

+++
Use of Design of Experiments
(DoE) software to quickly and
successfully develop
immunoassay methods

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EBF Young Scientist Symposium 2015

Agenda

- + Stat-Ease and Design-Expert Software
 - + Two Level Factorial Designs
 - + Response Surface Optimal Designs
- + Case Study – ADA assay development
 - + Factorial Design
 - + Optimal Design

Stat-Ease and Design-Expert Software

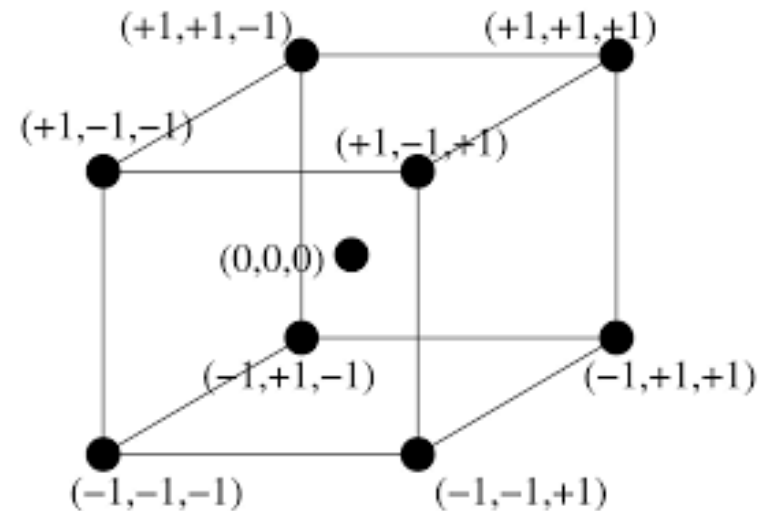


Introduction to Design of Experiments

- + Design of Experiments is a statistical approach to the development and optimisation of assays.
- + Investigates all factors that could affect the assay.
- + Tests a balanced proportion of all the combinations of these factors to cut down on assays
- + Better than a one factor at a time approach as all factors can be simultaneously tested

Introduction to Design of Experiments

- + Generates a model that predicts all responses to every combination of factors.
- + This model can then be used to decide
 - + the optimal conditions for you
 - + check robustness of a method
 - + decide on tolerance limits
 - + assist with assay troubleshooting.



Two Level Factorial Designs

- + Factorial designs are used to assess all the factors which you think might effect the assay.
- + They can be used to eliminate any factors that don't effect the assay.

Regular Two-Level Factorial Design
 Design for 2 to 21 factors where each factor is set to 2 levels. Useful for estimating main effects and interactions. Fractional factorials can be used for screening many factors to find the significant few. The color coding represents the design: White (Full Factorial), Yellow (Resolution III), Green (Resolution IV), Red (Ruggedness testing) - Res III.

Number of Factors

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4	2^2	2^{3-1}																		
8		2^3	2^{4-1}	2^{5-2}	2^{6-3}	2^{7-4}														
16			2^4	2^{5-1}	2^{6-2}	2^{7-3}	2^{8-4}	2^{9-5}	2^{10-6}	2^{11-7}	2^{12-8}	2^{13-9}	2^{14-10}	2^{15-11}						
32				2^5	2^{6-1}	2^{7-2}	2^{8-3}	2^{9-4}	2^{10-5}	2^{11-6}	2^{12-7}	2^{13-8}	2^{14-9}	2^{15-10}	2^{16-11}	2^{17-12}	2^{18-13}	2^{19-14}	2^{20-15}	2^{21-16}
64					2^6	2^{7-1}	2^{8-2}	2^{9-3}	2^{10-4}	2^{11-5}	2^{12-6}	2^{13-7}	2^{14-8}	2^{15-9}	2^{16-10}	2^{17-11}	2^{18-12}	2^{19-13}	2^{20-14}	2^{21-15}
128						2^7	2^{8-1}	2^{9-2}	2^{10-3}	2^{11-4}	2^{12-5}	2^{13-6}	2^{14-7}	2^{15-8}	2^{16-9}	2^{17-10}	2^{18-11}	2^{19-12}	2^{20-13}	2^{21-14}
256							2^8	2^{9-1}	2^{10-2}	2^{11-3}	2^{12-4}	2^{13-5}	2^{14-6}	2^{15-7}	2^{16-8}	2^{17-9}	2^{18-10}	2^{19-11}	2^{20-12}	2^{21-13}
512								2^9	2^{10-1}	2^{11-2}	2^{12-3}	2^{13-4}	2^{14-5}	2^{15-6}	2^{16-7}	2^{17-8}	2^{18-9}	2^{19-10}	2^{20-11}	2^{21-12}

Optimal Designs

- + Optimal designs are used once the significant factors have been decided.
- + An experimental design is created that can interpret the optimal conditions from only a few assays.
- + This design space can be used again and again to run through various scenarios using the original data set.

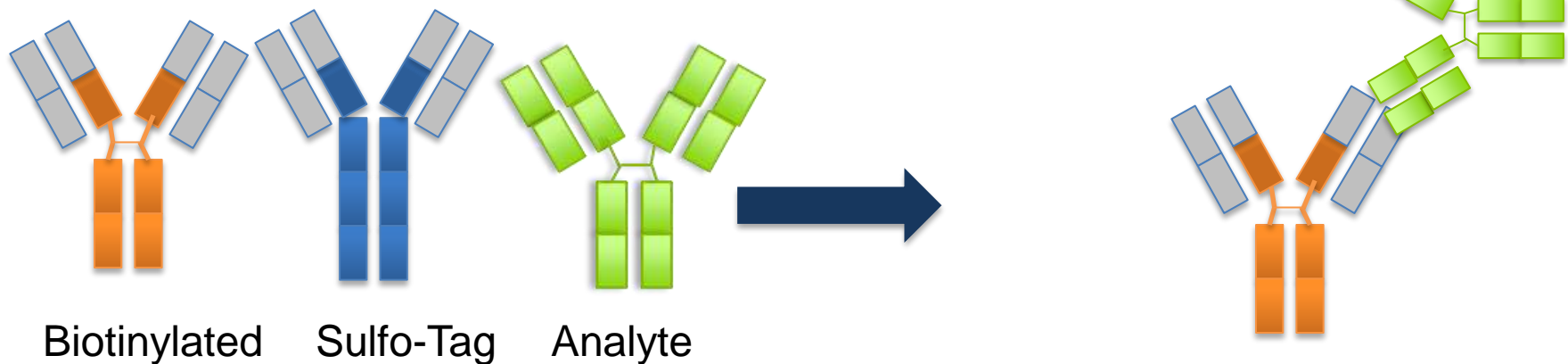
ADA assay development

ADA assay development

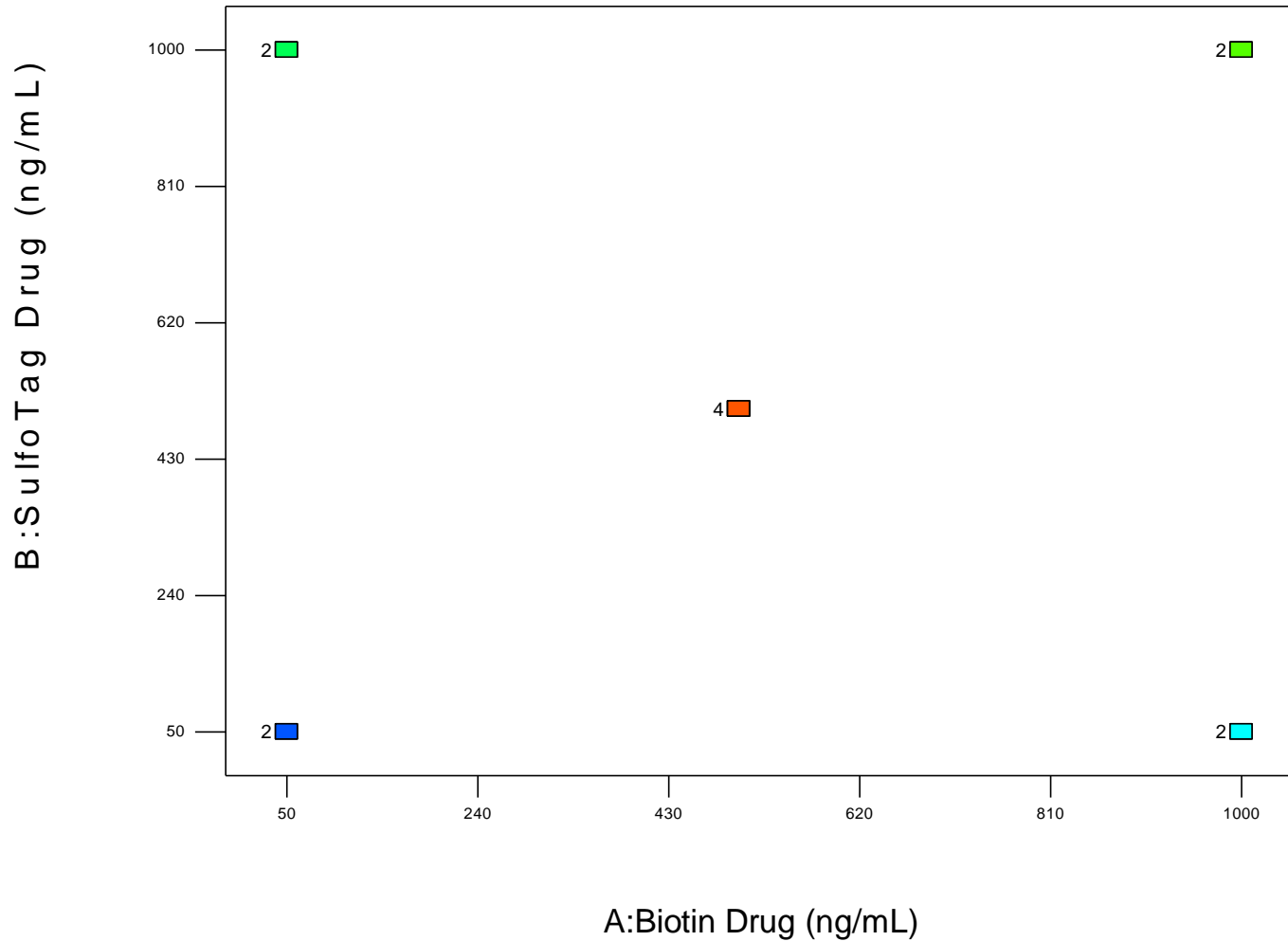
- + An anti-drug antibodies assay needed developing on the MSD platform.
- + Optimal concentrations were assessed for
 - + Biotinylated drug
 - + Sulfo-TAG drug
 - + Percentage of matrix (minimum required dilution (MRD)).

Mastermix procedure

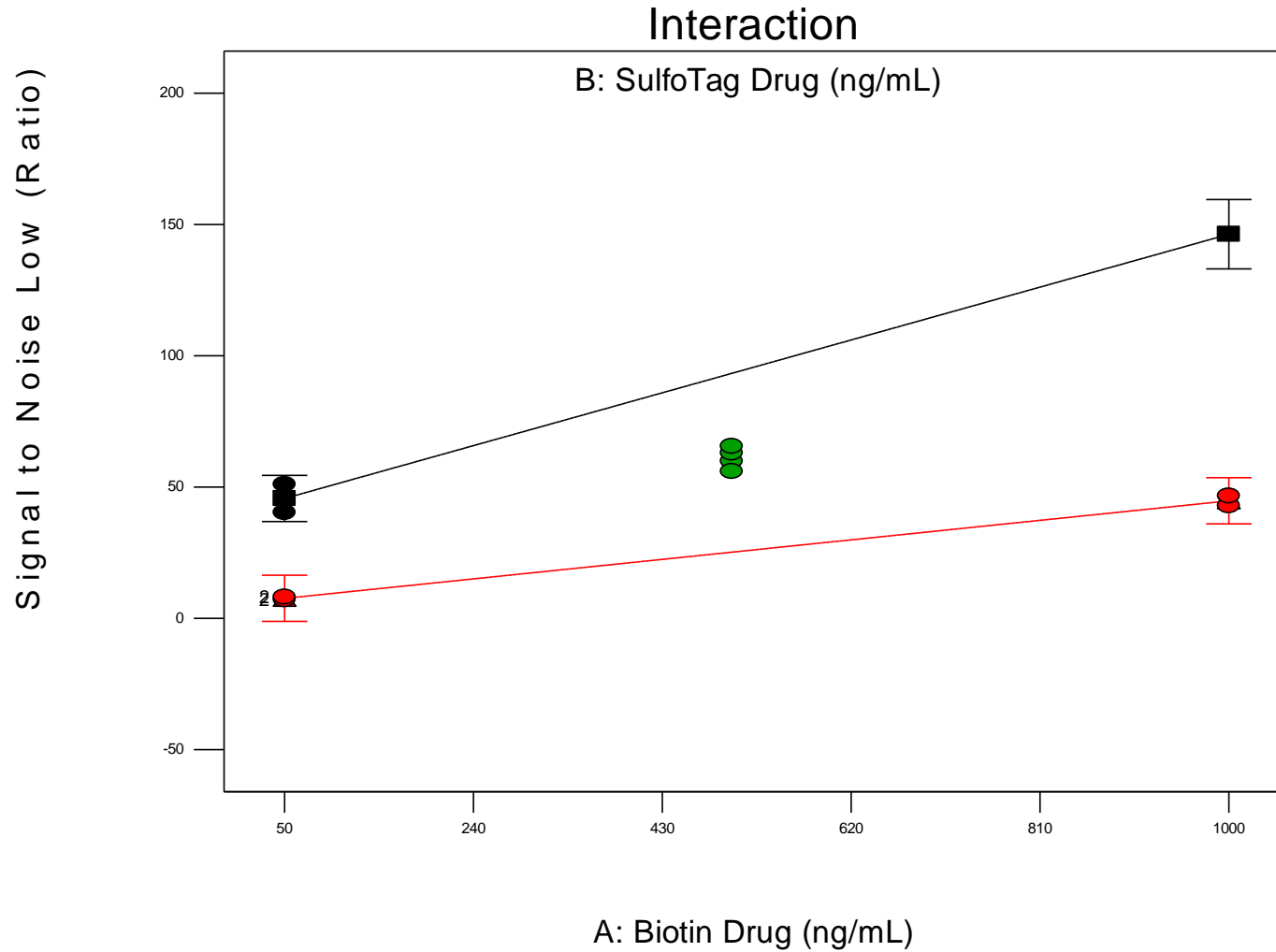
- + Create mastermix by incubating Biotinylated drug, Sulfo-Tagged drug and samples together overnight.
- + Block MSD streptavidin plate overnight.
- + Wash MSD plate and transfer mastermix.
- + Incubate for 2 hours before reading plate.



ADA factorial design space

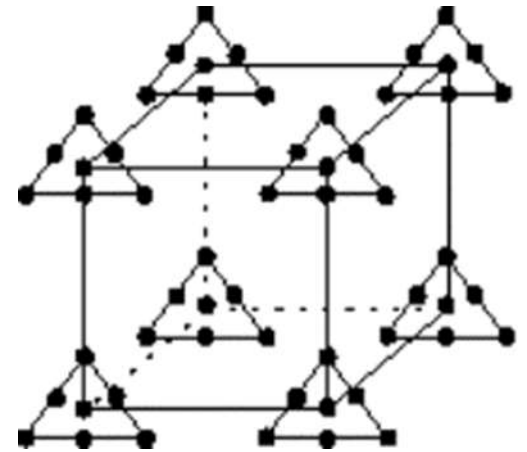


ADA signal to noise response



ADA Factorial design

- + The factorial design was suggesting the best signal to noise ratio was;
- + 50 ng/ml SulfoTag and 1000 ng/mL Biotinylated drug.
- + These were the limits of our original design space.

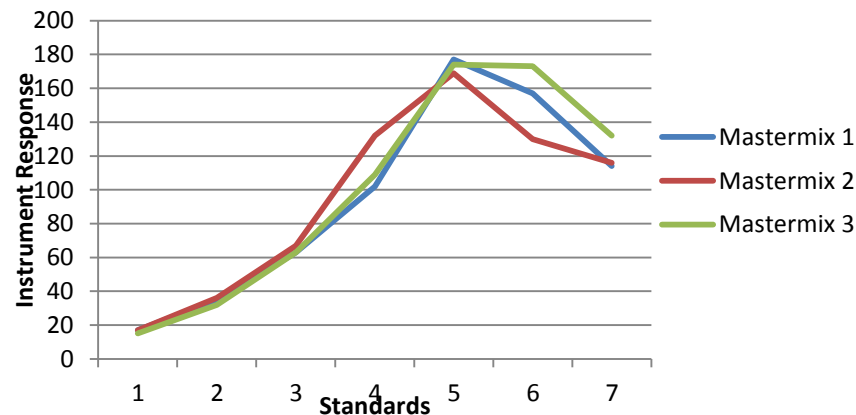


Prozone

Mastermix Assay

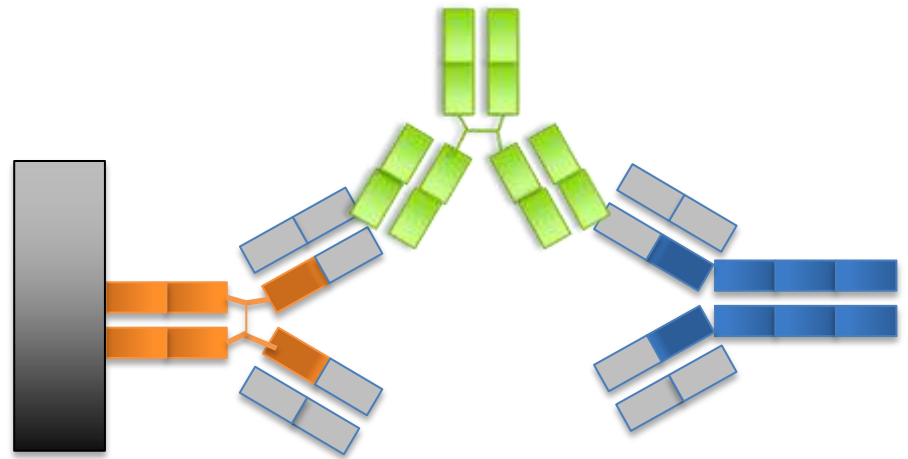
	Instrument response x1000		
	Response	Response	Response
STD.1	17	17	15
STD.2	35	36	32
STD.3	63	67	63
STD.4	102	132	109
STD.5	177	169	174
STD.6	157	130	173
STD.7	114	116	132
Biotin (ng/mL)	500	500	500
SulfoTag (ng/mL)	250	250	250

- + Prozone seen when SulfoTag concentrations are too low.
- + Mastermix assay not the appropriate procedure.



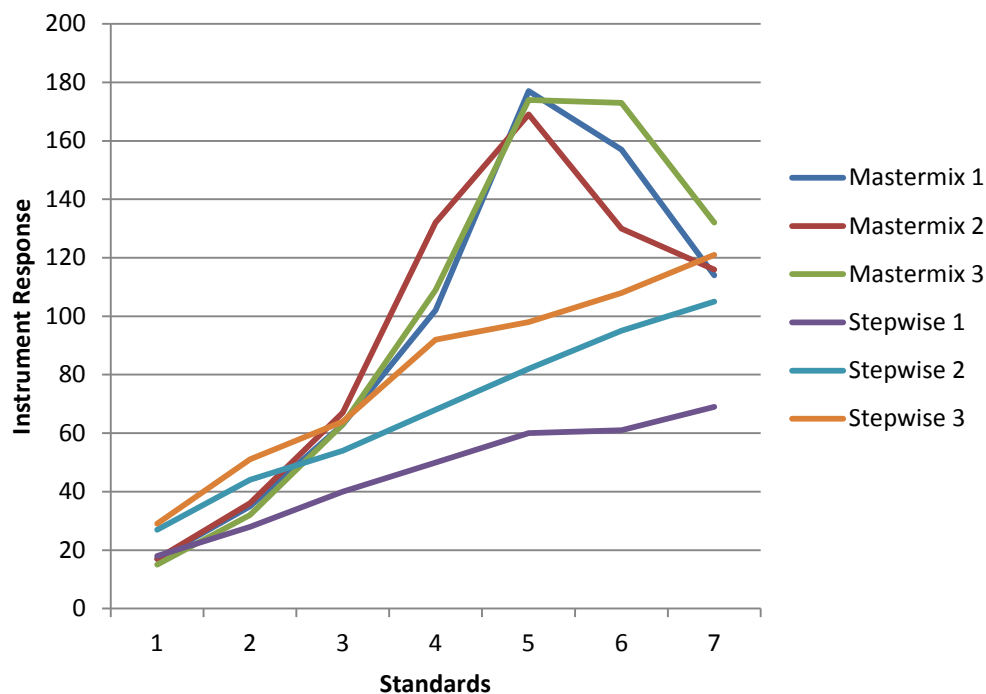
Stepwise procedure

- + Coat MSD streptavidin plate with Biotinylated drug. Incubate 1.5 hours.
- + Wash and block plate for 1.5 hours.
- + Wash and load samples. Incubate overnight (2-8°C).
- + Wash and add Sulfo-Tagged drug. Incubate for 1.5 hours.
- + Wash and add read buffer.



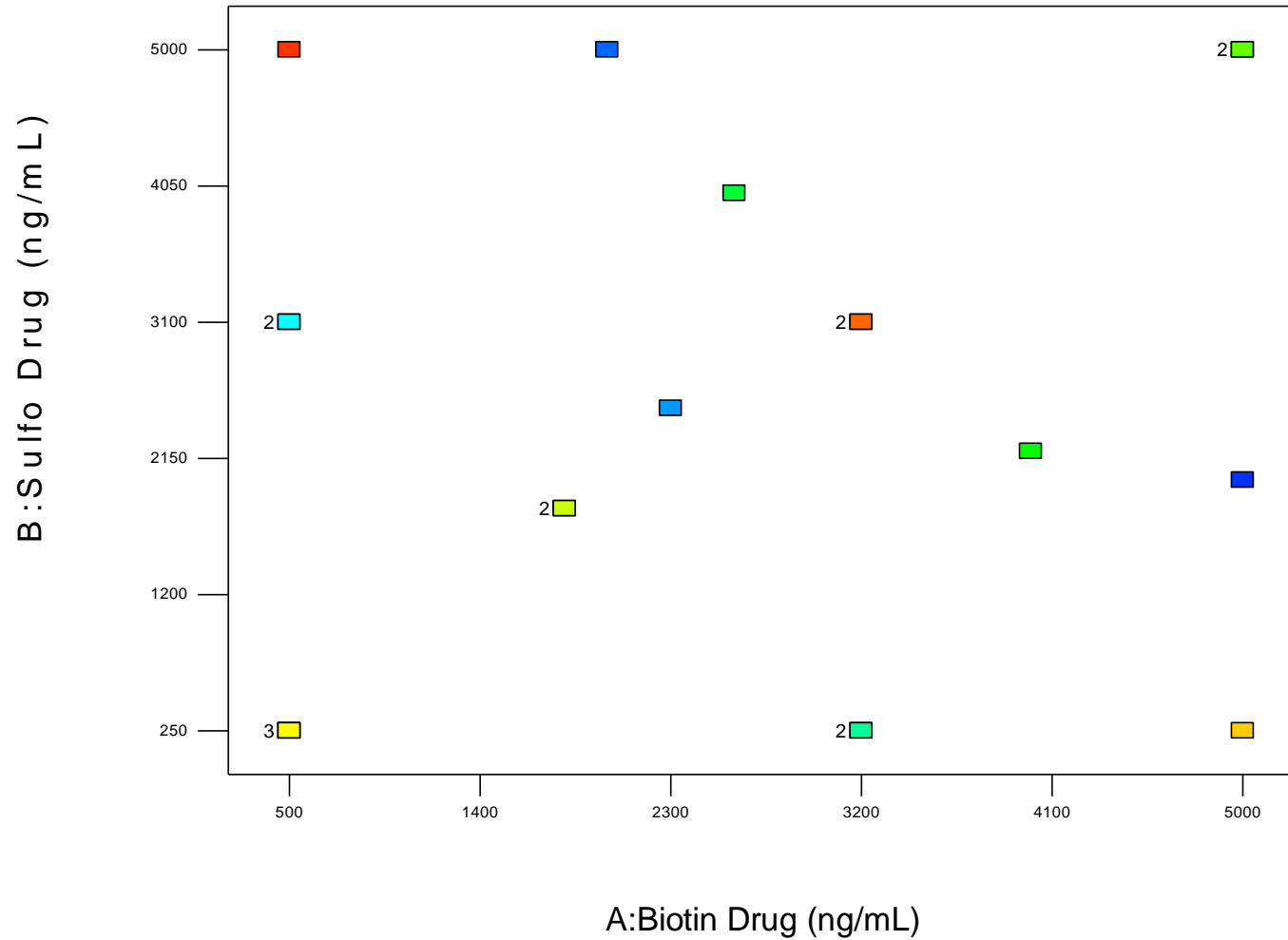
Prozone

Mastermix Assay vs Stepwise assay

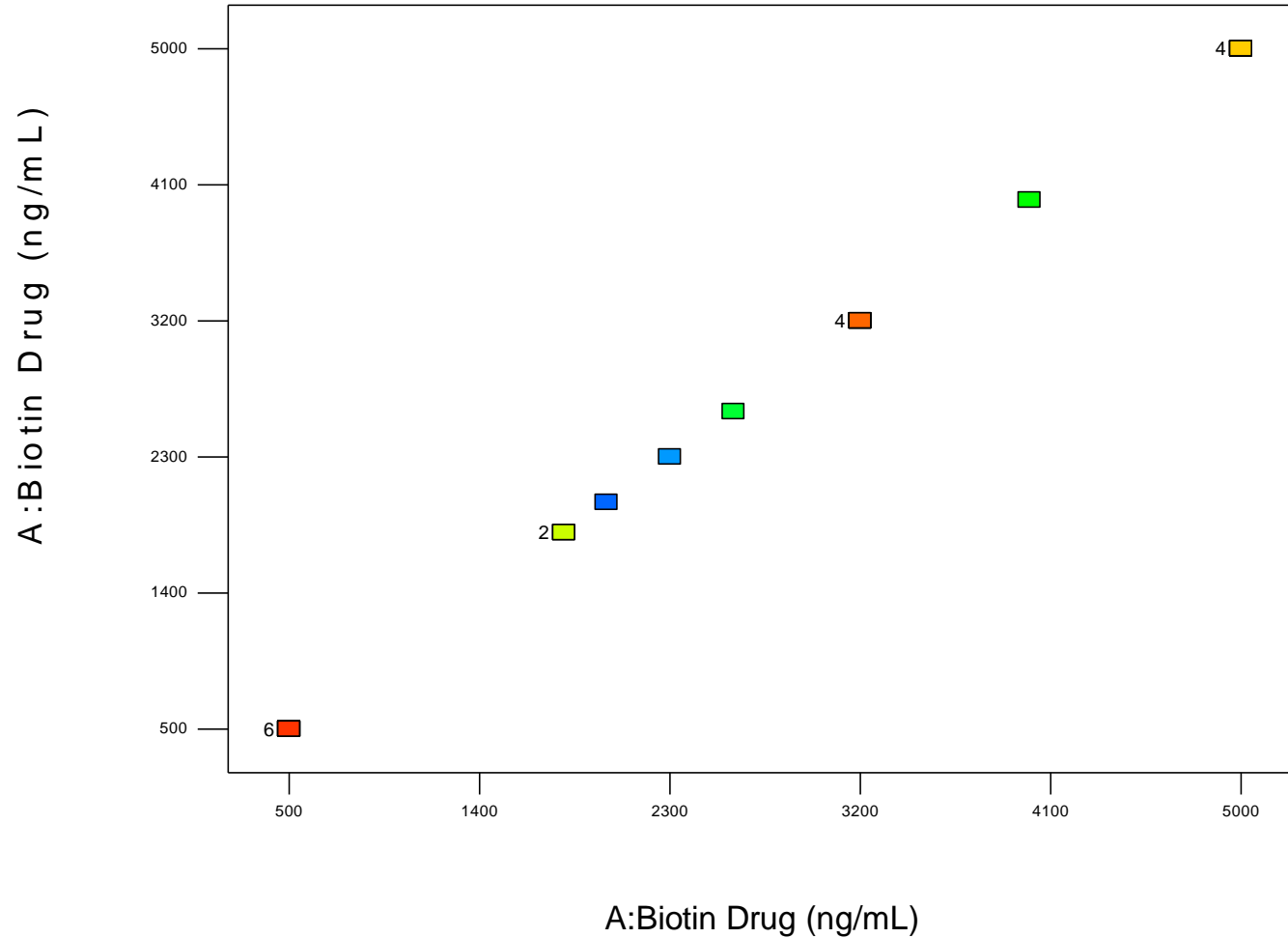


	Instrument response x1000		
	Response	Response	Response
STD.1	18	27	29
STD.2	28	44	51
STD.3	40	54	64
STD.4	50	68	92
STD.5	60	82	98
STD.6	61	95	108
STD.7	69	105	121
Biotin (ng/mL)	500	500	500
SulfoTag (ng/mL)	250	250	250

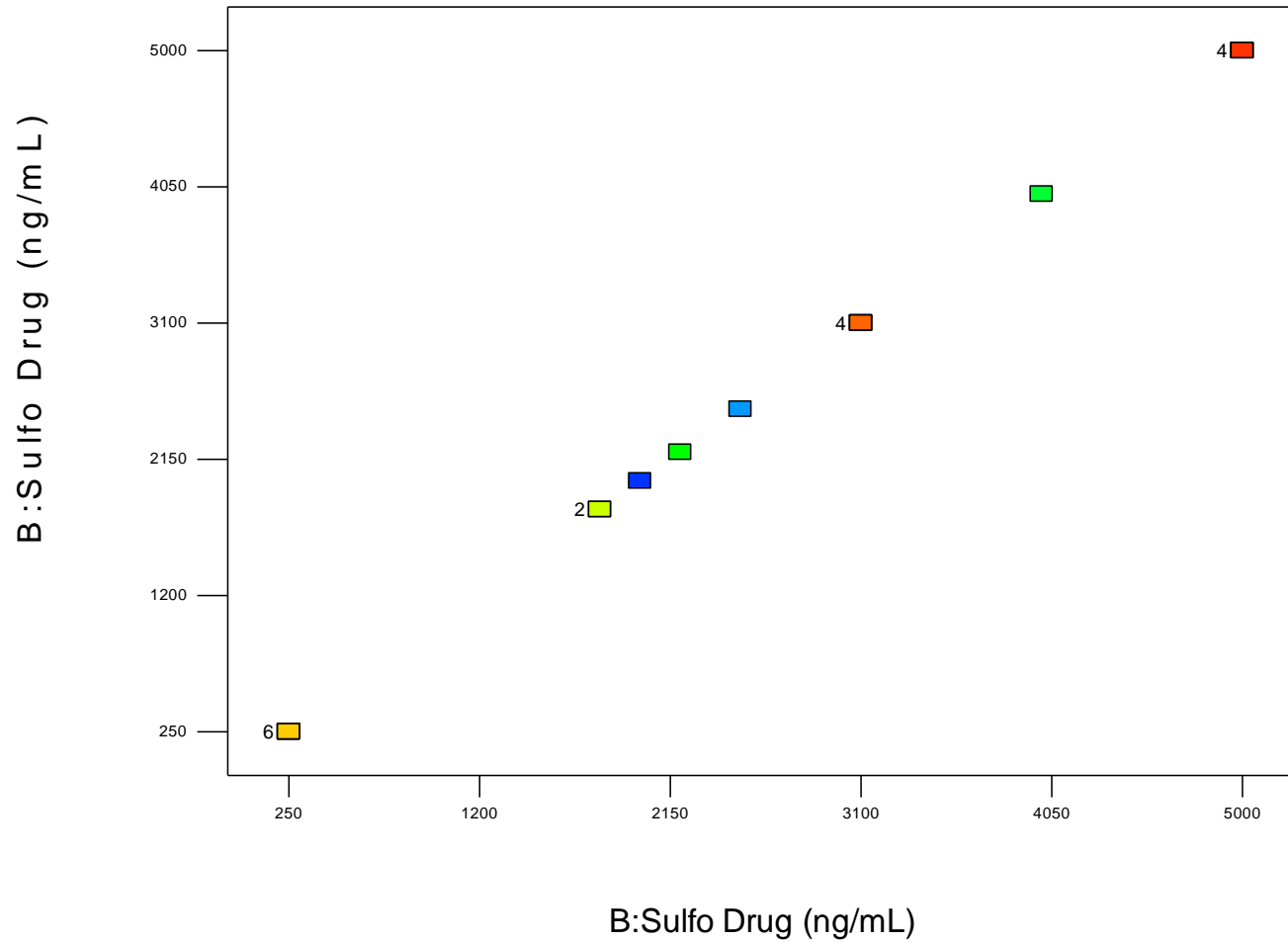
Stepwise optimal design space



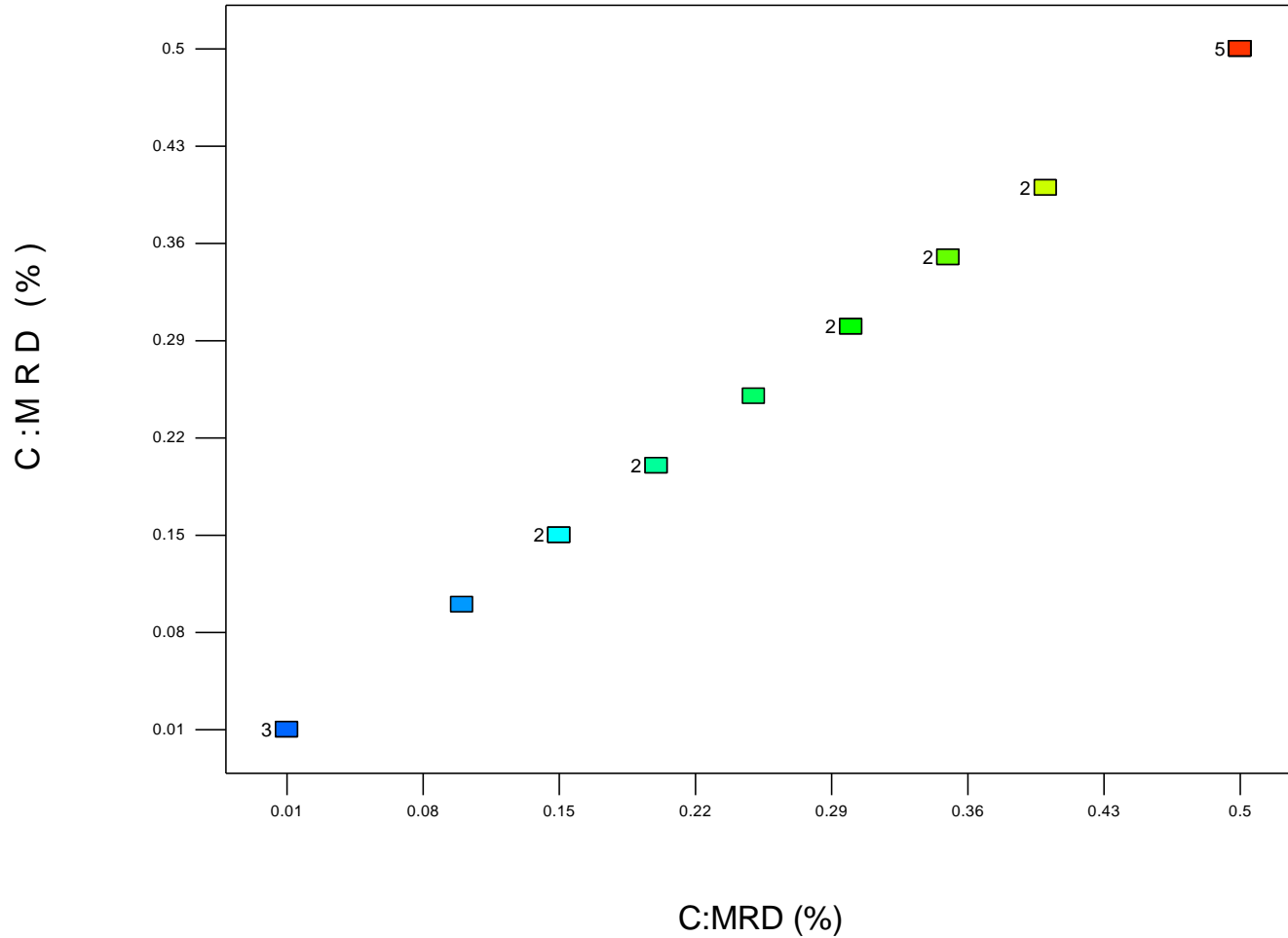
Stepwise optimal Biotinylated drug



Stepwise optimal Sulfo-TAG drug



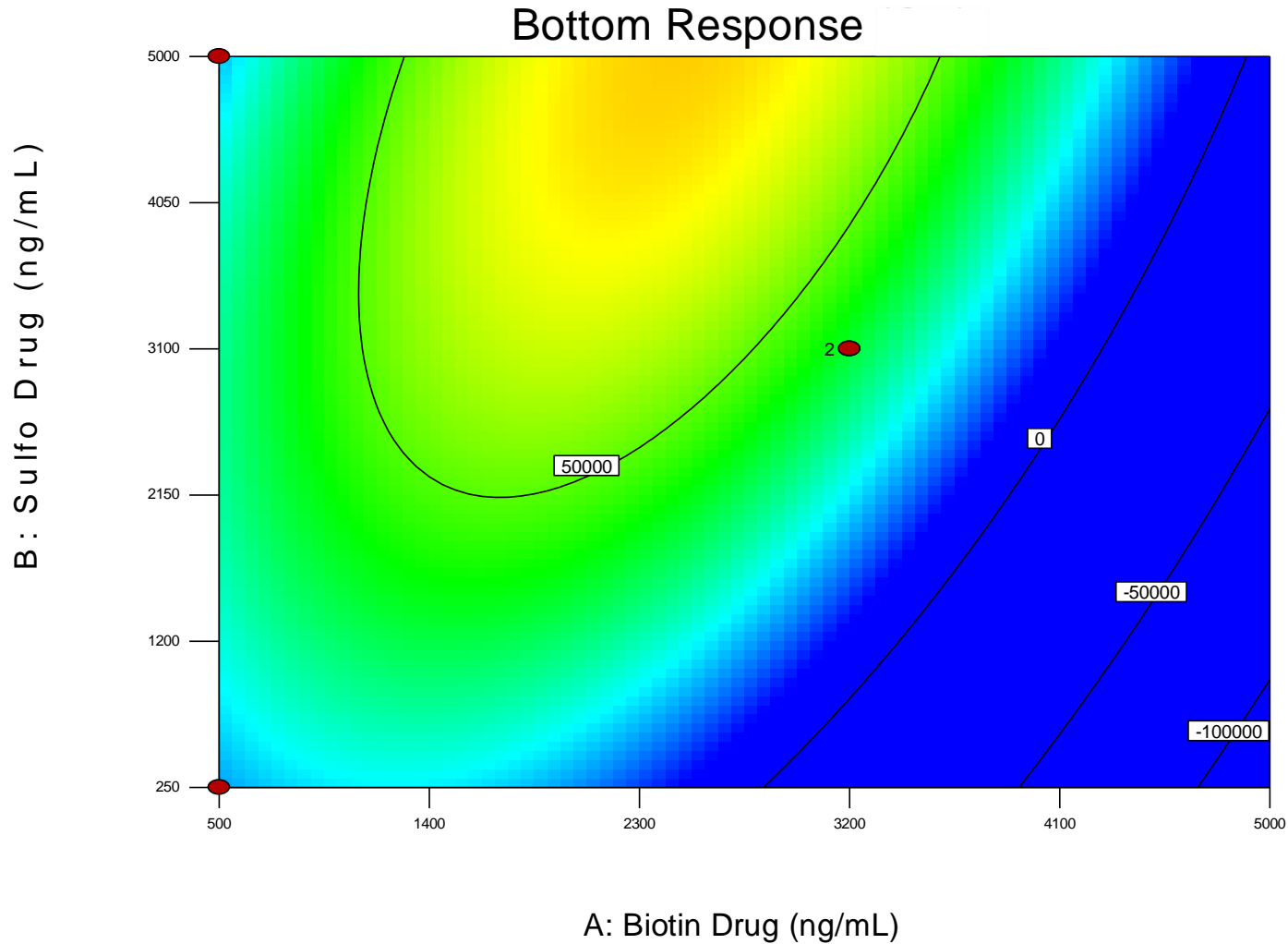
Stepwise optimal MRD



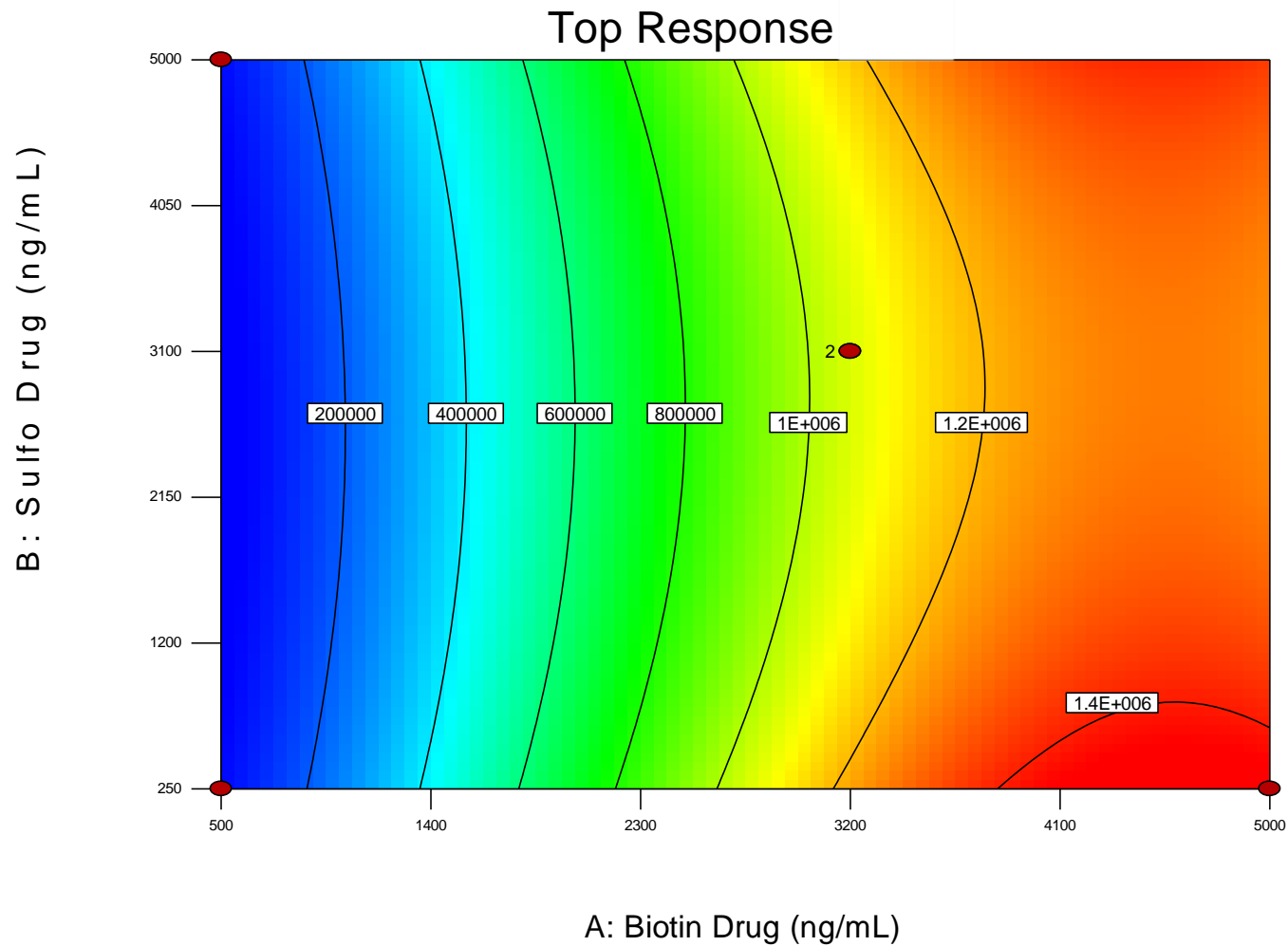
Summary of assays

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6
	A:Biotin Drug	B:Sulfo Drug	C:MRD	Background	Bottom Response	Top Response	Signal To Noise (low)	Signal To Noise (high)	Buffer Response
	ng/mL	ng/mL	%	OD	OD	OD	Ratio	Ratio	OD
1	2300	2500	0.1	216.625	77179	591232	356.279	2729.29	502
2	2600	4000	0.3	284.625	59478	900637	208.97	3164.29	323
3	3200	3100	0.5	251.75	44982	1.28069E+006	178.677	5087.14	309
4	500	250	0.5	71.25	18008.5	68952	252.751	967.747	69
5	500	5000	0.5	223.125	24398.5	72682	109.349	325.746	852.5
6	1800	1800	0.4	125.75	53980.5	381402	429.268	3033.01	176
7	3200	3100	0.5	276.75	36776	982437	132.885	3549.91	531
8	500	3100	0.15	182.375	25676	69938.5	140.787	383.487	207.5
9	5000	2000	0.01	628.375	11220.5	1.42715E+006	17.8564	2271.18	681
10	500	3100	0.15	156.25	33967.5	119263	217.392	763.28	159
11	3200	250	0.2	78.25	22379	1.39115E+006	285.994	17778.2	70
12	5000	250	0.5	150	7955	1.36418E+006	53.0333	9094.51	156
13	4000	2200	0.3	612	13016	1.42418E+006	21.268	2327.09	555
14	5000	5000	0.35	2041	16271.5	1.40212E+006	7.97232	686.975	2305
15	500	250	0.01	62.125	27685	105083	445.634	1691.48	58
16	3200	250	0.2	83.25	29065.5	1.35965E+006	349.135	16332.1	71
17	5000	5000	0.35	2704.38	17965.5	1.43745E+006	6.64312	531.526	3464
18	1800	1800	0.4	185.125	63085.5	733289	340.772	3961.05	226
19	500	250	0.25	64.5	29343.5	121188	454.938	1878.88	64
20	2000	5000	0.01	429	65672.5	883278	153.083	2058.92	364.5

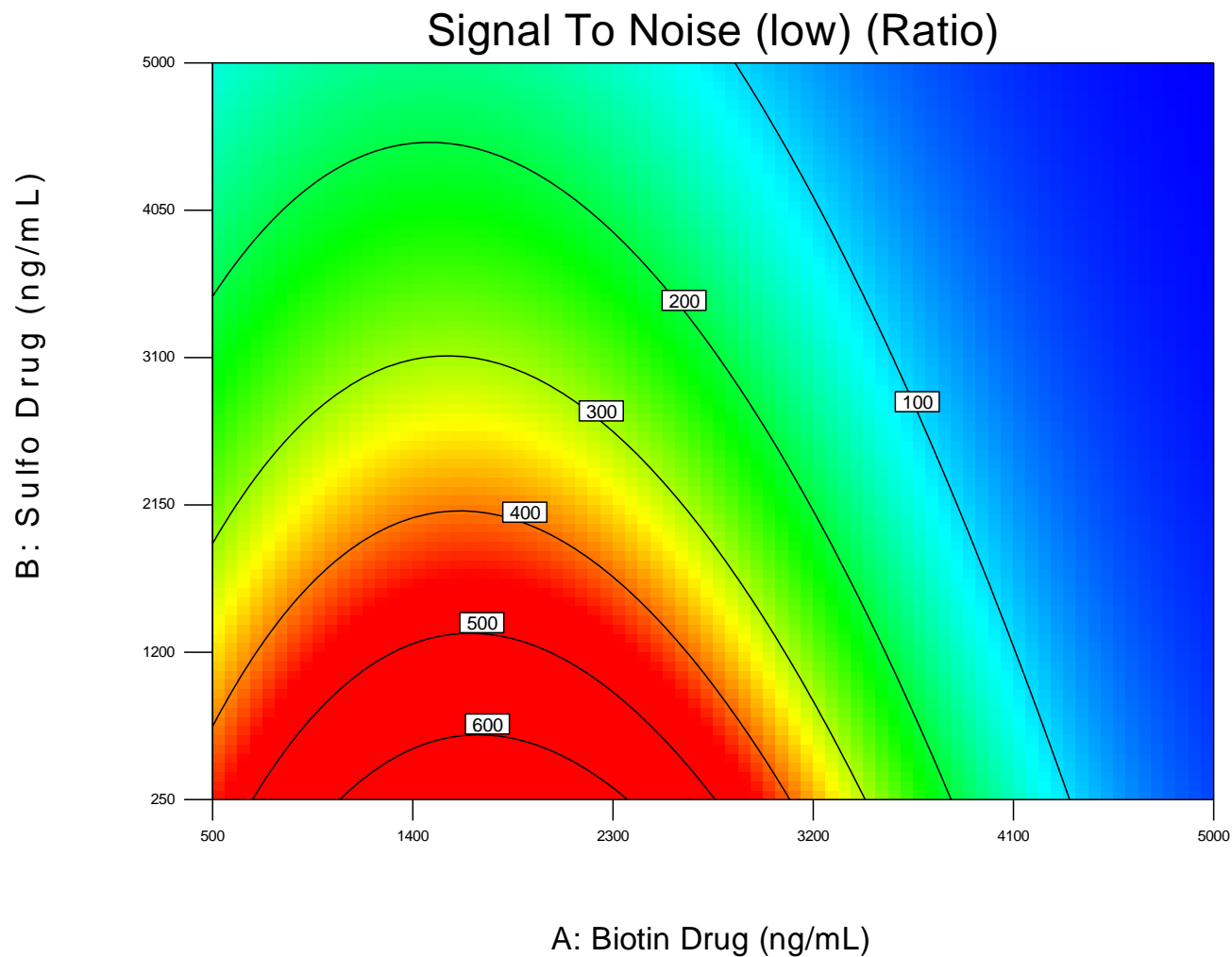
Step Wise Bottom response



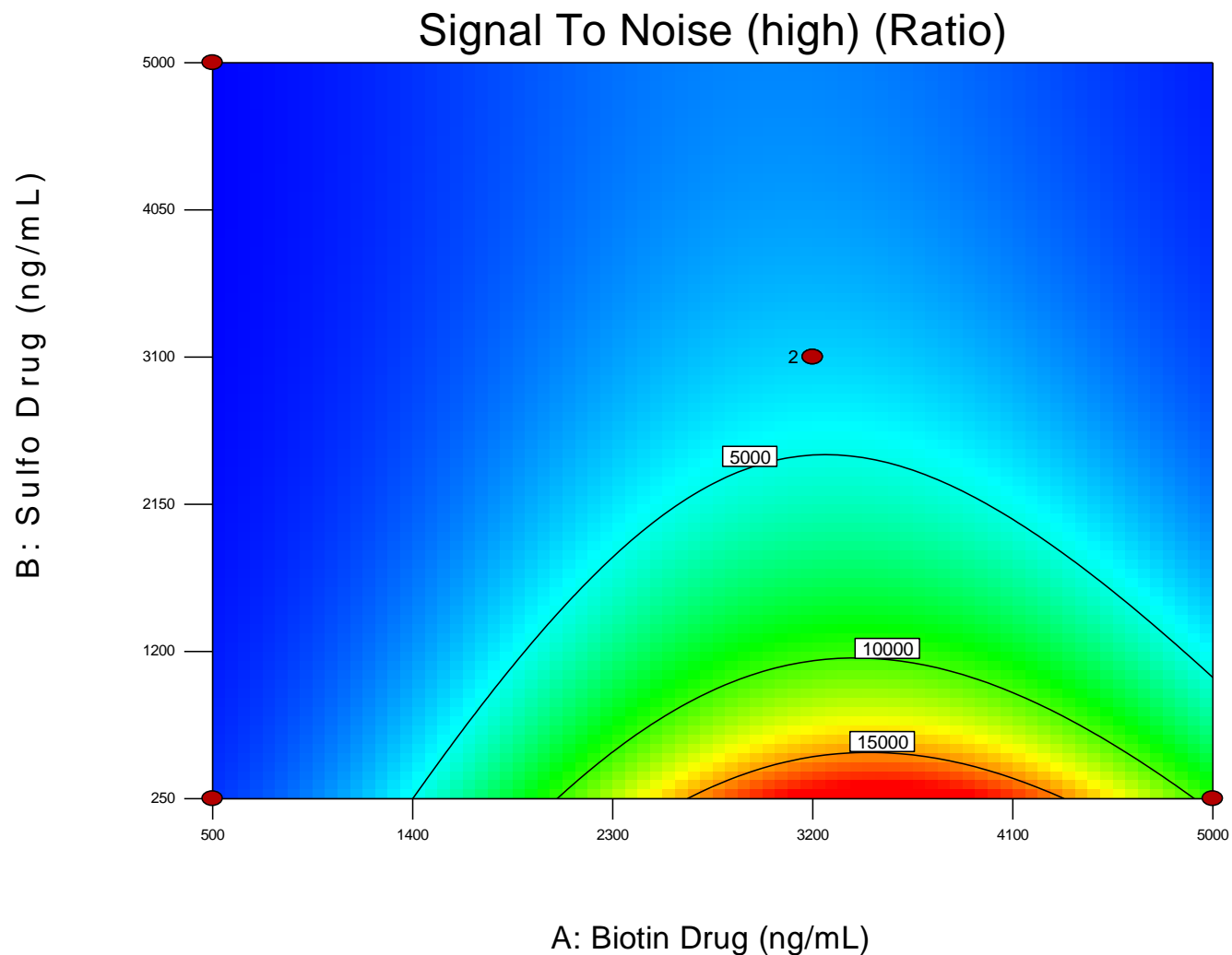
Step Wise Top response



Step Wise Signal to Noise (bottom response)



Step Wise Signal to Noise (top response)



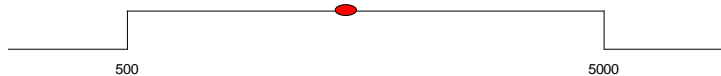
How to optimise the assay

The screenshot displays a software interface for assay optimization. The main window is titled "Signal To Noise (low)". The left sidebar shows a tree view of the assay design, including "Design (Actual)" and "Analysis" sections. The "Analysis" section is expanded, showing various response criteria like "R1: Background (An)", "R2: Bottom Respons", "R3: Top Response (/)", "R4: Signal To Noise (/)", "R5: Signal To Noise (/)", "R6: Buffer Response", and "R7: S/N Buffer/Blank".

The main panel shows the configuration for the "Signal To Noise (low)" criterion. The goal is set to "maximize". The limits are defined as Lower: 6.64312 and Upper: 454.938. The weights are set to 1 for both. The importance is set to "+++++", which is highlighted in the dropdown menu. The importance dropdown menu shows options: "+", "++", "+++", "++++", and "+++++", with "+++++" selected.

Below the configuration, a graph shows the "Signal To Noise (low)" response. The x-axis is labeled "Signal To Noise (low)" and has two vertical lines indicating the limits at 6.64312 and 454.938. A small square marker is positioned on the line between these two values.

Step Wise Solutions



A: Biotin Drug = 2572.73



B: Sulfo Drug = 884.763



C: MRD = 0.0100002



Background = 95.7181



Bottom Response = 63847.7



Top Response = 1.01022E+006



Signal To Noise (low) = 454.938



Buffer Response = 111.48

Desirability = 0.887

Outcome of ADA development

- + Suggested solution of
 - + Biotinylated drug (2573 ng/mL)
 - + SulfoTag drug (885 ng/mL)
 - + MRD (1 in 100)
- + Would result in
 - + Background of 96
 - + Bottom response of 63848
 - + Top response of over 1 million
 - + S/N (low) of 455

Summary

- + DoE a quick way to effectively develop an assay
 - + 20x assays performed by 1 person
 - + Alternative would take 5 times longer
- + One experiment can give you all the answers you need
- + Result predictions from the software tend to be accurate
- + Less repeat work since using Design Expert
- + Saves on reagents and cuts costs
- + Model can be re-used if assay requirements change or the assay needs troubleshooting