

## P100 Synthetic Heavy Mix Stock Creation

December 8, 2015 ~CF

Heavy synthetic versions of the 96 P100 peptides were obtained from New England Peptide. These peptides arrived in stocks of 1mM (1 nmol/uL) and were arranged in a 96-well plate as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A	bi5340	bi5341	bi5342	bi5343	bi5344	bi5345	bi5346	bi5347	bi5348	bi5349	bi5350	bi5351
B	bi5352	bi5353	bi5354	bi5355	bi5356	bi5357	bi5358	bi5359	bi5360	bi5361	bi5362	bi5363
C	bi5364	bi5365	bi5366	bi5367	bi5368	bi5369	bi5370	bi5371	bi5372	bi5373	bi5374	bi5375
D	bi5376	bi5377	bi5378	bi5379	bi5380	bi5381	bi5382	bi5383	bi5384	bi5385	bi5386	bi5387
E	bi5388	bi5389	bi5390	bi5391	bi5392	bi5393	bi5394	bi5395	bi5396	bi5397	bi5398	bi5399
F	bi5400	bi5401	bi5402	bi5403	bi5404	bi5405	bi5406	bi5407	bi5408	bi5409	bi5410	bi5411
G	bi5412	bi5413	bi5414	bi5415	bi5416	bi5417	bi5418	bi5419	bi5420	bi5421	bi5422	bi5423
H	bi5424	bi5425	bi5426	bi5427	bi5428	bi5429	bi5430	bi5431	bi5432	bi5433	bi5434	bi5435

After "1x Heavy Mix" concentrations had been determined, 1mM peptides were aliquoted into 5 stock plates with the following volumes:

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
B	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	3.71	2.00	2.00
C	2.00	2.00	2.00	2.00	7.50	2.00	2.00	2.00	15.75	2.00	2.00	2.00
D	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
E	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
F	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	6.81	2.00
G	6.13	8.88	2.00	2.00	3.47	2.00	7.50	2.00	2.00	2.00	2.00	2.00
H	2.00	2.00	2.00	2.00	2.00	2.29	2.00	2.00	2.00	2.00	2.00	2.00

The goal of this procedure is to dilute these peptides to varying intermediate concentrations so that they can be used to create a 50x concentration Heavy Mix Stock to be frozen. This procedure can be done manually or via automated protocols using a Perkin Elmer JANUS (Bender). The following instructions are for the automated procedure but a manual protocol should be inferred based on the automated steps.

1mM stock plates should be diluted with the following volumes (uL) of 3%ACN/5%FA to reach the desired concentrations in pmol/uL:

Volume of 3%ACN/5%FA (uL):

	1	2	3	4	5	6	7	8	9	10	11	12
A	198.00	18.00	18.00	198.00	198.00	198.00	18.00	18.00	18.00	18.00	18.00	18.00
B	18.00	18.00	18.00	198.00	198.00	198.00	198.00	198.00	198.00	3.71	198.00	18.00
C	18.00	18.00	18.00	18.00	7.50	198.00	198.00	198.00	0.00	18.00	198.00	198.00
D	18.00	198.00	18.00	18.00	18.00	198.00	198.00	18.00	198.00	18.00	198.00	18.00
E	18.00	198.00	198.00	198.00	18.00	18.00	198.00	18.00	198.00	18.00	198.00	198.00
F	198.00	198.00	198.00	198.00	18.00	198.00	198.00	198.00	18.00	18.00	6.81	18.00
G	6.13	8.88	18.00	198.00	3.47	18.00	7.50	18.00	18.00	18.00	198.00	18.00
H	18.00	198.00	18.00	18.00	198.00	2.29	18.00	198.00	198.00	18.00	198.00	18.00

Desired intermediate concentrations (pmol/uL):

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	100	100	10	10	10	100	100	100	100	100	100
B	100	100	100	10	10	10	10	10	10	500	10	100
C	100	100	100	100	500	10	10	10	1000	100	10	10
D	100	10	100	100	100	10	10	100	10	100	10	100
E	100	10	10	10	100	100	10	100	10	100	10	10
F	10	10	10	10	100	10	10	10	100	100	500	100
G	500	500	100	10	500	100	500	100	100	100	10	100
H	100	10	100	100	10	500	100	10	10	100	10	100

**Note:** All peptides with a desired intermediate concentration of 500 pmol/uL and above should have reagents added manually. These are labeled in **BLUE**.

Worklist 1. at the end of this protocol can be used with the Intermediate dilutions protocol to add the appropriate volumes of 3%ACN/5%FA to the correct samples.

**Protocol:**

C:\Packard\JANUS\Protocol Files\1\_IntermediateDilutionsForHeavyFormulation\_20151208

**Worklist:**

C:\Packard\JANUS\Worklists\Heavy Mix\1\_IntermediateDilutionWorklist

Worklist 2. at the end of this protocol can be used with the 50x Heavy Formulation protocol to create the 50x Heavy Mix in a 1.7mL epi tube. The table below shows volumes of diluted peptides that need to be added to the 50x Heavy Mix. Again, peptides with intermediate concentrations of 500 pmol/uL and above must be added to the mix manually. These are labeled in **BLUE**.

**Protocol:**

C:\Packard\JANUS\Protocol Files\2\_50xHeavyFormulation\_20151208

**Worklist:**

C:\Packard\JANUS\Worklists\2\_50XHeavyFormulationWorklist\_25Plates

Volume (uL) of peptide to add to 50x Heavy Mix

	1	2	3	4	5	6	7	8	9	10	11	12
A	6.22	10.22	8.25	6.71	10.83	13.75	3.81	4.95	8.56	6.87	2.20	2.09
B	4.07	2.24	2.82	1.65	2.92	8.42	4.72	4.41	8.25	4.95	4.70	1.53
C	4.37	4.13	2.49	2.70	11.00	6.57	4.91	4.63	13.75	2.21	6.57	4.46
D	2.02	9.82	6.81	5.61	1.49	2.96	6.92	3.32	8.79	4.13	10.13	2.54
E	11.00	6.18	3.27	7.73	1.85	4.13	13.75	2.75	5.90	4.31	6.92	10.38
F	6.46	8.75	4.18	5.32	5.89	5.01	6.37	8.47	1.63	2.78	9.63	6.48
G	8.25	13.75	8.11	4.48	4.63	6.27	11.00	2.40	3.41	6.79	11.45	3.41
H	1.60	3.14	1.93	2.82	13.34	3.06	3.18	3.28	3.99	1.67	3.00	4.13

Total Peptide Volume: 545.7uL

Balance Reagent: 4.3uL

The total volume of peptides will be 545.7uL. This must be brought up to 550uL by adding 4.3uL of 3%ACN/5%FA to the mix for the total mix to be at 50x concentration. Vortex and spin down the mix, then aliquot 22uL each into 25 separate 1.7mL epi tubes. These 22uL aliquots will be thawed and diluted to 1x concentration with 1078uL of 3%ACN/5%FA as needed to resuspend one full plate of samples (960uL required).

**Worklist 1. Intermediate Concentration Plate**

Well	Rack	900uL Dispense Volume (uL)	175uL Dispense Volume (uL)	50uL Dispense Volume (uL)
A01	Sample	198	0	0
F01	Sample	198	0	0
D02	Sample	198	0	0
E02	Sample	198	0	0
F02	Sample	198	0	0
H02	Sample	198	0	0
E03	Sample	198	0	0
F03	Sample	198	0	0
A04	Sample	198	0	0
B04	Sample	198	0	0
E04	Sample	198	0	0
F04	Sample	198	0	0
G04	Sample	198	0	0
A05	Sample	198	0	0
B05	Sample	198	0	0
H05	Sample	198	0	0
A06	Sample	198	0	0
B06	Sample	198	0	0
C06	Sample	198	0	0
D06	Sample	198	0	0
F06	Sample	198	0	0

B07	Sample	198	0	0
C07	Sample	198	0	0
D07	Sample	198	0	0
E07	Sample	198	0	0
F07	Sample	198	0	0
B08	Sample	198	0	0
C08	Sample	198	0	0
F08	Sample	198	0	0
H08	Sample	198	0	0
B09	Sample	198	0	0
D09	Sample	198	0	0
E09	Sample	198	0	0
H09	Sample	198	0	0
B11	Sample	198	0	0
C11	Sample	198	0	0
D11	Sample	198	0	0
E11	Sample	198	0	0
G11	Sample	198	0	0
H11	Sample	198	0	0
C12	Sample	198	0	0
E12	Sample	198	0	0
B01	Sample	0	0	18
C01	Sample	0	0	18
D01	Sample	0	0	18
E01	Sample	0	0	18
H01	Sample	0	0	18
A02	Sample	0	0	18
B02	Sample	0	0	18

C02	Sample	0	0	18
A03	Sample	0	0	18
B03	Sample	0	0	18
C03	Sample	0	0	18
D03	Sample	0	0	18
G03	Sample	0	0	18
H03	Sample	0	0	18
C04	Sample	0	0	18
D04	Sample	0	0	18
H04	Sample	0	0	18
D05	Sample	0	0	18
E05	Sample	0	0	18
F05	Sample	0	0	18
E06	Sample	0	0	18
G06	Sample	0	0	18
A07	Sample	0	0	18
H07	Sample	0	0	18
A08	Sample	0	0	18
D08	Sample	0	0	18
E08	Sample	0	0	18
G08	Sample	0	0	18
A09	Sample	0	0	18
F09	Sample	0	0	18
G09	Sample	0	0	18
A10	Sample	0	0	18
C10	Sample	0	0	18
D10	Sample	0	0	18
E10	Sample	0	0	18

F10	Sample	0	0	18
G10	Sample	0	0	18
H10	Sample	0	0	18
A11	Sample	0	0	18
A12	Sample	0	0	18
B12	Sample	0	0	18
D12	Sample	0	0	18
F12	Sample	0	0	18
G12	Sample	0	0	18
H12	Sample	0	0	18

Worklist 2. 50x Formulation

Well	Rack	900uL Dispense Volume (uL)	175uL Dispense Volume (uL)	50uL Dispense Volume (uL)
A01	Sample	0	0	6.22
B01	Sample	0	0	4.07
C01	Sample	0	0	4.37
D01	Sample	0	0	2.02
E01	Sample	0	0	11.00
F01	Sample	0	0	6.46
H01	Sample	0	0	1.60
A02	Sample	0	0	10.22
B02	Sample	0	0	2.24
C02	Sample	0	0	4.13
D02	Sample	0	0	9.82
E02	Sample	0	0	6.18
F02	Sample	0	0	8.75
H02	Sample	0	0	3.14
A03	Sample	0	0	8.25

B03	Sample	0	0	2.82
C03	Sample	0	0	2.49
D03	Sample	0	0	6.81
E03	Sample	0	0	3.27
F03	Sample	0	0	4.18
G03	Sample	0	0	8.11
H03	Sample	0	0	1.93
A04	Sample	0	0	6.71
B04	Sample	0	0	1.65
C04	Sample	0	0	2.70
D04	Sample	0	0	5.61
E04	Sample	0	0	7.73
F04	Sample	0	0	5.32
G04	Sample	0	0	4.48
H04	Sample	0	0	2.82
A05	Sample	0	0	10.83
B05	Sample	0	0	2.92
D05	Sample	0	0	1.49
E05	Sample	0	0	1.85
F05	Sample	0	0	5.89
H05	Sample	0	0	13.34
A06	Sample	0	0	13.75
B06	Sample	0	0	8.42
C06	Sample	0	0	6.57
D06	Sample	0	0	2.96
E06	Sample	0	0	4.13
F06	Sample	0	0	5.01
G06	Sample	0	0	6.27



A07	Sample	0	0	3.81
B07	Sample	0	0	4.72
C07	Sample	0	0	4.91
D07	Sample	0	0	6.92
E07	Sample	0	0	13.75
F07	Sample	0	0	6.37
H07	Sample	0	0	3.18
A08	Sample	0	0	4.95
B08	Sample	0	0	4.41
C08	Sample	0	0	4.63
D08	Sample	0	0	3.32
E08	Sample	0	0	2.75
F08	Sample	0	0	8.47
G08	Sample	0	0	2.40
H08	Sample	0	0	3.28
A09	Sample	0	0	8.56
B09	Sample	0	0	8.25
D09	Sample	0	0	8.79
E09	Sample	0	0	5.90
F09	Sample	0	0	1.63
G09	Sample	0	0	3.41
H09	Sample	0	0	3.99
A10	Sample	0	0	6.87
C10	Sample	0	0	2.21
D10	Sample	0	0	4.13
E10	Sample	0	0	4.31
F10	Sample	0	0	2.78
G10	Sample	0	0	6.79

H10	Sample	0	0	1.67
A11	Sample	0	0	2.20
B11	Sample	0	0	4.70
C11	Sample	0	0	6.57
D11	Sample	0	0	10.13
E11	Sample	0	0	6.92
G11	Sample	0	0	11.45
H11	Sample	0	0	3.00
A12	Sample	0	0	2.09
B12	Sample	0	0	1.53
C12	Sample	0	0	4.46
D12	Sample	0	0	2.54
E12	Sample	0	0	10.38
F12	Sample	0	0	6.48
G12	Sample	0	0	3.41
H12	Sample	0	0	4.13