

## Product Information

### MS PhosphoMix 3 Heavy

Catalog Number **MSP3H**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

#### Product Description

Within mammalian systems, cellular processes have been shown to be largely reliant upon signaling via post-translational modifications (PTMs) such as protein phosphorylation and dephosphorylation. The complete elucidation of these signaling pathways will therefore require a full understanding of the roles of kinases, phosphatases, and most importantly, their substrates. However, several factors have hindered the analysis of protein phosphorylation.<sup>1</sup> For example, in the analysis of phosphopeptides via mass spectrometry, ion suppression<sup>2</sup> phenomena can lead to a loss in signal and, therefore, to an underestimation of the role that phosphorylation plays in biological processes. Additionally, loss of phosphopeptides during handling has been noted, which has been attributed to adsorption of the species onto metal surfaces, leading to a diminished signal for phosphorylated molecules.<sup>3</sup>

Newly developed methods to aid in the analyses of phosphorylated species, including the capture and enrichment of phosphopeptides and phosphoproteins, have been moderately successful in allowing deeper analyses of the phosphoproteome. However, these techniques are prone to bias, leading to selective enrichment of certain phosphorylated species over others. For example, immobilized affinity chromatography (IMAC) has been noted for its tendency to enrich selectively multiple phosphorylated species compared to single phosphorylated species.<sup>4</sup>

The PhosphoMix line of products has been designed to allow for the testing of the strengths and weaknesses of phosphopeptide sample processing, analysis protocols, and instrumental configurations. The mixes are produced from synthetic phosphopeptides with sequences derived from naturally occurring peptides identified in HeLa cells.<sup>5</sup> Because the sequences are derived from mammalian cells, many natural phosphorylation motifs, such as those presenting an abundance of proline, are represented.<sup>6</sup> Additionally, the phosphopeptide distribution in each mix has been chosen to present a broad range of characteristics, including ionizability, LC retention time, charge state, and isoelectric point.

PhosphoMix 1, 2, and 3 were designed in a complementary fashion, as highlighted in Table 1. For example, all three mixes contain peptides of the same sequence with different sites of phosphorylation. Production of the phosphopeptides within these mixes is accomplished using standard solid phase protocols, after which the phosphopeptides are purified via HPLC and quantified with amino acid analysis (AAA). Although AAA is considered to be one of the most accurate methods for protein and peptide quantitation, the inherent complexities in the process can lead to discrepancies of 10–20% in quantitative values.<sup>7</sup>

Each of the three phosphopeptides mixes is available in a naturally occurring isotopic abundances version (light) or as a stable isotope-enriched version (heavy), making the set of products highly amenable to quantitative analyses, to allow users to compare recovery between workflows or techniques. However, as previously detailed, slight differences in AAA measurements can lead to discrepancies in actual concentration values. Therefore, *if this product is intended to be used in a quantitative manner, it is strongly recommended normalization of the light and heavy samples be performed as a control.* This can most easily be accomplished by mixing a portion the light and heavy mixtures in a 1:1 fashion prior to any sample manipulation and experimentally determining the actual heavy to light ratio.

#### Component

MS PhosphoMix 3 Heavy, 1 vial  
40 pmoles each of 5 phosphopeptides  
(200 pmoles total) dried in a 0.5 mL vial.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

The preparation should be compatible with the analysis to be run. For LC/MS analysis, it is suggested the peptides be suspended in acidic aqueous solution (e.g., 0.1% formic acid) containing 20% acetonitrile, and vortexed well to ensure complete dissolution. *Vortexing of the solution upon standing is also recommended.*

**Storage/Stability**

The vial ships in wet ice and storage at –20 °C is recommended.

**References**

1. Thingholm, T.E. *et al.*, *Mol. Cell. Proteomics.*, **7(4)**, 661-671 (2008).
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3. Aebersold, R., and Goodlett, D.R., *Chem. Rev.*, **101(2)**, 269-295 (2001).
4. Bodenmiller, B. *et al.*, *Nat. Methods*, **4(3)**, 231-237 (2007).
5. Olsen, J.V. *et al.*, *Cell*, **127(3)**, 635-648 (2006).
6. Schwartz, D., and Gygi, S.P., *Nat. Biotech.*, **23(11)**, 1391-1398 (2005).
7. Alternman, M. *et al.*, ABRF AAARG2003 study, "Quantitation of proteins by Amino Acid Analysis and Colorimetric Assays", 2003, poster available from ABRF AAARG page ([https://abrf.org/sites/default/files/temp/RGs/Old/abrf2003\\_poster.pdf](https://abrf.org/sites/default/files/temp/RGs/Old/abrf2003_poster.pdf))

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TD,FF,JLT,HD,KR,GCY,MAM 04/16-1

**Table 1.**  
Composition of PhosphoMix Products

Peptide*	FASTA Abbreviation**	Complementary Peptide	Total # of Phosphates	MW Light (Monoisotopic)	MW Heavy (Monoisotopic)	Relative Signal Intensity***	# of Phosphates per amino acid		
							S	T	Y
<b>PhosphoMix 1</b>									
VLHSGpS[R]	1.1		1	834.37	844.38	Weak	1		
RSpYpSRS[R]	1.2	2.2	2	1070.41	1080.41	Weak	1		1
RDSLGPtYSS[R]	1.3		1	1220.52	1230.53	Medium		1	
pTKLlpTQLRDA[K]	1.4		2	1445.70	1453.72	Strong		2	
EVQAEQPSSpSSP[R]	1.5		1	1480.62	1490.63	Medium	1		
ADEPpSSEESDLEID[K]	1.6	1.7, 2.6, 3.5	1	1742.68	1750.69	Strong	1		
ADEPpSSEEpSDLEID[K]	1.7	1.6, 2.6, 3.5	2	1822.64	1830.66	Medium	2		
FEDEGAGFEESpSETGDYEE[K]	1.8		1	2333.84	2341.85	Strong	1		
ELSNpSPLRENSFGpSPLEF[R]	1.9	2.9	2	2338.00	2348.01	Medium	2		
SPTEYHEPvpYANPFYRPTpTPQ[R]	1.10		2	2809.19	2819.20	Strong		1	1
<b>PhosphoMix 2</b>									
LPQEpTA[R]	2.1		1	893.40	903.41	Weak		1	
RypSpSRS[R]	2.2	1.2	2	1070.41	1080.41	Weak	2		
EpTQSPQV[K]	2.3		1	1124.48	1132.49	Weak		1	
VIDNEpYTA[R]	2.4		1	1288.53	1298.54	Medium			1
pSRSpSPPELNN[K]	2.5		2	1474.59	1482.60	Medium	2		
ADEPSSEEpSDLEID[K]	2.6	1.6, 1.7, 3.5	1	1742.68	1750.69	Strong	1		
HQYSDYDpYHSSpSE[K]	2.7		2	1904.63	1912.64	Medium	1		1
NTPpSQHSHpSIQHSPE[R]	2.8		2	2000.79	2010.80	Medium	2		
ELpSNpSPLRENSFGSPLEF[R]	2.9	1.9	2	2338.00	2348.01	Medium	2		
LGpGRPLPTFPpTSE(CAM)TSDVEPDT[R]	2.10		1	2708.22	2718.22	Strong		1	
<b>PhosphoMix 3</b>									
SLpSpYpSP[V]ER	3.1		3	1276.42	1282.43	Weak	2		1
LQGpSGVpS[L]ApSK	3.2		3	1285.48	1292.49	Medium	3		
PPpYpSRV[I]pTQR	3.3		3	1455.57	1462.59	Strong	1	1	1
pSRS[R]pSYpTPEpYR	3.4		4	1720.54	1730.55	Weak	2	1	1
ADEPpSpSEEpSDLE[I]DK	3.5	1.6, 1.7, 2.6	3	1902.61	1909.63	Medium	3		

\* Amino acid in [brackets] denotes site of label incorporation for heavy mixes as follows:  
 [K],  $^{13}\text{C}_6\text{ }^{15}\text{N}_2$  [R],  $^{13}\text{C}_6\text{ }^{15}\text{N}_4$  [V],  $^{13}\text{C}_5\text{ }^{15}\text{N}_1$  [L],  $^{13}\text{C}_6\text{ }^{15}\text{N}_1$  [I],  $^{13}\text{C}_6\text{ }^{15}\text{N}_1$   
 (CAM) denotes carbamidomethyl cysteine

\*\* A FASTA file with all of the phosphopeptide sequences in the PhosphoMix product line is available for free download on the product display page at [sigma.com/phosphomix](http://sigma.com/phosphomix).

\*\*\* As determined using electrospray ionization following standard reverse phase chromatography

**Table 2.**  
Accurate mass to charge (m/z) values for peptides within the PhosphoMix Products

Peptide*	FASTA Abbreviation	Mono (M)	(M+1H) <sup>1+</sup>	(M+2H) <sup>2+</sup>	(M+3H) <sup>3+</sup>
<b>PhosphoMix 1</b>					
VLHSGpSR	1.1 Light	834.3749	835.3827	418.1953	279.1328
VLHSGpS[R]	1.1 Heavy	844.3832	845.3910	423.1994	282.4689
RSpYpSRSR	1.2 Light	1070.4060	1071.4138	536.2108	357.8098
RSpYpSRS[R]	1.2 Heavy	1080.4142	1081.4221	541.2149	361.1459
RDSLGPpTYSSR	1.3 Light	1220.5187	1221.5265	611.2672	407.8474
RDSLGPpTYSS[R]	1.3 Heavy	1230.5269	1231.5348	616.2713	411.1835
pTKLipTQLRDAK	1.4 Light	1445.7044	1446.7123	723.8600	482.9093
pTKLipTQLRDA[K]	1.4 Heavy	1453.7186	1454.7265	727.8671	485.5807
EVQAEQPSpSSPR	1.5 Light	1480.6195	1481.6273	741.3176	494.5477
EVQAEQPSpSSP[R]	1.5 Heavy	1490.6278	1491.6356	746.3217	497.8838
ADEPpSSEESDLEIDK	1.6 Light	1742.6772	1743.6850	872.3464	581.9002
ADEPpSSEESDLEID[K]	1.6 Heavy	1750.6913	1751.6992	876.3535	584.5716
ADEPpSSEEpSDLEIDK	1.7 Light	1822.6435	1823.6513	912.3296	608.5557
ADEPpSSEEpSDLEID[K]	1.7 Heavy	1830.6577	1831.6655	916.3367	611.2271
FEDEGAGFEESpSETGDYEEK	1.8 Light	2333.8373	2334.8451	1167.9265	778.9536
FEDEGAGFEESpSETGDYEE[K]	1.8 Heavy	2341.8515	2342.8593	1171.9336	781.6250
ELSNpSPLRENSFGpSPLEFR	1.9 Light	2338.0032	2339.0110	1170.0094	780.3422
ELSNpSPLRENSFGpSPLEF[R]	1.9 Heavy	2348.0115	2349.0193	1175.0136	783.6783
SPTHEYHEPvpyANPFYRTPpTPQR	1.10 Light	2809.1939	2810.2017	1405.6048	937.4058
SPTHEYHEPvpyANPFYRTPpTPQ[R]	1.10 Heavy	2819.2021	2820.2100	1410.6089	940.7419
<b>PhosphoMix 2</b>					
LPQEpTAR	2.1 Light	893.4008	894.4086	447.7082	298.8081
LPQEpTA[R]	2.1 Heavy	903.4091	904.4169	452.7124	302.1442
RYpSpSRSR	2.2 Light	1070.4060	1071.4138	536.2108	357.8098
RYpSpSRS[R]	2.2 Heavy	1080.4142	1081.4221	541.2149	361.1459
EpTQSPeQVK	2.3 Light	1124.4751	1125.4829	563.2454	375.8329
EpTQSPeQV[K]	2.3 Heavy	1132.4893	1133.4971	567.2525	378.5043
VIEDNEpYTAR	2.4 Light	1288.5337	1289.5415	645.2747	430.5190
VIEDNEpYTA[R]	2.4 Heavy	1298.5419	1299.5498	650.2788	433.8551
pSRSpSSPELNK	2.5 Light	1474.5855	1475.5933	738.3006	492.5363
pSRSpSSPELN[K]	2.5 Heavy	1482.5997	1483.6075	742.3077	495.2077
ADEPpSSEEpSDLEIDK	2.6 Light	1742.6772	1743.6850	872.3464	581.9002
ADEPpSSEEpSDLEID[K]	2.6 Heavy	1750.6913	1751.6992	876.3535	584.5716
HQYSDYDpYHSSpSEK	2.7 Light	1904.6292	1905.6370	953.3224	635.8842
HQYSDYDpYHSSpSE[K]	2.7 Heavy	1912.6434	1913.6512	957.3295	638.5556
NTPpSQHSHpSIQHSPEK	2.8 Light	2000.7891	2001.7970	1001.4024	667.9375
NTPpSQHSHpSIQHSPE[R]	2.8 Heavy	2010.7974	2011.8052	1006.4065	671.2736
ELpSNpSPLRENSFGpSPLEFR	2.9 Light	2338.0032	2339.0110	1170.0094	780.3422
ELpSNpSPLRENSFGpSPLEF[R]	2.9 Heavy	2348.0115	2349.0193	1175.0136	783.6783
LGpGRPLPTFFpTSE(CAM)TSDVEPDTR	2.10 Light	2708.2153	2709.2231	1355.1155	903.7463
LGpGRPLPTFFpTSE(CAM)TSDVEPDTR[R]	2.10 Heavy	2718.2236	2719.2314	1360.1196	907.0824
<b>PhosphoMix 3</b>					
SLpSpYpSPVER	3.1 Light	1276.4179	1277.4257	639.2168	426.4805
SLpSpYpSP[V]ER	3.1 Heavy	1282.4317	1283.4395	642.2237	428.4851
LQGPpSGVpSLApSK	3.2 Light	1285.4758	1286.4836	643.7457	429.4997
LQGPpSGVpSLApSK	3.2 Heavy	1292.4929	1293.5008	647.2543	431.8388
PPpYpSRVlpTQR	3.3 Light	1455.5714	1456.5792	728.7935	486.1983
PPpYpSRVlpTQR	3.3 Heavy	1462.5886	1463.5964	732.3021	488.5373
pSRSpSYpTPEpYR	3.4 Light	1720.5450	1721.5528	861.2803	574.5228
pSRSpSYpTPEpYR	3.4 Heavy	1730.5533	1731.5611	866.2845	577.8589
ADEPpSpSSEEpSDLEIDK	3.5 Light	1902.6098	1903.6176	952.3127	635.2111
ADEPpSpSSEEpSDLEI[IK]	3.5 Heavy	1909.6270	1910.6348	955.8213	637.5502