Supporting Information for

Enhanced Enrichment Performance of Nickel oxide Nanoparticles via Fabrication of Nanocomposite with Graphene Template

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Preparation of HeLa Cell Extract

HeLa cells were cultured in high glucose Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin. The cells were cultivated on 150 mm tissue culture plastic dishes at 37 °C in 5% CO_2 and 98% humidity. For total cell lysate preparation, cells were washed twice in ice cold PBS, harvested and centrifuged at 500xg for 4 min at 4 °C. Cells collected from 15 cm dish were re-suspended in 1 mL of lysis buffer (50 mM Tris-HCl, pH 8.0), 0.5% Triton X100, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 10 mg/mL leupeptin, 10 mg/mL aprotinin and 1 mM PMSF. Lysis was performed for 30 min at 4 °C followed by the centrifugation at 16000xg for 10 min at 4 °C. Protein concentration was determined using Coomassie Plus Protein Assay (Thermo Scientific).

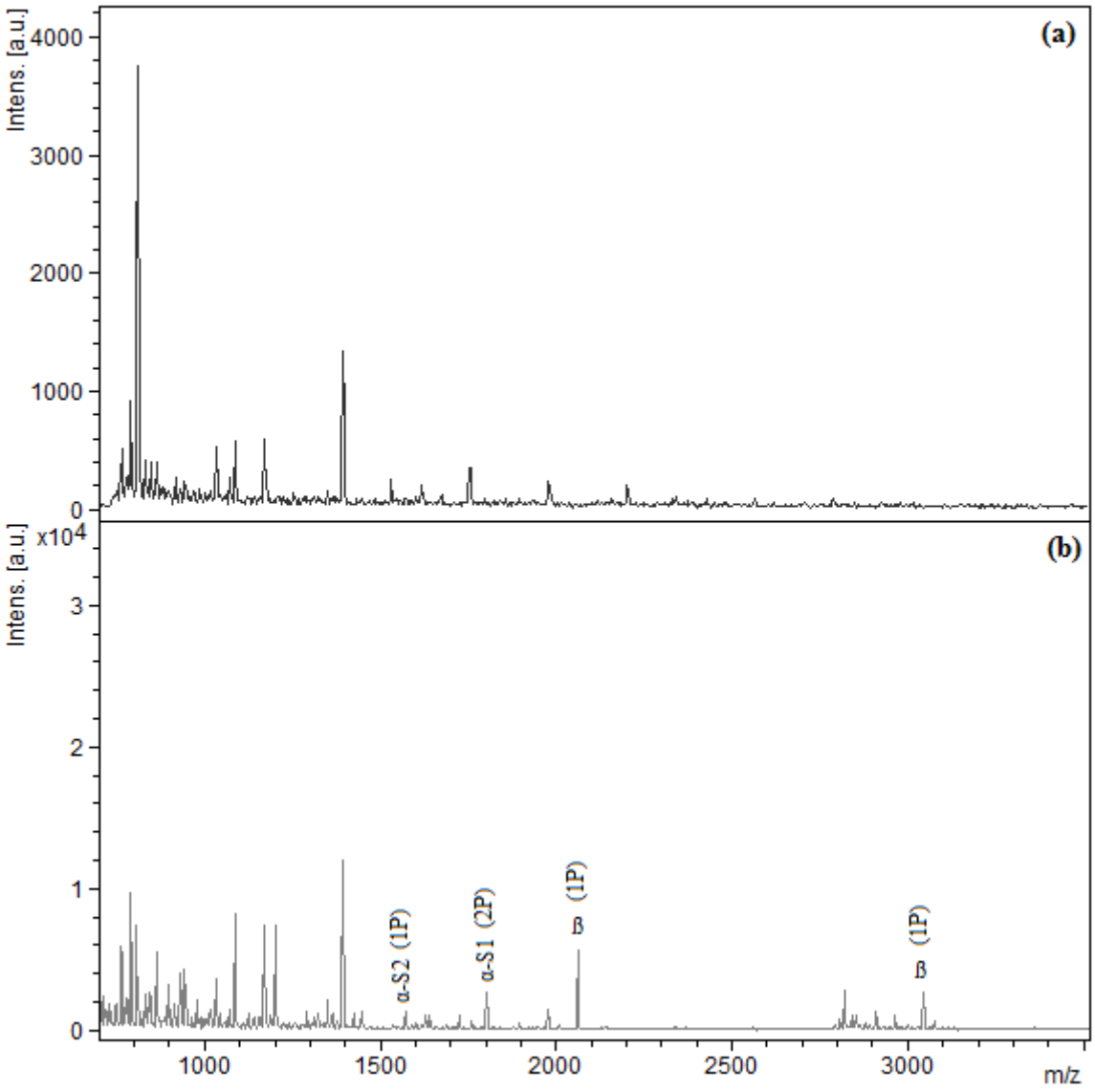

Fig. S1 MALDI-MS spectra eluted fractions after applying β-casein digest to (a) graphene nanofoam and (b) derivatized graphene nanofoam with orthosilicate. αS1, αS2 and β reprents the detected phosphopeptides with status of phosphorylation given in brackets.

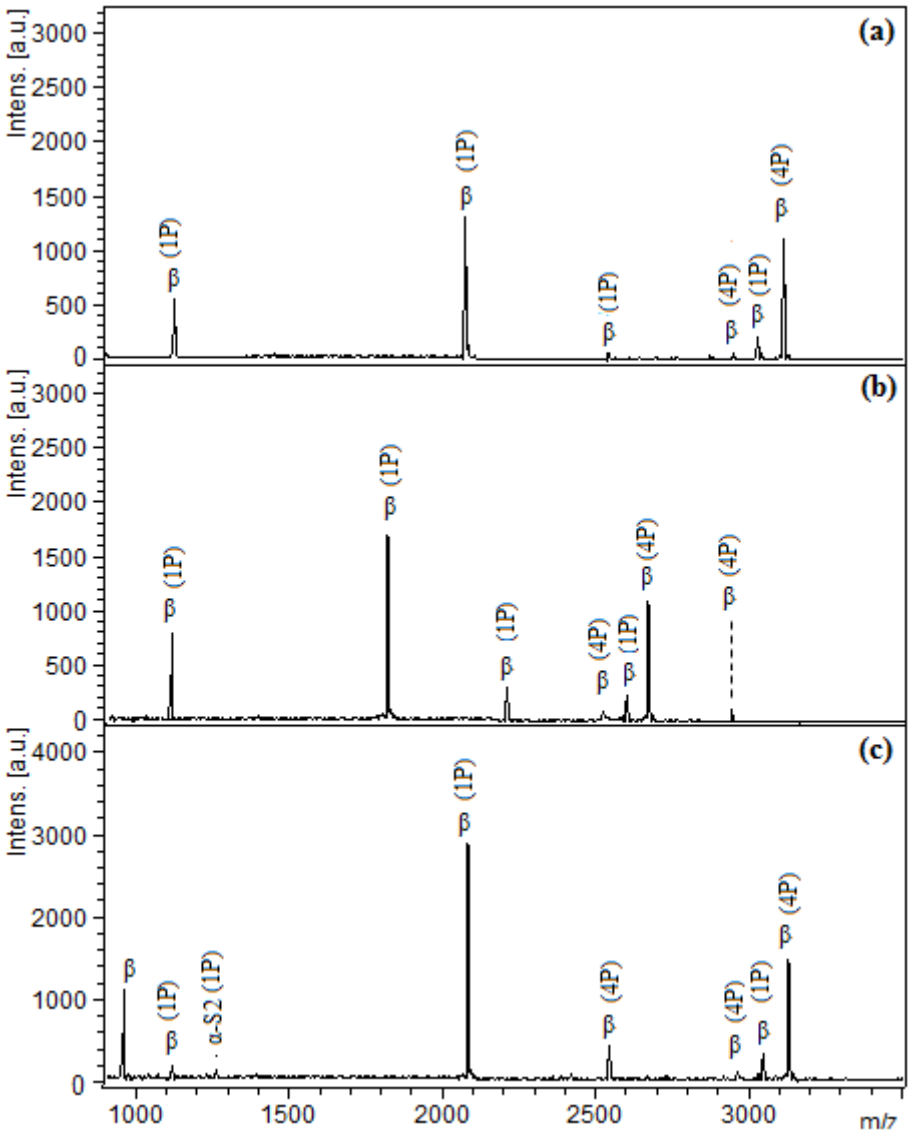

Fig. S2 Comparison of metal oxides nanoparticles using β-casein digest for (a) zirconia NPs (b) titania NPs and (c) nickel oxide NPs. αS2 and β reprents the detected phosphopeptides with status of phosphorylation given in brackets.

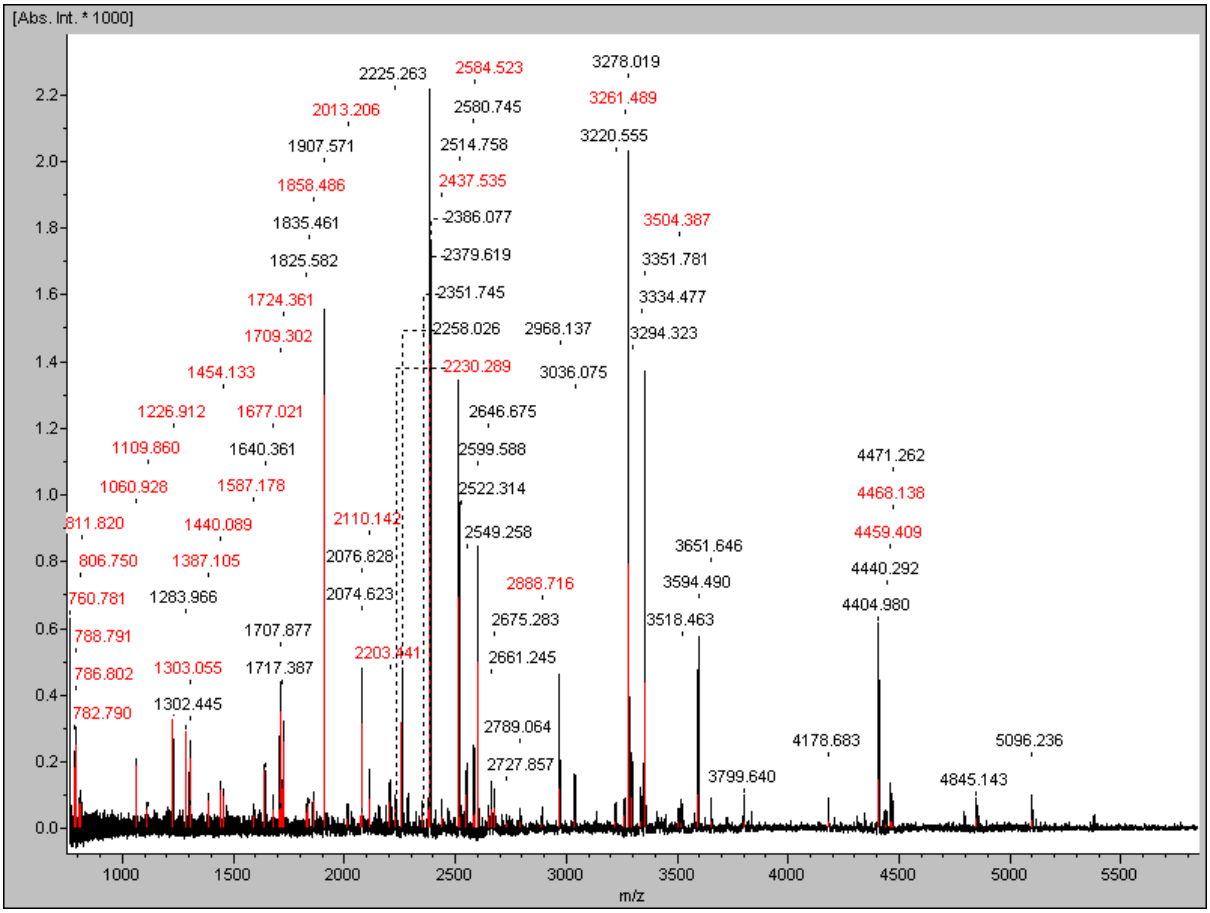

Fig. S3 Serum phosphopeptides analysis using graphene-NiO nanocomposite.

Table S1 Serum analysis using graphene-NiO nanocomposite using MALDI-MS. Search parameters are Charge=1+, MS Tol.:0.200000 Da, Trypsin, Mascot 2.4.1, SwissProt database, modifications: Global: Carboxymethyl (C), Optional: Oxidation (M), Phospho (ST), Phospho (Y).

Tree hierarchy	Meas. M/z	Calc. MH+	Int.	Dev. (Da)	Dev. (ppm)	Range	P	Sequence
Apoptosis-associated speck-like protein containing a CARD OS=Homo sapiens GN=PYCARD PE=1 SV=2 ASC_HUMAN								
peak 37	2386.077	2385.976	1458.721	0.1	42.079	140 - 158	1	VLTDEQYQAVRAEPTNPSK 3: Phospho (ST) 7: Phospho (Y) 15: Phospho (ST)
peak 46	2661.245	2661.293	54.613	-0.048	-18.072	162 - 182	1	LFSFTPAWNWTCKDLLLQALR 3: Phospho (ST) 12: Carboxymethyl (C)
peak 64	4178.683	4178.625	22.425	0.058	13.896	42 -74	1	GALLSMDALDLTDKLVSFYLETYGAELTANVLR 5: Phospho (ST) 6: Oxidation (M) 12: Phospho (ST) 17: Phospho (ST) 19: Phospho (Y) 22: Phospho (ST) 23: Phospho (Y) 28: Phospho (ST)
peak 70	4845.143	4845.146	27.769	-0.003	-0.61	75 -119	0	DMGLQEMAGQLQAATHQGSGAAPAGIQAPPQSAAKPGLHFIDQHR 2: Oxidation (M) 15: Phospho (ST) 19: Phospho (ST) 32: Phospho (ST)
Voltage-dependent calcium channel gamma-2 subunit OS=Homo sapiens GN=CACNG2 PE=1 SV=1 CCG2_HUMAN								
peak 21	1717.387	1717.446	118.951	-0.058	-34.009	239 - 250	1	SSSRSTEPSHSR 1: Phospho (ST) 2: Phospho (ST) 3: Phospho (ST) 5: Phospho (ST) 6: Phospho (ST)
peak 26	1907.571	1907.734	1303.449	-0.163	-85.504	243 - 258	1	STEPSHSRDASPVGIK 1: Phospho (ST) 2: Phospho (ST) 5: Phospho (ST)
peak 29	2076.828	2076.875	314.598	-0.047	-22.811	259 - 275	0	GFNTLPSTEISMYTLSR 4: Phospho (ST) 13: Phospho (Y)
peak 61	3594.49	3594.627	101.445	-0.137	-38.076	100 - 129	1	AVRASSIFPILSVILLFMGGLCIAASEFYK 5: Phospho (ST) 6: Phospho (ST) 12: Phospho (ST) 29: Phospho (Y) 22: Carboxymethyl (C)
IQ domain-containing protein K OS=Homo sapiens GN=IQCK PE=2 SV=1 IQCK_HUMAN								
peak 41	2549.258	2549.086	103.974	0.172	67.467	162 - 180	1	TKFIACDFLTEWLYNQNPK 1: Phospho (ST) 14: Phospho (Y) 6: Carboxymethyl (C)
peak 47	2675.283	2675.275	60.218	0.008	3.174	14 - 37	1	LKPSCSTDSSFTRTPVPTVSLASR 4: Phospho (ST) 5: Carboxymethyl (C)
peak 58	3351.781	3351.585	436.235	0.196	58.582	125 - 152	1	TCSPKEYLETFIFPVLLPGMASLLHQAK 1: Phospho (ST) 7: Phospho (Y) 2: Carboxymethyl (C)
peak 71	5096.236	5096.122	24.316	0.114	22.37	77 - 117	1	QEPVITVAPVEEMLFHGFSAEHYFPVSHFTMISRTPCPQDK 6: Phospho (ST) 13: Oxidation (M) 19: Phospho (ST) 23: Phospho (Y) 27: Phospho (ST) 37: Carboxymethyl (C)
Histatin-1 OS=Homo sapiens GN=HTN1 PE=1 SV=2 HIS1_HUMAN								
peak 51	2968.137	2968.326	118.621	-0.19	-63.971	24-Jan	1	MKFFVFALVLALMISMISADSHEK 15: Phospho (ST) 18: Phospho (ST) 21: Phospho (ST)

Leucine zipper putative tumor suppressor 2 OS=Homo sapiens GN=LZTS2 PE=1 SV=2 LZTS2_HUMAN								
peak 23	1825.582	1825.76	47.586	-0.178	-97.664	616 - 627	1	QLQHNYIQMYRR 6: Phospho (Y) 9: Oxidation (M) 10: Phospho (Y)
Dynactin subunit 5 OS=Homo sapiens GN=DCTN5 PE=1 SV=1 DCTN5_HUMAN								
peak 49	2789.064	2788.991	22.117	0.073	26.111	21-Jan	1	MELGELLYNKSEYIETASGNK 8: Phospho (Y) 11: Phospho (ST) 13: Phospho (Y) 16: Phospho (ST) 18: Phospho (ST)
Mitoferrin-1 OS=Homo sapiens GN=SLC25A37 PE=2 SV=2 MFRN1_HUMAN								
peak 19	1707.877	1707.728	54.734	0.149	87.104	15-Jan	1	MELRSGSVGSQAVAR 5: Phospho (ST) 7: Phospho (ST)
peak 65	4404.98	4404.981	145.837	-0.001	-0.188	130 - 168	1	RTLNDVFHHQGNSHLANGIAGSMATLLHDAVMNPAEVVK 2: Phospho (ST) 13: Phospho (ST) 22: Phospho (ST)
Photoreceptor-specific nuclear receptor OS=Homo sapiens GN=NR2E3 PE=1 SV=1 NR2E3_HUMAN								
peak 56	3294.323	3294.45	91.493	-0.127	-38.603	126 - 155	0	STAQVHLDSMESNTESRPESLVAPPAPAGR 1: Phospho (ST) 2: Phospho (ST)
Protein Mpv17 OS=Homo sapiens GN=MPV17 PE=1 SV=1 MPV17_HUMAN								
peak 40	2522.314	2522.197	32.04	0.117	46.272	49 - 71	1	GRTLTMVSLGCGFVGPVVGGWYK 22: Phospho (Y) 11: Carboxymethyl (C)
Mid1-interacting protein 1 OS=Homo sapiens GN=MID1IP1 PE=1 SV=1 M1IP1_HUMAN								
peak 53	3220.555	3220.481	18.235	0.074	23.028	67 - 95	1	TPPVPDSGSANGSFFAPSRDMYSHYVLLK 22: Phospho (Y)
Melanoma-associated antigen 9 OS=Homo sapiens GN=MAGEA9 PE=2 SV=1 MAGA9_HUMAN								
peak 34	2258.026	2257.993	321.492	0.033	14.758	243 - 258	1	KLLTQDWVQENYLEYR 12: Phospho (Y) 15: Phospho (Y)
peak 42	2580.745	2580.942	37.307	-0.197	-76.309	296 - 315	0	EPICYPSLYEEVLGEEQEGV 5: Phospho (Y) 7: Phospho (ST) 9: Phospho (Y) 4: Carboxymethyl (C)
Rab11 family-interacting protein 1 OS=Homo sapiens GN=RAB11FIP1 PE=1 SV=3 RFIP1_HUMAN								
peak 11	1302.445	1302.551	60.579	-0.106	-81.455	666 - 676	1	GTEDSLMGRTR 2: Phospho (ST)
peak 24	1835.461	1835.513	67.502	-0.052	-28.152	337 - 350	1	DSSPSSSPSPKGFR 2: Phospho (ST) 3: Phospho (ST) 5: Phospho (ST) 6: Phospho (ST) 7: Phospho (ST)
peak 28	2074.623	2074.774	39.488	-0.151	-72.784	658 - 674	1	QPSFPANKGTEDSLMGR 3: Phospho (ST) 10: Phospho (ST) 13: Phospho (ST)
peak 49	2789.064	2789.083	22.117	-0.019	-6.99	949 - 971	0	SPIMADLNLSLPSIPEVASDDER 1: Phospho (ST) 10: Phospho (ST) 13: Phospho (ST) 19: Phospho (ST)
Protein Wnt-2 OS=Homo sapiens GN=WNT2 PE=1 SV=1 WNT2_HUMAN								
peak 48	2727.857	2727.95	16.255	-0.093	-34.18	208 - 228	1	CHGVSGSCTLRTCWLAMADFR 5: Phospho (ST) 7: Phospho (ST) 9: Phospho (ST) 1: Carboxymethyl (C) 8: Carboxymethyl (C) 13: Carboxymethyl (C)
peak 66	4440.292	4440.186	9.216	0.106	23.898	Jan-38	1	MNAPLGGIWLWLPLLLTWLTPEVNSSWWYMRATGGSSR 29: Phospho (Y)
UDP-glucose 4-epimerase OS=Homo sapiens GN=GALE PE=1 SV=2 GALE_HUMAN								
peak 32	2225.263	2225.116	28.293	0.147	65.873	107 - 125	1	VNLTGTIQLLEIMKAHGVK 4: Phospho (ST) 6: Phospho (ST)
Centriole, cilia and spindle-associated protein OS=Homo sapiens GN=CCSAP PE=1 SV=2 CCSAP_HUMAN								

peak 45	2646.675	2646.818	33.085	-0.143	-54.053	251 - 269	1	ASSSENPWMTEYMRCYSAR 2: Phospho (ST) 3: Phospho (ST) 12: Phospho (Y) 16: Phospho (Y) 15: Carboxymethyl (C)
Protein FAM131A OS=Homo sapiens GN=FAM131A PE=2 SV=1 F131A_HUMAN								
peak 62	3651.646	3651.504	20.979	0.141	38.705	264 - 295	0	SLGPLEAQDSLYNSPLTESCLSPAEEEPAPCK 1: Phospho (ST) 12: Phospho (Y) 20: Carboxymethyl (C) 31: Carboxymethyl (C)
Olfactory receptor 52E5 OS=Homo sapiens GN=OR52E5 PE=3 SV=2 O52E5_HUMAN								
peak 60	3518.463	3518.297	17.744	0.165	47.038	236 - 262	0	ALSTCGSHVCVMLAFYLPALFSFMTHR 3: Phospho (ST) 4: Phospho (ST) 7: Phospho (ST) 16: Phospho (Y) 22: Phospho (ST) 5: Carboxymethyl (C) 10: Carboxymethyl (C)
Multifunctional protein ADE2 OS=Homo sapiens GN=PAICS PE=1 SV=3 PUR6_HUMAN								
peak 37	2386.077	2386.209	1458.721	-0.132	-55.311	54 - 74	1	AAISNKITSCIFQLLQEAGIK 4: Phospho (ST) 10: Carboxymethyl (C)
peak 63	3799.64	3799.688	21.877	-0.048	-12.563	368 - 401	0	LPSGLGCSTVLSPEGSAQFAAQIFGLSNHLVWSK 3: Phospho (ST) 8: Phospho (ST) 9: Phospho (ST) 7: Carboxymethyl (C)
BTB/POZ domain-containing protein KCTD14 OS=Homo sapiens GN=KCTD14 PE=1 SV=2 KCD14_HUMAN								
peak 52	3036.075	3036.274	24.735	-0.199	-65.714	28 - 52	0	RPTMSTVVELNVGGEFHTTTLGTLR 3: Phospho (ST) 5: Phospho (ST) 6: Phospho (ST) 18: Phospho (ST)
N-acetyllactosaminide beta-1,6-N-acetylglucosaminyl-transferase, isoform B OS=Homo sapiens GN=GCNT2 PE=2 SV=1 GNT2B_HUMAN								
peak 69	4471.262	4471.126	8.72	0.136	30.384	74 - 110	1	EYLTQSHYITAPLSKEEADFPLAYIMVIHHHFDTFAR 2: Phospho (Y)
Putative uncharacterized protein encoded by LINC00474 OS=Homo sapiens GN=LINC00474 PE=5 SV=2 CI027_HUMAN								
peak 55	3278.019	3278.101	794.277	-0.082	-24.926	26 - 51	1	TSNWGSSFSEKSGCMQTHPSMNLDCR 1: Phospho (ST) 2: Phospho (ST) 6: Phospho (ST) 15: Oxidation (M) 21: Oxidation (M) 14: Carboxymethyl (C) 25: Carboxymethyl (C)
Tumor protein D53 OS=Homo sapiens GN=TPD52L1 PE=1 SV=1 TPD53_HUMAN								
peak 68	4468.1380	4467.1307	18.812		0.1914	1-40	1	MEAQAQGLLETEPLQGTDEDAVASADFSSMLSEEEKEELKA : Phospho (ST); 2 Oxidation (M)
peak 20	1709.3020	1708.2947	352.65		-0.5337	41-54	0	**KAELVQLEDEITTLRQ: Phospho (ST)**
peak 22	1724.3610	1723.3537	258.407		-0.1854	86-79	0	KSWHDMQTTTAYKK : 2 Phospho (ST); Oxidation (M); Phospho (Y)
peak 18	1677.0210	1676.0137	57.74		0.3072	86-98	1	KSWHDMQTTTAYKKT : Phospho (Y)
peak 27	2013.2060	2012.1987	27.66		0.6320	86-98	1	KSWHDMQTTTAYKKT : 4 Phospho (ST); Oxidation (M); Phospho (Y)
peak 10	1283.9660	1282.9587	290.374		0.4170	124-133	1	KKFGDMSYSIRH: Phospho (Y)
peak 38	2437.5350	2436.5277	32.159		-0.2868	125-142	1	KFGDMSYSIRHSISMPAMRN: 3 Phospho (ST); 2 Oxidation (M); Phospho (Y)
peak 8	1109.860	1108.8527	57.87		0.3969	134-142	0	RHSISMPAMRN + Phospho (ST)
peak 57	3334.4770	3333.4697	21.522		0.0033	163-195	1	KTKVGGTNPNGGSFEEVLSSTAHASAQSLAGGSRR + 2 Phospho (ST)