

Supporting Information
for

**In-Tip Fabrication of Lanthanum oxide Monolith for Enrichment of
Phosphorylated Biomolecules**

Fahmida Jabeen^{1,2}, Muhammad Najam-ul-Haq*^{1,2}, Matthias Rainer², Christian W. Huck²,
Guenther K. Bonn²

¹: Division of Analytical Chemistry, Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan.

²: Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Innsbruck 6020, Austria.

* Corresponding Author

Prof. Dr. M. Najam-ul-Haq
Institute of Chemical Sciences
Bahauddin Zakariya University
Multan 60800
Pakistan
Tel.: +92 306 7552653
Email: najamulhaq@bzu.edu.pk

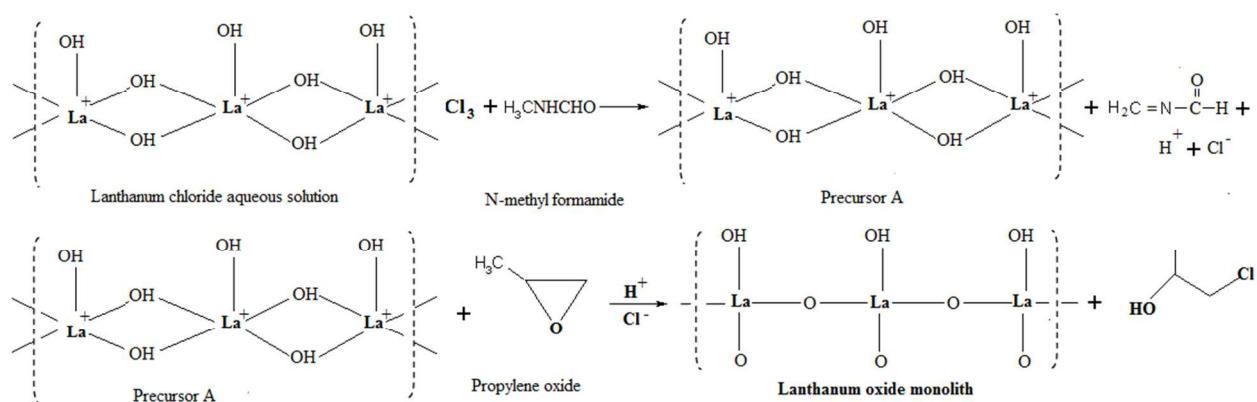


Figure S1: Mechanism of monolithic synthesis using lanthanum chloride as precursor, n-methyl formamide as porogen and propylene oxide as initiator.

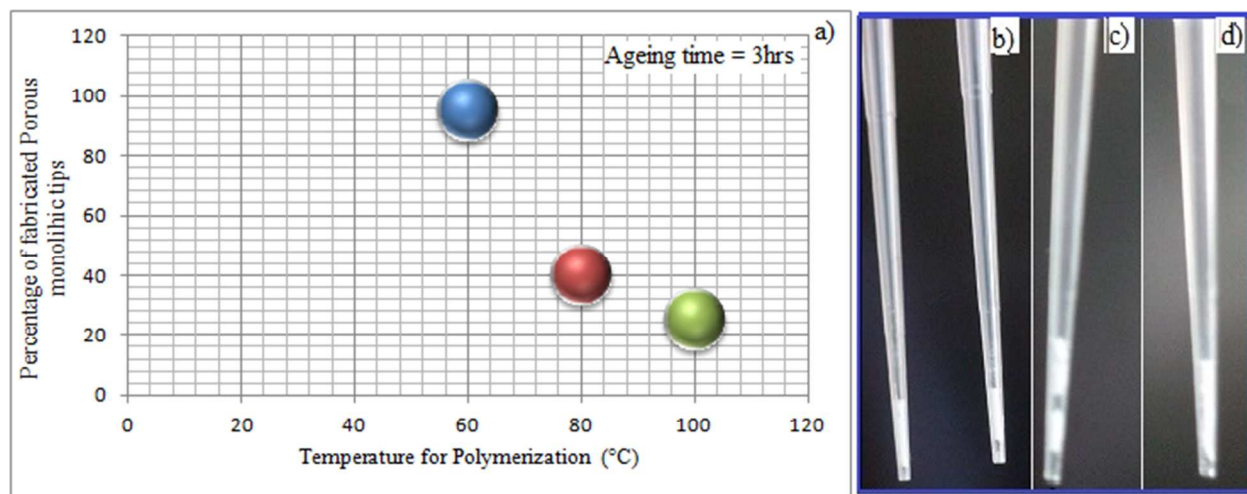


Figure S2: (a) Graphical representation of experiments performed for temperature optimization, (b) monolithic tip at 60 °C with stable bed and porosity, (c) at 80 °C with breakage in the bed and (d) at 100 °C with no porosity.

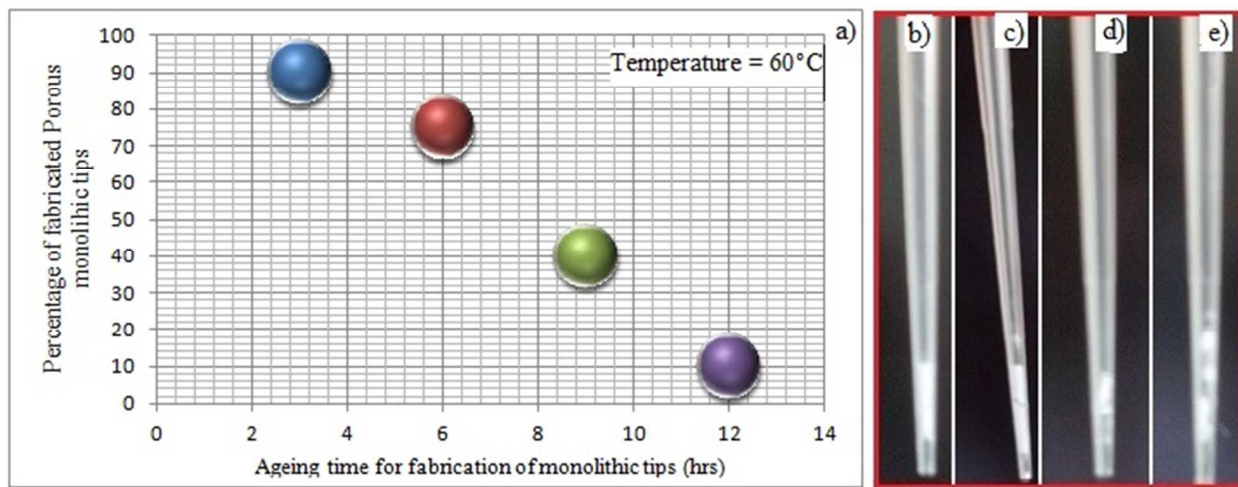


Figure S3: (a) Graphical representation of experiments performed for ageing time optimization (b) Monolithic tip aged for 3 hours (stable and porous), (c) aged for 6 hours (d) aged for 9 hours (unstable bed) and (e) aged for 12 hours (unstable bed).

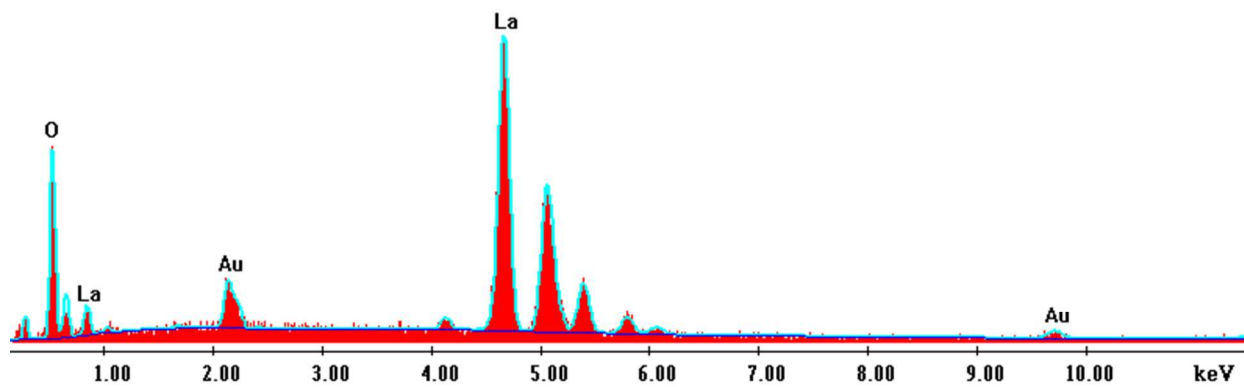


Figure S4: EDX analysis of lanthanum oxide monolithic tip. The contamination from Au is from the instrument sampling.

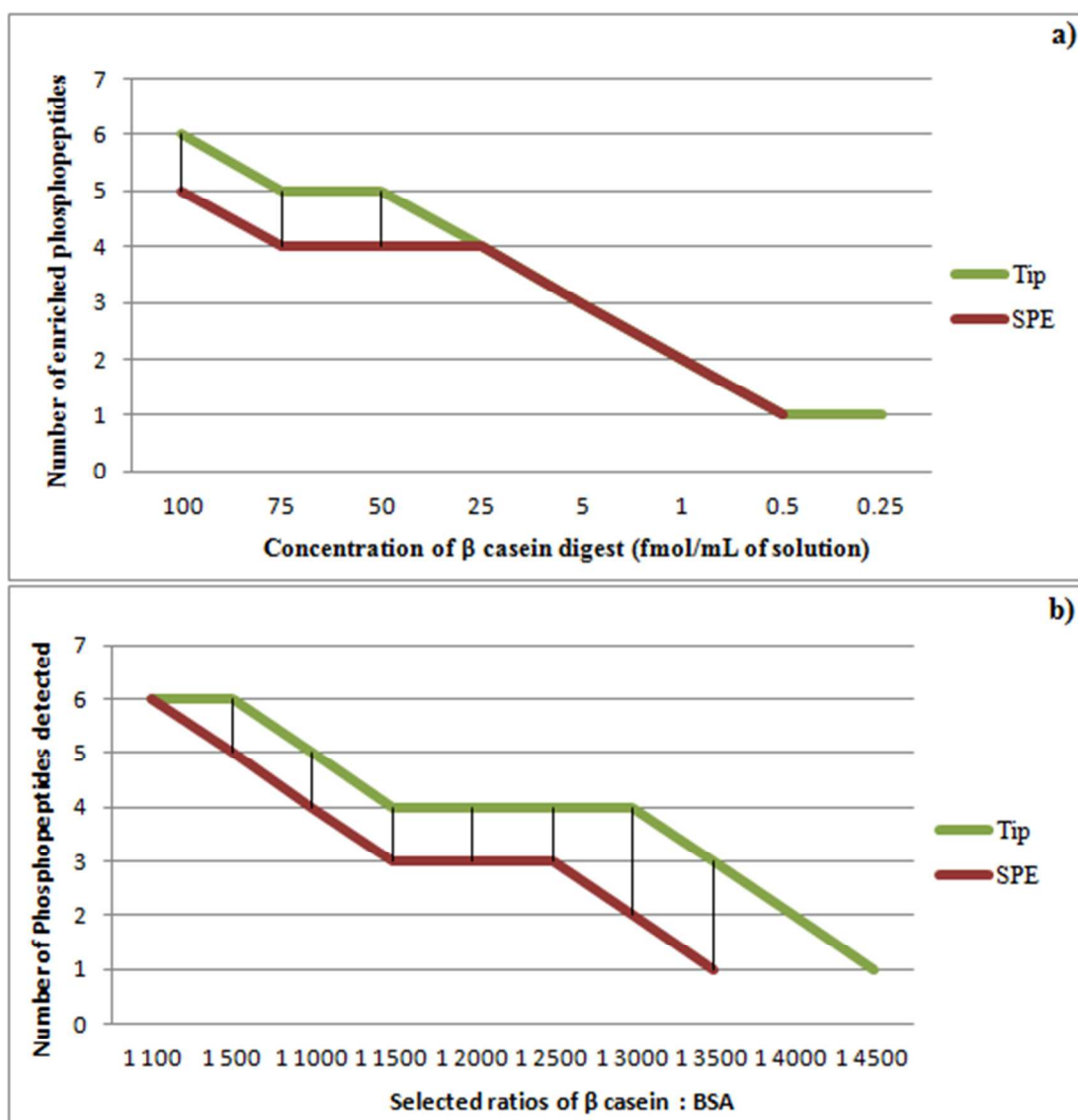


Figure S5: Graphical representation of data collected from (a) sensitivity and (b) selectivity studies performed using La_2O_3 monolithic tip and La_2O_3 as SPE. The experiments are performed using β -casein digest diluted to different concentrations for sensitivity and spiked in BSA digest for selectivity studies.

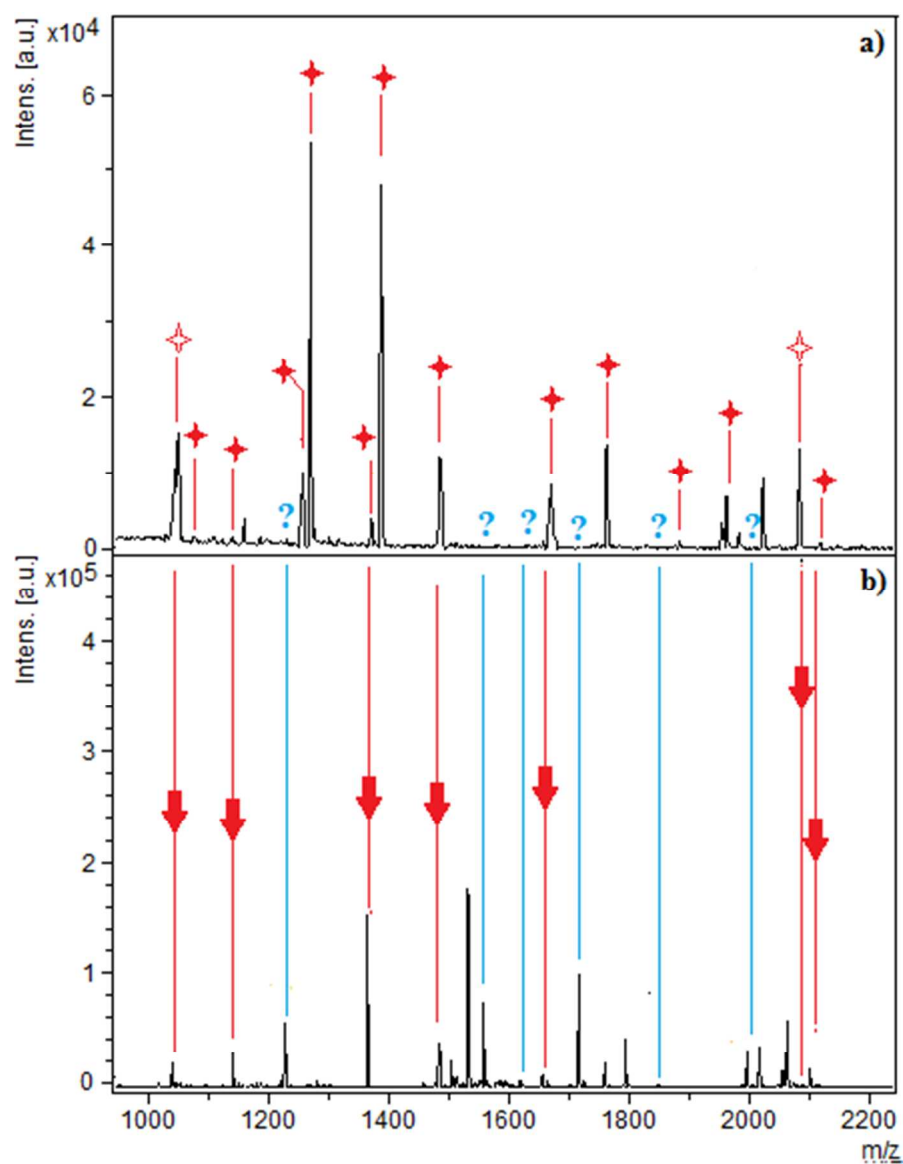


Figure S6: Comparative MS analysis of egg yolk digest applied to La_2O_3 as SPE material (a) first elution and (b) La_2O_3 spotted after first elution. The retained phosphopeptides from phosvitin and lipovitellin onto the SPE material after first elution are shown with blue lines. Red lines with arrows and red stars indicate phosphopeptides before and after elution.

Table S1: Comparative view of enrichment performance by in tip embedded polymers with metal oxides (TiO₂, ZrO₂ and La₂O₃) and in-tip lanthanum oxide after enrichment using β -casein digest.

m/z	Peptide Sequence	Krokhin_NET Hydrophobicity	poly(GMA/ DVB)	poly(GMA/ DVB)TiO ₂	poly(GMA/ DVB)ZrO ₂	poly(GMA/ DVB)La ₂ O ₃	in- tip La ₂ O ₃
1104.3	KFQSEEQQQT	0.04997731	*	-	*	*	*
1473.6	KIEKFQSEEQQQT	0.1176232	-	-	-	*	*
2061.7	FQSEEQQQTDELQDK	0.09920053	-	*	*	*	*
2432.8	IEKFQSEEQQQTEDLQDK	0.1750709	-	-	-	*	*
2556.4	FQSEEQQQTDELQDKIHPF	0.282194	-	*	*	*	*
2779.7	KIEKFQSEEQQQTDELQDKIHPF	0.3397476	-	-	-	-	*
2965.8	RELEELNVPGEIVESLSSEESITR	0.46311	-	*	*	*	*
3054.3	KIEKFQSEEQQQTDELQDKIHPF	0.04226933	-	*	*	*	*
3122.9	RELEELNVPGEIVESLSSEESITRI	0.5137824	-	*	*	*	*
3477.8	RELEELNVPGEIVESLSSEESITRINKK	0.4662831	-	*	-	*	*
3605.9	RELEELNVPGEIVESLSSEESITRINKKI	0.5142704	-	-	-	-	*
3837.9	RINKKIEKFQSEEQQQTDELQDKIHPFAQTQS	0.3349487	-	-	-	-	*
3975.8	RELEELNVPGEIVESLSSEESITRINKKIEKF	0.5510378	-	-	-	-	*
1057.8	VPPFLQPEVMG	0.3422324	*	-	*	-	-
1151.6	LGPVPGPFPIIV	0.4278075	*	-	-	-	-
1591.9	VLPVPQKAVPYPQR	0.215165	*	-	*	*	-
1698.1	SWMHQPHQPLPPTVMF	0.4366232	*	*	*	*	-
1881.7	LYQEPVLGPVPGPFPIIV	0.5428776	*	-	-	-	-
1994.4	LLYQEPVLGPVPGPFPIIV	0.6010243	*	-	-	-	-
2107.3	FLLYQEPVLGPVPGPFPIIV	0.6804723	*	-	-	-	-
2390.2	LVYPPFGPIPNSLPQNIPPLTQTP	0.4986497	*	-	-	-	-
2682.8	LQSSWMHQPHQPLPPTVMFPPQSVLS	0.5166875	*	*	-	-	-
2877.6	LSLSQSKVLPVPQKAVPYPQRDMPIQAF	0.5032384	*	-	-	-	-
2997.6	AVPYPQRDMPIQAFLLYQEPVLGPVR	0.5825111	*	-	-	-	-

Table S2: Selected m/z of β -casein phosphopeptides to determine the batch to batch reproducibility of lanthanum oxide monolithic tips. Standard deviation (SD) is calculated for each batch to statistically evaluate the given data.

Selected Peaks	Tip Batch I	Tip Batch II	Tip Batch III	Tip Batch IV	SD
β-1	2062.508	2062.387	2062.113	2062.068	0.21274
β-2	2556.277	2556.715	2556.926	2556.178	0.35534
β-3	2779.687	2779.095	2778.784	2779.345	0.38262
β-4	3054.266	3053.483	3054.011	3054.624	0.47981
β-5	3122.021	3121.932	3122.509	3123.574	0.75394
β-6	3179.296	3180.137	3179.509	3179.596	0.35792

Table S3: Phosphopeptides detected from egg yolk digest after enrichment with La₂O₃ monolithic tip and SPE strategy. Characteristic phosphopeptides are derived from three domains of phosvitin (PV) and lipovitellin (LP).

	Position	Sequence	Mr (Expt)	Mr (Calc)	Tip	SPE
Domain-I	1112-1145	AEFGTEPDAKTSSSSSSASSTATSSSSSSASSPN (PV 1-34)				
	1112-1131	AEFGTEPDAKTSSSSSSASS	1931.9	1931.8	yes	no
	1122-1145	TSSSSSSASSTATSSSSSSASSPN	2137.9	2137.9	yes	yes
Domain-III	1301-1322	SGHLEDDSSSSSSSVLSKIWG (PV 190-211)				
	1308-1320	SSSSSSSVLSKI	1254.6	1254.6	yes	yes
	1306-1318	DDSSSSSSSVLS	1243.5	1243.5	yes	yes
	1301-1319	SGHLEDDSSSSSSSVLSK	1894.9	1894.8	yes	yes
	1304-1317	LEDDSSSSSSSVL	1398.6	1398.6	yes	yes
	1306-1319	DDSSSSSSSVLSK	1371.6	1371.6	yes	yes
	1310-1320	SSSSSVLSKI	1080.6	1080.6	yes	yes
	1301-1318	SGHLEDDSSSSSSSVLS	1766.8	1766.7	yes	yes
	1305-1322	EDDSSSSSSSVLSKIWG	1856.8	1856.8	yes	no
	1304-1322	LEDDSSSSSSSVLSKIWG	1969.9	1969.9	yes	yes
	1301-1320	SGHLEDDSSSSSSSVLSKI	2007.9	2007.9	yes	no
	1306-1320	DDSSSSSSSVLSKI	1484.7	1484.7	yes	yes
	1304-1319	LEDDSSSSSSSVLSK	1613.7	1613.7	yes	no
	1301-1317	SGHLEDDSSSSSSSVL	1679.7	1679.7	yes	yes
	1304-1320	LEDDSSSSSSSVLSKI	1726.8	1726.8	yes	no
	1306-1317	DDSSSSSSSVL	1156.5	1156.5	yes	yes
Domain-IV	1056-1076	IITEVNPESEEEEDESSPYEDI (LP)				
	1059-1076	EVNPESEEEEDESSPYEDI	2095.8	2095.8	yes	yes
	1068-1076	DESSPYEDI	1053.4	1053.4	yes	yes
	1059-1072	EVNPESEEEEDESSP	1575.6	1575.6	yes	no

Table S4: Phosphoproteins identified from serum digest after enrichment with La₂O₃ monolithic tip using the bottom up approach. Phosphorylation at serine, threonine and tyrosine is represented as S*, T* and Y* respectively. Oxidation at methionine (Oxi M) is given as M#. Phosphorylation sites are confirmed from Phosphosite plus.

Phosphoprotein	Accession number	Phosphopeptide	[M+H] ⁺	Swiss Prot Phosphorylation site	Confirmation from Phosphosite
Heat shock protein HSP 90-alpha	P07900	K.ESEDKPEIEDVGS*DEEEK.K	2272.8	Ser 263	Yes Yes
Prostaglandin E synthase 3 (Cytosolic prostaglandin E2 synthase)	Q15185	K.DWEDDS*DEDMSNFDR.F	1955.6	Ser 113	Yes
		K.DWEDDS*DEDM#SNFDR.F	1971.6	Oxi (M), Ser 113	
Catenin delta-1 (p120 catenin)	O60716	R.LRS*YEDMIGEEVPSDQYYWAPLAQHER.G	3362.4	Ser 321	Yes
		R.S*YEDMIGEEVPSDQYYWAPLAQHER.G	3093.2	Ser 321	Yes
		R.S*GDLGDMEPLK.G	1241.5	Ser 920	No
		R.S*MGYDDL DYGMMSDYGTAR.R	2227.8	Ser 288	Yes
		R.T*LDRSGDLGDMEPLK.G	1726.7	Thr 916	Yes
Nuclear factor 1 C-type (Nuclear factor 1/C)	P08651	R.NWTEDMEGGISS*PVKK.T	1857.8	Ser 323	Yes
Cytoplasmic dynein 1 light intermediate chain 1	Q9Y6G9	K.PVT*VS*PTTPTSPTGEAS*	1997.7	Ser 510	Thr 508 Ser 510, Ser 523
Mitogen-activated protein kinase kinase kinase 2	Q9Y2U5	R.AQS*YPD NHQEFSDYDNPIFEK.F	2624.0	Ser 239	Yes
		K.RLS*IIGP TSR.D	1179.6	Ser 153	Yes
Zinc finger Ran-binding domain-containing protein 2	O95218	R.ENVEYIEREES*DGEYDEFGR.K	2545.0	Ser 120 (m1)	
		R.EES*DGEYDEFGR.K	1512.5	Ser 120	Yes
		K.EVEDKES*EGEEDEDEDLSK.Y	2419.9	Ser 153	Yes
Kinesin light chain 4	Q9NSK0	R.AAS*LN YLNQPSAAPLQVSR.G	2080.0	Ser 590	Yes
Armadillo repeat protein deleted in velo-cardio-facial syndrome	O00192	R.S*LAADDEGGPELEPDYGTATR.R	2243.9	Ser 267	Yes
		R.NFDT*LDLPK.R	1142.5	Thr 642	Yes
K0310_HUMAN Uncharacterized protein KIAA0310	O15027	R.FTGS*FDDDPDPHRDPYGEEVDR.R	2646.0	Ser 1149	No
Src substrate cortactin	Q14247	R.LPSS*PVYEDAASF.K	1590.7	Ser 418	Yes

(Amplaxin) (Oncogene EMS1)		A			
		K.TQT*PPVS*PAPQPTE ER.L	1894.7	Thr 401 , Ser 405	Yes & Yes
		R.VDKSAVGFDY*QGKT *EK.H	1931.8	No	Tyr 178, Thr 182
		K.GRY*GLFPANY*VEL R.Q	1814.8	No	Tyr 538, Tyr 545
RNA-binding protein 39	Q14498	K.DKS*PVREPIDNLTPE ER.D	2074.9	Ser 136	Yes
		R.YRS*PYSGPK.F	1134.4	Ser 97	Yes
Bcl-2-associated transcription factor 1 (Btf)	Q9NYF8	K.KAEGEPQEEs*PLK.S	1521.6	Ser 177	Yes
		K.DLFDYS*PPLHK.N	1411.6	Ser 512	Yes
		K.LKDLDYS*PPLHK.N	1652.8	Ser 512 (m1)	Yes
Lamina-associated polypeptide 2, isoforms beta/gamma	P42167	R.EQGTEs*RSST*PLPTI SSSAENTR.Q	2595.1	Ser 156, Thr 160	Yes
Ubiquitin carboxyl-terminal hydrolase 14	P54578	R.AS*GEMASAQYITAA LR.D	1719.7	Ser 143	Yes
		R.AS*GEM#ASAQYITA ALR.D	1735.7	Oxi (M)	Yes
		K.S*S*KISRLPAYLTIQ MVR.F	2123.6	Ser 314and Ser 315	Yes
Polyhomeotic-like protein 3	Q8NDX5	R.MDRT*PPPPTLS*PAA ITVGR.G	2136.9	Thr 609, Ser 616.	Yes
Serine/arginine repetitive matrix protein 1	Q8IYB3	K.KETES*EAEDNLDDL EK.H	1944.7	Ser 874	Yes
		K.KPPAPPS*PVQSQS*P STNWS*PAVPVK.K	2823.2	Ser 769, Ser 775, Ser 781	Yes
		K.EKT*PELPEPSVK.V	1433.6	Thr 220	Yes
		R.HRPS*PPAT*PPPK.T	1441.6	Ser 402, Thr 406	Yes & Yes
Nucleophosmin	P06748	K.DELHIVEAEAMNYEG S*PIK.V	2224.9	Ser 70	Yes
		K.DELHIVEAEAMNYEG S*PIKVTLATLK.M	2951.4	Ser 70 (m1)	Yes
Stathmin (Phosphoprotein p19)	P16949	K.ESVPEFPLS*PPK.K	1406.6	Ser 38	Yes
Hematological and neurological expressed 1 protein	Q9UK76	R.RNS*SEASSGDFLDLK .G	1705.7	Ser 87	Yes
		R.NS*SEASSGDFLDLK. G	1549.6	Ser 87	Yes
Transcription intermediary factor 1-beta (TIF1-beta)	Q13263	R.STAPSAASASASAA ASS*PAGGGAEALELLE HCGVCR.E	3408.5	Ser 50	Yes
		R.SRS*GEGEVSGLMR.K	1444.5	Ser 473	Yes
La-related protein 1	Q6PKG0	R.SLPTTVPEs*PNYR.N	1540.7	Ser 774	Yes
		R.EHRPTASISSS*PSEG TPT*VGSYGCTPQSLPK. F	3474.5	Ser 851, Thr 858	Yes

		K.QEVENFKKVN#IS* R.E	1817.8	Ser 716 , Oxi (M)	Yes
2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial precursor	P12694	R.IGHHS*TSDDSSAYR. S	1612.6	Ser 337	Yes
Zinc finger protein KIAA1802	Q96JM3	R.KPSGS*PDLWK.L	1194.5	Ser 445	Yes
		K.TAPTLS*PEHWK.A	1346.6	Ser 405	Yes
		R.RPAPAVS*PGSWK.P	1332.6	Ser 308	Yes
		R.KT*SPASLDFPES*QK S*SR.G	2104.8	No	Ser 458, Ser 468 and Ser 471
Serine/threonine-protein kinase TAO2	Q9UL54	R.HS*LDQDLLR.E	1176.5	Ser 656	Yes
Serine/threonine-protein kinase D1	Q15139	R.T*SS*AELST*SAPDEP LLQKSPSEFIGREK.R	3331.4	No	Thr 217, Ser 219 and Thr 224
STE20/SPS1-related proline- alanine-rich protein kinase	Q9UEW 8	K.ADMWSFGITAIELAT GAAPY*HKY*PPMK.V	3126.3	No	Tyr 273, Tyr 276
Kinesin light chain 2 (KLC 2)	Q9H0B6	R.RDS*APYGEYGSWYK .A	1758.7	No	Ser 428
		R.ASS*LNFLNK.S	1073.5	Ser 582	Yes
CTP synthase 1	P17812	R.SGSSS*PDSEITELK.F	1516.6	Ser 575	Yes
Transcriptional repressor protein YY1	P25490	K.DIDHETVVEEQIIGEN S*PPDYSEYMTGK.K	3275.3	Ser 247	Yes
Serine/threonine-protein kinase PAK 2 (p21-activated kinase 2)	Q13177	K.YLS*FTPPEK.D	1161.5	Ser 141	Yes
Splicing factor, arginine/serine-rich 9	Q13242	R.GSPHYFS*PFRPY	1534.6	Ser 216	Yes
		R.STS*YGYSR.S	1000.3	Ser 189	Yes
MORC family CW-type zinc finger protein 2	Q9Y6X9	R.SVAVS*DEEEVEEEA ER.R	1886.7	Ser 743	Yes
Bcl2 antagonist of cell death (BAD) (Bcl-2-binding component 6)	Q92934	R.RMS*DEFVDSFK.K	1440.5	Ser 118	Yes
		R.HSS*YPAGTEDDEGM GEEPSPFR.G	2474.9	Ser 75	Yes
		R.S*APPNLWAAQR.Y	1290.5	Ser 99	Yes
Proline-rich AKT1 substrate 1 (40 kDa proline-rich AKT substrate)	Q96B36	K.S*LPVSVPVWGFK.E	1395.7	Ser 183	Yes
		R.LNT*SDFQK.L	1032.4	Thr 246	Yes
		R.AAT*AARPPAPPPAPQ PPS*PT*PSPPR.P	2666.1	Ser 88	Thr 73, Ser 88, Thr 90
Serine/arginine repetitive matrix protein 2	Q9UQ35	R.HGGS*PQPLATTPLSQ EPVNPPSEAS*PTR.D	3012.3	Ser 377, Ser 398	Yes & Yes
RNA-binding protein 10	P98175	R.Y*GATDRSQDDGGEN RS*R.D	2043.7	No	Tyr 16, Ser 31
Protein kinase C and casein kinase substrate in neurons protein 3	Q9UKS6	R.DGTAPPPQSPGSPGTG QDEEWS*DEESPR.K	2990.1	Ser 354	Yes

Coiled-coil domain-containing protein 86	Q9H6F5	RLGGLRPES*PESLTSVSR	2020.0	Ser 18	Yes
Kinesin-like protein KIF23	Q02241	GGGQSVQFTDIETLKQES*PNGSR	2514.1	Ser 902	Yes
Protein AHNAK2	Q8IVF2	DAHDVSPTS*TDTEAQLTVER	2250.9	Ser 294	Yes
Ras GTPase-activating protein-binding protein 1 (G3BP-1)	Q13283	R.YQDEVFGGFVTEPQES*EEVEEPEER.Q	3296.4	Ser 149	Yes
Rap1 GTPase-activating protein 1 (Rap1GAP)	P47736	R.RSS*AIGIENIQEVQEK.R	1881.6	Ser 499	Yes
5'-AMP-activated protein kinase subunit beta-1	Q9Y478	R.S*HNNFVAILDLPEGEHQYK.F	2291.4	Ser 108	Yes
RAC-alpha serine/threonine-protein kinase (RAC-PK-alpha)	P31749	R.RPHFPQFS*YSASGTA	1732.74	Ser 473	Yes
Arginine/serine-rich-splicing factor 10 (Transformer-2-beta)	P62995	K.RPHT*PTPGIYMGR.P	1562.7	Thr 201	Yes
PHD finger protein 6 (PHD-like zinc finger protein)	Q8IWS0	K.TAHNSEADLEESFNEHELEPSS*PK.S	2777.1	Ser 122	Yes
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	O60825	R.RNS*FTPLSSSNTIR.R	1659.7	Ser 446	Yes
Myelin expression factor 2 (MyEF-2) (MST156)	Q9P2K5	K.AEVPGATGGDS*PHLQPAEPPGEPR.R	2446.1	Ser 17	Yes
Serine/threonine-protein kinase PAK 4 (p21-activated kinase 4)	O96013	R.PFNT*YPR.A	974.4	Ser 207	Yes
Fibrinogen alpha chain	P02671	DSGEGDFLAEGGGV	1389.5	Ser 22	Yes
		DSGEGDFLAEGGGVR	1545.6	Ser 22 (hydrolytic product)	
		ADSGEGDFLAEGGGVR	1616.8	Ser 22 (hydrolytic product)	