

# PROTOCOL: GCP Equimix and 2x Mastermix Formulation (STANDARDS)

## Purpose

Heavy synthetic standards are used as internal controls during mass spec analysis. Mastermix is a formulation of all peptides targeted in the assay, with labeled arginine's that are 10 Da's heavier than the endogenous peptide. All peptides are combined in their theoretical cellular concentrations, but at a 2x concentration. Equimix is comprised of the same heavy standards used to formulate the Mastermix, but with each peptide at a 30 fmol/uL concentration. Equimix samples are used to schedule the targeted GCP assay on the mass spec (see protocol "GCP Mass Spec Analysis")

## Preparation

1. Label 0.6 mL/1.7mL microcentrifuge tubes for 2 pmol/uL and 8 pmol/uL peptide stocks that will be created from the parental stocks. Look at volumes in table under "STANDARDS-MIX01: 2 pmol/uL and 8 pmol/uL peptide stocks" to determine which size tube to use
2. Collect and thaw all resuspended parental peptide stocks on ice

## Materials

- HPLC-grade water {STANDARDS-M01}
- Parental peptide stocks (BI numbers 1-65, 85-87) {STANDARDS-M02}
- Acetonitrile (ACN) {STANDARDS-M03}
- Formic Acid (FA) {STANDARDS-M04}
- 0.6 mL microcentrifuge tubes
- 1.7 mL microcentrifuge tubes
- 4 mL tubes
- 5 mL tube
- HPLC vials

## Assets

- Single channel pipettes {STANDARDS-A01}

## Reagent Mixes

ID	Name	Step	Composition	Stock Volume	Use
STANDARD S-MIX01	2pmol/uL and 8pmol/uL peptide stocks	STANDARD S	parental peptide stocks {STANDARDS-M02} at 2 pmol/uL or 8 pmol/uL in {STANDARDS-M01}	Varies-see procedure	Creation of 2x Mastermix and Equimix
STANDARD S-MIX02	2x Mastermix	MSA	Peptides in {STANDARDS-MIX01} in theoretical cellular concentrations	4.248 mL in 250 uL aliquots	internal standard for assay

STANDARD S-MIX03	Equimix	MSA	Peptides in {STANDARDS-MIX01} at 30fmol/uL concentration in a total 3%ACN/5%FA		scheduling of GCP assay
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## Reagent Mixture Preparation

### STANDARDS-MIX01: 2 pmol/uL and 8 pmol/uL peptide stocks

- To each pre-labeled tube:
  - Add volume HPLC-grade water {STANDARDS-M01} determined by table below
  - Add volume of parental stock determined by table below
  - Vortex and spin down/
  - Keep on ice to create 2x MasterMix {STANDARDS-MIX02} and/or Equimix {STANDARDS-MIX03}. Store remaining volumes at Store at -80°C

Peptide	Peptide BI Number	Parental Stock Concentration (pmol/uL)	Pipette Volume of Parental Stock (uL)	Pipette Volume HPLC-grade Water {STANDARDS-M01} (uL)
H3R2me2Sym	BI10001	368	2	366
H3R2Assym	BI10002	336	2	334
H3K4me0	BI10003	274	4	544
H3K4me1	BI10004	349	2	347
H3K4me2	BI10005	353	2	351
H3K4me3	BI10006	334	2	332
H3K4ac1	BI10007	489	2	487
H3K9me0K14ac0	BI10008	381	2	379
H3K9me1K14ac0	BI10009	354	2	352
H3K9me2K14ac0	BI10010	387	2	385
H3K9me3K14ac0	BI10011	395	2	393
H3K9ac1K14ac0	BI10012	388	2	386
H3K9me0K14ac1	BI10013	447	2	445
H3K9me1K14ac1	BI10014	427	2	425
H3K9me2K14ac1	BI10015	359	2	357
H3K9me3K14ac1	BI10016	266	2	264

H3K9ac1K14ac1	BI10017	479	2	477
H3K9me0S10ph1K14ac0	BI10018	473	2	471
H3K9me1S10ph1K14ac0	BI10019	296	2	294
H3K9me2S10ph1K14ac0	BI10020	304	2	302
H3K9me3S10ph1K14ac0	BI10021	359	2	357
H3K9ac1S10ph1K14ac0	BI10022	416	2	414
H3K9me0S10ph1K14ac1	BI10023	309	2	307
H3K9me1S10ph1K14ac1	BI10024	321	2	319
H3K9me2S10ph1K14ac1	BI10025	228	2	226
H3K9me3S10ph1K14ac1	BI10026	301	2	299
H3K9ac1S10ph1K14ac1	BI10027	297	2	295
H3K18ac0K23ac0	BI10028	259	4	514
H3K18ac1K23ac0	BI10029	260	2	258
H3K18ac0K23ac1	BI10030	397	2	395
H3K18ac1K23ac1	BI10031	244	2	242
H3K18ub1K23ac0	BI10032	253	2	251
H3K18ac0K23ub1	BI10033	401	2	399
H3.1K27me0K36me0	BI10034	286	2	284
H3.1K27me0K36me1	BI10085	693	2	691
H3.1K27me0K36me2	BI10086	733	2	731
H3.1K27me0K36me3	BI10087	814	2	812
H3.1K27me1K36me0	BI10035	338	2	336
H3.1K27me1K36me1	BI10036	349	2	347
H3.1K27me1K36me2	BI10037	359	2	357
H3.1K27me1K36me3	BI10038	331	2	329
H3.1K27me2K36me0	BI10039	395	2	393
H3.1K27me2K36me1	BI10040	359	2	357
H3.1K27me2K36me2	BI10041	341	2	339
H3.1K27me2K36me3	BI10042	316	2	314
H3.1K27me3K36me0	BI10043	281	2	279

H3.1K27me3K36me1	BI10044	277	2	275
H3.1K27me3K36me2	BI10045	439	2	437
H3.1K27me3K36me3	BI10046	412	2	410
H3.1K27ac1K36me0	BI10047	426	2	424
H3.1K27ac1K36me1	BI10048	387	2	385
H3.1K27ac1K36me2	BI10049	360	2	358
H3.1K27ac1K36me3	BI10050	350	2	348
H3.3K27me0K36me0	BI10051	287	2	285
H3Y41ph0_NORM	BI10052	327	4	650
H3Y41ph1	BI10053	332	2	330
H3K56me0	BI10054	315	16	614
H3K56me1	BI10055	312	8	304
H3K56me2	BI10056	314	8	306
H3K56me3	BI10057	381	8	373
H3K56ac1	BI10058	330	8	322
H3K56ub1	BI10059	332	8	324
H3K79me0	BI10060	416	8	408
H3K79me1	BI10061	422	8	414
H3K79me2	BI10062	313	8	305
H3K79me3	BI10063	309	8	301
H3K79ac1	BI10064	452	8	444
H3K79ub1	BI10065	304	8	296

### STANDARDS-MIX01: 2x MasterMix

- To a 5 mL tube add:
  - 274 uL HPLC-grade water {STANDARDS-M01}
  - Volumes of 2 pmol/uL or 8 pmol/uL stock peptides listed in table below
  - Vortex and spin down
  - Aliquot in 250 uL amounts
  - Store at -80°C

Peptide	Peptide BI Number	Stock Concentration (pmol/uL)	Pipette Volume (uL)	Concentration in 2x MasterMix (fmol/uL)
H3R2me2Sym	BI10001	2	2	0.94
H3R2Assym	BI10002	2	2	0.94
H3K4me0	BI10003	2	400	188.32
H3K4me1	BI10004	2	40	18.83
H3K4me2	BI10005	2	4	1.88
H3K4me3	BI10006	2	4	1.88
H3K4ac1	BI10007	2	4	1.88
H3K9me0K14ac0	BI10008	2	100	47.08
H3K9me1K14ac0	BI10009	2	100	47.08
H3K9me2K14ac0	BI10010	2	100	47.08
H3K9me3K14ac0	BI10011	2	200	94.16
H3K9ac1K14ac0	BI10012	2	2	0.94
H3K9me0K14ac1	BI10013	2	40	18.83
H3K9me1K14ac1	BI10014	2	40	18.83
H3K9me2K14ac1	BI10015	2	100	47.08
H3K9me3K14ac1	BI10016	2	20	9.42
H3K9ac1K14ac1	BI10017	2	4	1.88
H3K9me0S10ph1K14ac0	BI10018	2	4	1.88
H3K9me1S10ph1K14ac0	BI10019	2	4	1.88
H3K9me2S10ph1K14ac0	BI10020	2	4	1.88
H3K9me3S10ph1K14ac0	BI10021	2	4	1.88
H3K9ac1S10ph1K14ac0	BI10022	2	4	1.88
H3K9me0S10ph1K14ac1	BI10023	2	4	1.88
H3K9me1S10ph1K14ac1	BI10024	2	4	1.88
H3K9me2S10ph1K14ac1	BI10025	2	4	1.88
H3K9me3S10ph1K14ac1	BI10026	2	4	1.88

H3K9ac1S10ph1K14ac1	BI10027	2	4	1.88
H3K18ac0K23ac0	BI10028	2	400	188.32
H3K18ac1K23ac0	BI10029	2	10	4.71
H3K18ac0K23ac1	BI10030	2	200	94.16
H3K18ac1K23ac1	BI10031	2	10	4.71
H3K18ub1K23ac0	BI10032	2	2	0.94
H3K18ac0K23ub1	BI10033	2	2	0.94
H3.1K27me0K36me0	BI10034	2	20	9.42
H3.1K27me0K36me1	BI10085	2	4	1.88
H3.1K27me0K36me2	BI10086	2	20	9.42
H3.1K27me0K36me3	BI10087	2	40	18.83
H3.1K27me1K36me0	BI10035	2	20	9.42
H3.1K27me1K36me1	BI10036	2	20	9.42
H3.1K27me1K36me2	BI10037	2	200	94.16
H3.1K27me1K36me3	BI10038	2	20	9.42
H3.1K27me2K36me0	BI10039	2	100	47.08
H3.1K27me2K36me1	BI10040	2	10	4.71
H3.1K27me2K36me2	BI10041	2	100	47.08
H3.1K27me2K36me3	BI10042	2	40	18.83
H3.1K27me3K36me0	BI10043	2	40	18.83
H3.1K27me3K36me1	BI10044	2	10	4.71
H3.1K27me3K36me2	BI10045	2	10	4.71
H3.1K27me3K36me3	BI10046	2	4	1.88
H3.1K27ac1K36me0	BI10047	2	2	0.94
H3.1K27ac1K36me1	BI10048	2	2	0.94
H3.1K27ac1K36me2	BI10049	2	2	0.94
H3.1K27ac1K36me3	BI10050	2	2	0.94
H3.3K27me0K36me0	BI10051	2	4	1.88
H3Y41ph0_NORM	BI10052	2	400	188.32
H3Y41ph1	BI10053	2	2	0.94

H3K56me0	BI10054	8	400	753.30
H3K56me1	BI10055	8	10	18.83
H3K56me2	BI10056	8	10	18.83
H3K56me3	BI10057	8	10	18.83
H3K56ac1	BI10058	8	10	18.83
H3K56ub1	BI10059	8	2	3.77
H3K79me0	BI10060	8	400	753.30
H3K79me1	BI10061	8	200	376.65
H3K79me2	BI10062	8	20	37.66
H3K79me3	BI10063	8	2	3.77
H3K79ac1	BI10064	8	10	18.83
H3K79ub1	BI10065	8	2	3.77

#### STANDARDS-MIX03- Equimix:

- In a 4 mL conical tube:
  - Add 110.86 uL ACN {STANDARDS-M03}
  - Add 184.48 uL FA {STANDARDS-M02}
  - Add Volumes listed in table below. 50 uL of each peptide 2 pmol/uL or 8 pmol/uL stock is added
  - Vortex to mix
  - Aliquot into HPLC vials, 50 uL each
  - Store at -80°C

Peptide	Peptide BI Number	Stock Concentration (pmol/uL)	Pipette Volume (uL)	Concentration in Equimix (fmol/uL)
H3R2me2Sym	BI10001	2	50	27.06
H3R2Assym	BI10002	2	50	27.06
H3K4me0	BI10003	2	50	27.06
H3K4me1	BI10004	2	50	27.06
H3K4me2	BI10005	2	50	27.06
H3K4me3	BI10006	2	50	27.06
H3K4ac1	BI10007	2	50	27.06
H3K9me0K14ac0	BI10008	2	50	27.06

H3K9me1K14ac0	BI10009	2	50	27.06
H3K9me2K14ac0	BI10010	2	50	27.06
H3K9me3K14ac0	BI10011	2	50	27.06
H3K9ac1K14ac0	BI10012	2	50	27.06
H3K9me0K14ac1	BI10013	2	50	27.06
H3K9me1K14ac1	BI10014	2	50	27.06
H3K9me2K14ac1	BI10015	2	50	27.06
H3K9me3K14ac1	BI10016	2	50	27.06
H3K9ac1K14ac1	BI10017	2	50	27.06
H3K9me0S10ph1K14ac0	BI10018	2	50	27.06
H3K9me1S10ph1K14ac0	BI10019	2	50	27.06
H3K9me2S10ph1K14ac0	BI10020	2	50	27.06
H3K9me3S10ph1K14ac0	BI10021	2	50	27.06
H3K9ac1S10ph1K14ac0	BI10022	2	50	27.06
H3K9me0S10ph1K14ac1	BI10023	2	50	27.06
H3K9me1S10ph1K14ac1	BI10024	2	50	27.06
H3K9me2S10ph1K14ac1	BI10025	2	50	27.06
H3K9me3S10ph1K14ac1	BI10026	2	50	27.06
H3K9ac1S10ph1K14ac1	BI10027	2	50	27.06
H3K18ac0K23ac0	BI10028	2	50	27.06
H3K18ac1K23ac0	BI10029	2	50	27.06
H3K18ac0K23ac1	BI10030	2	50	27.06
H3K18ac1K23ac1	BI10031	2	50	27.06
H3K18ub1K23ac0	BI10032	2	50	27.06
H3K18ac0K23ub1	BI10033	2	50	27.06
H3.1K27me0K36me0	BI10034	2	50	27.06
H3.1K27me0K36me1	BI10085	2	50	27.06
H3.1K27me0K36me2	BI10086	2	50	27.06
H3.1K27me0K36me3	BI10087	2	50	27.06
H3.1K27me1K36me0	BI10035	2	50	27.06



H3.1K27me1K36me1	BI10036	2	50	27.06
H3.1K27me1K36me2	BI10037	2	50	27.06
H3.1K27me1K36me3	BI10038	2	50	27.06
H3.1K27me2K36me0	BI10039	2	50	27.06
H3.1K27me2K36me1	BI10040	2	50	27.06
H3.1K27me2K36me2	BI10041	2	50	27.06
H3.1K27me2K36me3	BI10042	2	50	27.06
H3.1K27me3K36me0	BI10043	2	50	27.06
H3.1K27me3K36me1	BI10044	2	50	27.06
H3.1K27me3K36me2	BI10045	2	50	27.06
H3.1K27me3K36me3	BI10046	2	50	27.06
H3.1K27ac1K36me0	BI10047	2	50	27.06
H3.1K27ac1K36me1	BI10048	2	50	27.06
H3.1K27ac1K36me2	BI10049	2	50	27.06
H3.1K27ac1K36me3	BI10050	2	50	27.06
H3.3K27me0K36me0	BI10051	2	50	27.06
H3Y41ph0_NORM	BI10052	2	50	27.06
H3Y41ph1	BI10053	2	50	27.06
H3K56me0	BI10054	8	50	108.24
H3K56me1	BI10055	8	50	108.24
H3K56me2	BI10056	8	50	108.24
H3K56me3	BI10057	8	50	108.24
H3K56ac1	BI10058	8	50	108.24
H3K56ub1	BI10059	8	50	108.24
H3K79me0	BI10060	8	50	108.24
H3K79me1	BI10061	8	50	108.24
H3K79me2	BI10062	8	50	108.24
H3K79me3	BI10063	8	50	108.24
H3K79ac1	BI10064	8	50	108.24
H3K79ub1	BI10065	8	50	108.24

