	21989	5906	12313	16035	13983	13164	20141	16942	14233	13282	16168	9771
Avg	42082	86689	85858	89398	87817	65738	70307	89310	90677	86976	68845	41944
**Blank Corrected Avg		44606	43776	47315	45735	23656	28225	47227	48595	44894	26763	-138
STDEV	10110	6380	13300	10549	7860	14219	21755	8455	6790	8413	17463	4128
SE	4127	2411	5027	4307	3209	5374	8223	3452	2772	3435	6600	1685

() Denote outliers

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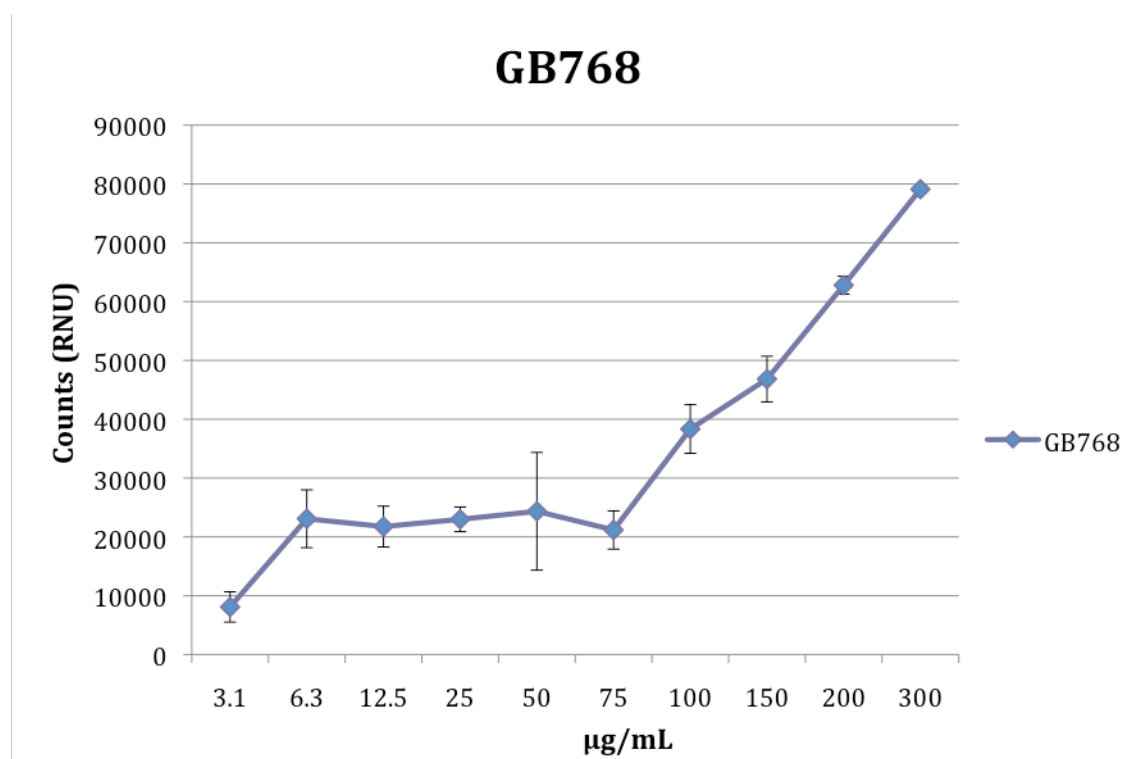
*Truncated graph (minus highest concentrations)

Raw Data

Well N°:	GB630 (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	78524	98117	99003	96948	99982	91499	91118	99982	99982	99982	90749	63818
2	48858	93022	99982	99982	99986	93327	99982	99982	99982	99982	93083	73985
3	75297	99982	99982	99982	99982	78862	87524	99982	99982	99982	94185	62134
4	45768	97023	99982	99982	99982	70315	57290	99982	99982	99982	79827	63518
5	67398	99982	99982	99982	99986	99982	97943	99982	99982	99982	82733	99982
6	41702	99564	99982	99982	99982	94375	90437	99837	99982	95623	81628	45747
7	52970	91748	74108	75086	99982	69600	53062	(55695)	(67354)	(69278)	(62113)	35974
Avg	58645	97063	96146	95992	99983	85423	82479	93635	95321	94973	83474	63594
STDEV	13784	3139	9003	8599	2	11422	17763	15489	11417	10597	10237	18957
Avg	58645	97063	96146	95992	99983	85423	82479	99958	99982	99256	87034	63594
**Blank Corrected Avg		38417	37501	37347	41338	26778	23834	41313	41337	40610	28389	4949
STDEV	14888	3391	9725	9288	2	12337	19187	59	0	1780	6343	20476
SE	5627	1282	3676	3510	1	4663	7252	24	0	727	2590	7739

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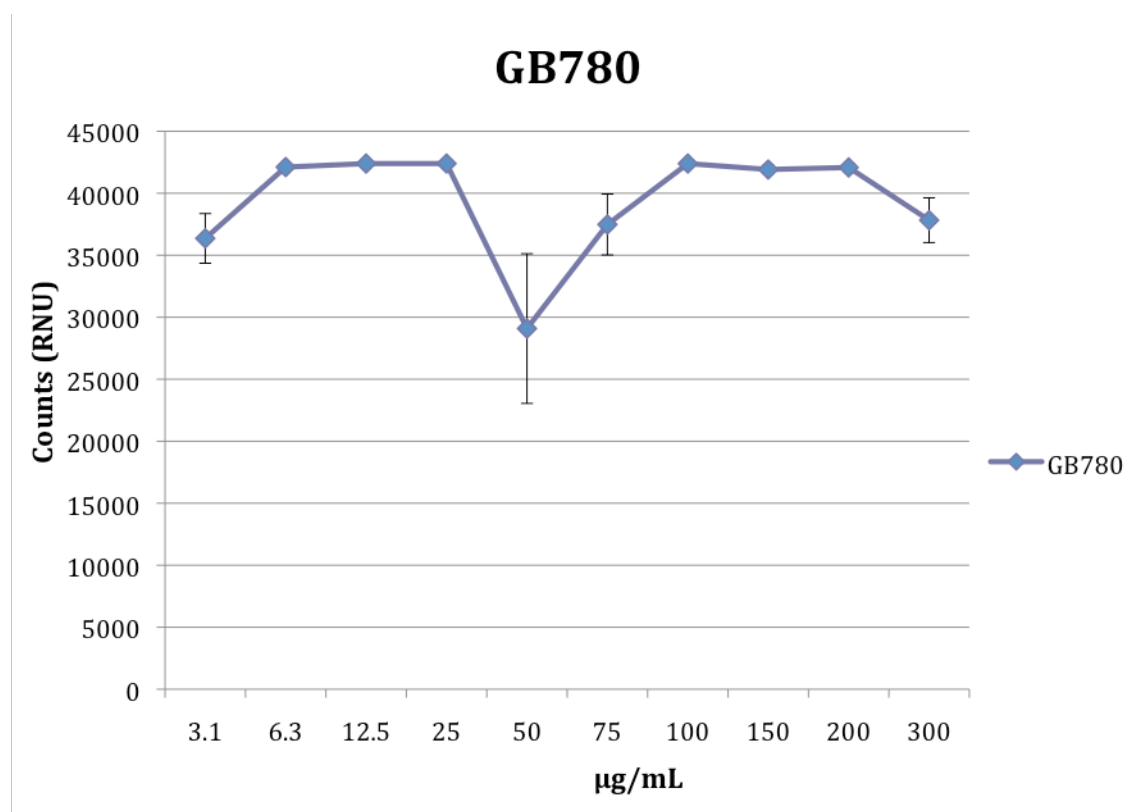
*Truncated graph (minus highest concentrations)

Raw Data

Well N ^o :	GB768B (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	19872	96647	77566	57599	55087	51787	37331	43095	46312	40896	32018	23359
2	19191	96165	79240	67772	51928	46616	48434	49119	47325	67048	32200	25159
3	25133	99203	81898	72286	60240	37204	34614	47328	50650	47791	31371	29514
4	15319	99982	83526	67459	63340	31803	24352	38692	39233	38180	(50502)	18544
5	25012	98144	88320	79780	76139	(99382)	99982	38410	45134	42292	25936	18918
6	14534	99963	81675	69400	55880	42472	39078	36757	33946	35191	26737	19831
7	15645	(83099)	(61795)	48253	40501	32630	21394	(19706)	24407	24908	15728	12143
Avg	19244	96172	79146	66078	57588	48842	43598	39015	41001	42329	30642	21067
STDEV	4121	5517	7754	9500	10124	21703	24514	8994	8508	12039	9703	5148
Avg	19244	98351	82038	66078	57588	40419	43598	42234	41001	42329	27332	21067
**Blank Corrected Avg		79107	62794	46835	38344	21175	24354	22990	21757	23086	8088	1823
STDEV	4451	1656	3732	10261	10935	7964	26478	5124	9189	13004	6308	5560
SE	1682	676	1524	3878	4133	3251	10008	2092	3473	4915	2575	2102

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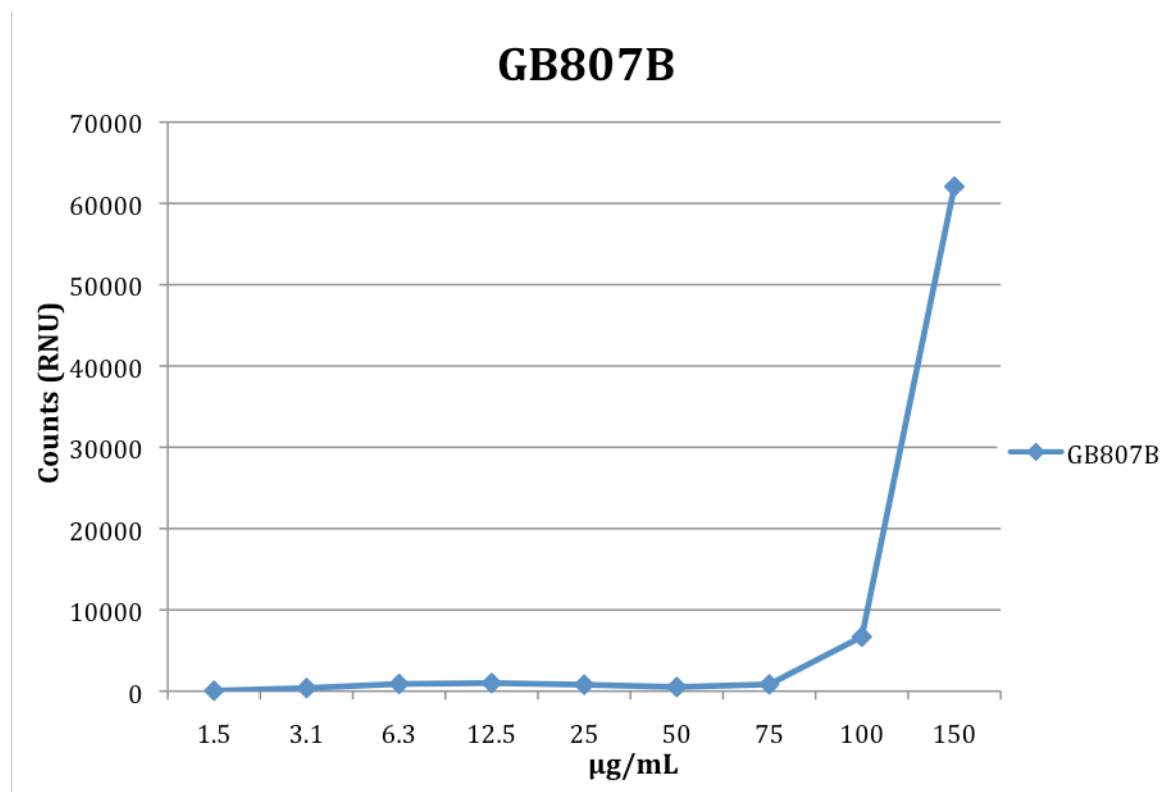
*Truncated graph (minus highest concentrations)

Raw Data

Well N ^o :	GB780 (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	81532	96799	98106	98826	99982	92097	93024	99982	99982	99982	93695	58029
2	47428	90600	99982	99982	99982	99058	99982	99982	99982	99982	92081	83749
3	59179	97534	99982	99982	99982	84696	91523	99982	99982	99982	96573	67469
4	45519	96209	99982	99982	99982	(72859)	68898	99982	99982	99982	99982	67717
5	70297	99624	99982	99982	99982	99982	99982	99982	99982	99978	85569	50559
6	43197	99982	99978	99982	99982	94604	93927	99982	99982	98292	95775	50134
7	55959	87163	(81407)	97783	99982	99978	59397	(59057)	(73216)	(76939)	(71454)	37855
Avg	57587	95416	97060	99503	99982	91896	86676	94136	96158	96448	90733	59359
STDEV	13071	4422	6423	807	0	9286	14787	14321	9366	7986	8896	13924
Avg	57587	95416	99669	99503	99982	95069	86676	99982	99982	99700	93946	59359
**Blank Corrected Avg		37829	42081	41915	42395	37482	29089	42395	42395	42112	36359	1772
STDEV	14119	4776	766	872	0	6013	15972	0	0	690	4907	15040
SE	5336	1805	313	330	0	2455	6037	0	0	282	2003	5685

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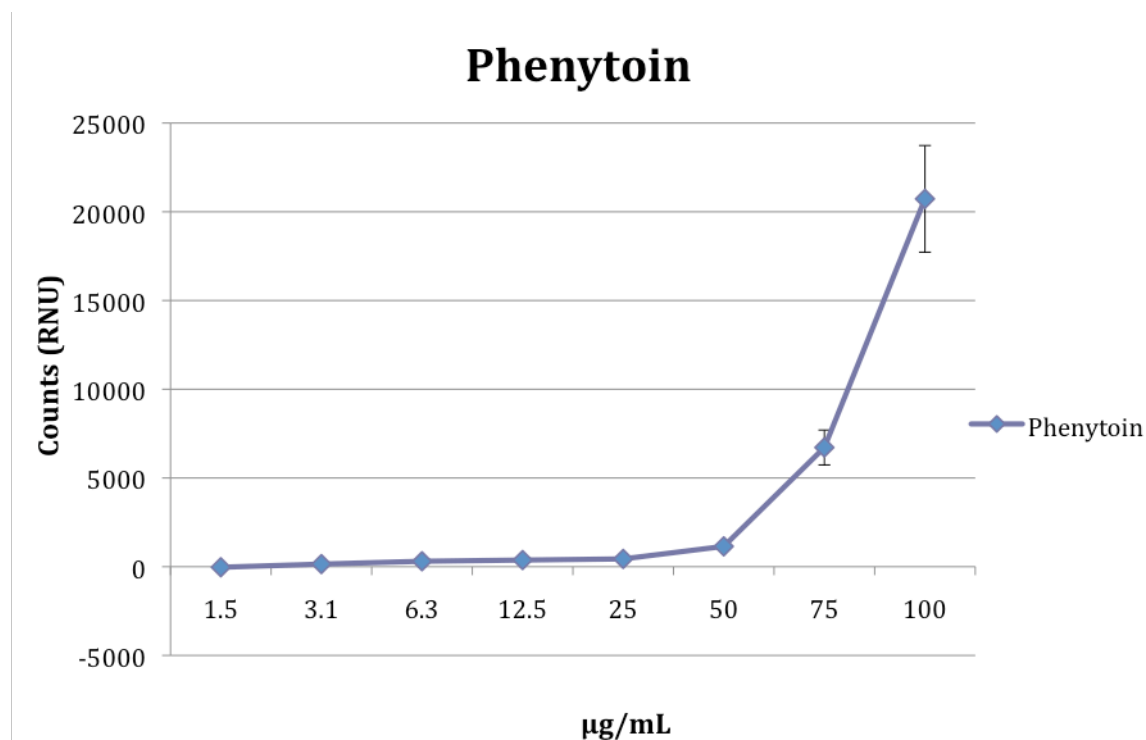
*Truncated graph (minus highest concentrations)

Raw Data

Well N ^o :	GB807B (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	761	99982	99982	69982	1378	1182	1180	1570	1660	1682	1257	642
2	636	99982	99982	90043	(48951)	(4619)	1578	1895	1902	1602	1152	961
3	(1029)	99982	99982	99982	4502	1657	1066	1988	1761	1977	(2519)	670
4	581	(81059)	54832	48894	1673	862	741	1457	1790	1831	1152	785
5	825	99982	99982	45836	8554	1719	1202	1462	1603	1856	1054	(4355)
6	689	99982	43790	47356	17821	2850	1399	1293	1404	1224	1129	670
7	620	99982	99982	37161	10380	868	(3857)	734	(871)	881	670	(8657)
Avg	734	97279	85505	62751	13323	1965	1575	1486	1570	1579	1276	2391
STDEV	143	6622	23080	22530	15479	1254	963	384	321	363	536	2847
Avg	685	99982	85505	62751	7385	1523	1194	1486	1687	1579	1069	746
**Blank Corrected Avg		99297	84819	62065	6699	838	509	800	1001	894	384	60
STDEV	93	0	24930	24335	6265	748	287	414	173	392	206	132
SE	38	0	9422	9198	2558	305	117	157	71	148	84	59

() Denote outliers

**Denotes raw data corrected for outliers by Grubb's T-statistic for detecting outliers.



*Truncated graph (minus highest concentrations)

Raw Data

Well N ^o :	Phenytoin (µg/mL)											
	Blank	300	200	150	100	75	50	25	12.5	6.3	3.1	1.5
1	400	66130	56211	44221	21019	4776	792	599	639	556	433	207
2	218	93604	62926	48675	14294	8479	1712	831	699	598	412	300
3	250	81142	64213	52556	18358	11206	1321	761	732	660	447	230
4	232	65950	61642	51451	28507	6872	1092	600	564	542	389	254
5	325	86249	77594	53305	18147	3892	1522	555	683	613	389	235
6	192	97932	84493	(64664)	12110	5105	1600	903	543	491	431	204
7	287	79728	75051	49373	34559	8586	1870	438	409	(344)	(1650)	175
Avg	272	81534	68876	52035	20999	6988	1416	670	610	543	593	229
STDEV	66	11467	9471	5869	7361	2408	347	154	104	96	432	37
Avg	270	81534	68876	49930	20999	6988	1416	708	643	577	417	238
**Blank Corrected Avg		81264	68606	49661	20730	6719	1146	439	374	307	147	-31
STDEV	72	12385	10230	3319	7951	2601	375	166	113	59	24	40
SE	27	4681	3867	1355	3005	983	142	63	43	24	10	15

() Denote outliers

**Denotes raw data corrected for outliers by Grubb's T-statistic for detecting outliers.