# PTMScan<sup>®</sup> Direct: Ser/Thr Kinases Final Report Results



### Report Table of Contents

PTMScan® Direct Report • Ser/Thr Kinases

Project Overview (slides 3-6)

- Significant Changes Identified (3, 4)
- Summary of Results (5)
- PTMScan<sup>®</sup> Direct Method and Method Results (6)

Qualitative Results Summary (slides 7, 8)

Quantitative Results (slides 9-26)

- Table Overview (9-14)
- Result Summary(15-26)

Informatics Table (slide 27)

Summary of Available Services (slide 28)





#### Significant Changes: HeLa Cells –/+ EGF

PTMScan® Direct Report • Ser/Thr Kinases

		Normalized Fold Change	Average Raw Intensity		
Protein Name	Site	EGF : Control	Control	EGF	
Akt3	§472, 474	87.4	2,030	192,189	
Akt3	473, 474	87.4	2,030	192,189	
JNK2; JNK2 iso2; JNK2 iso3	§183, §185; §183, §185; §183, §185	8.1	4,851,520	42,785,466	
JNK3; JNK3 iso2; JNK1; JNK1 iso2	§221, §223; §221, §223; §183, §185; §183, §185	7.6	3,582,605	29,401,470	
GSK3A	§20	4.9	149,014	786,790	
RSK2	§386	4.0	46,383	199,498	
AMPKA1	§496	3.9	1,381,845	5,872,825	
ERK1	§202, §204	3.6	76,024,108	299,779,023	
p70S6K; p70S6K iso2	§447, §452; §424, §429	3.4	364,500	1,355,000	
ERK5; ERK5 iso2	§218, §220; §219, §221	3.4	502,653	1,866,092	
p70S6K; p70S6K iso2	§444, §447; §421, §424	3.4	12,474,667	45,809,266	
GSK3A	§21	3.2	1,164,082	4,048,066	
MEK1; MEK2	§218; §222	3.1	164,000	544,500	
GSK3B; GSK3B iso2	§9; §9	2.9	890,792	2,758,587	
MEK1; MEK2	§222; §226	2.8	1,150,040	3,542,525	
MSK2; MSK2 iso2	§360; §360	2.7	40,527	119,674	
MKK4	§257	2.7	3,001,038	8,639,953	
ERK2	§185, §187	2.6	32,927,950	91,062,000	
ERK2	§187, §190	2.6	32,927,950	91,062,000	
MEK1; MEK2	§218, §222; §222, §226	2.5	48,996	131,359	
OSR1; STLK3	§185; §233	-2.5	1,007,269	436,462	
OSR1; STLK3	189; §237	-2.5	1,007,269	436,462	
Chk1	307	-3.2	54,278	18,500	
CDK2; CDK3	§14; §14	-3.7	1,021,685	296,307	

Selected peptides that changed in abundance at least 2.5-fold with EGF treatment in HeLa cells. Green signifies fold change increases greater than 2.5, red signifies decreases. Red/Purple intensity denotes multiple identifications for 1 m/z due to ambiguous phosphorylation site localization. Blue intensity = peak area measurement. Blue = CST antibody available.

#### Significant Changes: HeLa Cells -/+ UV

PTMScan<sup>®</sup> Direct Report • Ser/Thr Kinases

		Fold Change	Average Raw Intensity		
Protein Name	Site	UV : Control	Control	UV	
JNK3; JNK3 iso2; JNK1; JNK1 iso2	§221, §223; §221, §223; §183, §185; §183, §185	55.9	61,971	3,464,900	
JNK3; JNK3 iso2; JNK1; JNK1 iso2	217, §223; 217, §223; 179, §185; 179, §185	53.4	35,000	1,870,000	
JNK2; JNK2 iso2; JNK2 iso3	§183, §185; §183, §185; §183, §185	44.9	3,280,290	147,311,119	
MKK4	§257, §261	28.4	4,835	137,191	
MKK4	§257	24.6	2,701,006	66,311,558	
MSK1	§376	23.3	6,697	156,288	
MKK4	§261	11.6	4,131	47,885	
JNK2; JNK2 iso2; JNK2 iso3	§183, 188; §183, 188; §183, 188	10.0	2,380	23,790	
MKK3	212, §222	8.3	12,672	104,977	
VRK1	§378	8.1	4,075	32,985	
ZAK	§648, §649	7.9	614,387	4,874,404	
MKK6; MKK6 iso2	§207, §211; §151, §155	7.9	648,974	5,102,919	
МАРКАРКЗ	§313	7.7	404,399	3,093,923	
ATM; LOC651610	§1981; 1859	7.6	1,148,442	8,724,508	
MSK2; MSK2 iso2	§360; §360	7.6	10,138	76,800	
dCK	§13	7.3	3,910	28,597	
MAPKAPK2	§334	6.5	10,424,377	67,766,696	
p70S6Kb	219	6.0	7,600	45,968	
BRD2	256	5.6	243,509	1,352,156	
p70S6K; p70S6K iso2	§441, §444, §447; §418, §421, §424	5.3	310,458	1,652,885	
p70S6K; p70S6K iso2	§441, §444, §452; §418, §421, §429	5.3	310,458	1,652,885	
p70S6K; p70S6K iso2	§441, §447, §452; §418, §424, §429	5.3	310,458	1,652,885	
p70S6K; p70S6K iso2	§444, §447, §452; §421, §424, §429	5.3	310,458	1,652,885	
p70S6K; p70S6K iso2	§444, §447; §421, §424	5.2	507,763	2,654,545	

Selected peptides that increased in abundance at least 2.5-fold with UV treatment in HeLa cells. Green signifies fold change increases greater than 2.5. Red intensity denotes multiple identifications for 1 m/z due to ambiguous phosphorylation site localization. Blue intensity = peak area measurement. Blue = CST antibody available.

## Summary of Results

PTMScan<sup>®</sup> Direct Report • Ser/Thr Kinases

HeLa cells with and without EGF treatment, H1650 cells with and without Phenformin treatment, and HeLa cells with and without UV treatment were subjected to PTMScan<sup>®</sup> analysis using the PTMScan<sup>®</sup> Direct Ser/Thr Kinases Reagent.

#### 136 non-redundant peptides (400 redundant peptides) were identified in HeLa cells –/+ EGF.

26 of the 136 peptides changed in abundance at least 2.5-fold with EGF treatment.

#### 181 non-redundant peptides (594 redundant peptides) were identified in H1650 cells –/+ Phenformin.

43 of the 181 peptides changed in abundance at least 2.5-fold with compound treatment.

#### 186 non-redundant peptides (592 redundant peptides) were identified in HeLa cells –/+ UV.

52 of the 186 peptides changed in abundance at least 2.5-fold with UV treatment.





### PTMScan<sup>®</sup> Direct Method



Method Summary

Phosphorylated peptide enrichment using the following antibodies: PTMScan® Direct: Ser/Thr Kinases Reagent LC-MS/MS analysis (LTQ-Orbitrap-CID): Sorcerer search results

Label-free quantitation from phosphorylated peptide intensities (Tables 1 & 2).

### Qualitative Results Summary

PTMScan® Direct Report • Ser/Thr Kinases



<u>Analysis</u>

LTQ-Orbitrap LC-MS/MS CID

2 samples, duplicate runs = 4 LC-MS/MS experiments

						Phosphopeptide Assignments		
Experiment	Cell Type	Treatment	Antibody	cs	Protease	Redundant	Non-Redundant	
1	HeLa	Control	S/T Kinase	11945	trypsin	827	545	
2	HeLa	Control	S/T Kinase	11946	trypsin	817	536	
3	HeLa	EGF	S/T Kinase	11947	trypsin	799	492	
4	HeLa	EGF	S/T Kinase	11948	trypsin	783	481	

A total of **400** redundant peptide assignments (Table 1 Details Tab) to **136** non-redundant peptides (Table 1 Summary Tab) using the PTMScan<sup>®</sup> Direct: Ser/Thr Kinases Reagent.

A 5% default false positive rate was used to filter the SORCERER results.

7

### Qualitative Results Summary

PTMScan® Direct Report • Ser/Thr Kinases

LTQ-Orbitrap

LC-MS/MS CID

Analysis

<u>Cells</u>	<u>Treatments</u>	Curation Sets (CS)
Hela	Control	<ul> <li>CS 11953, 11954</li> </ul>
IICEG	UV	<ul> <li>CS 11955, 11956</li> </ul>

2 samples, duplicate runs = 4 LC-MS/MS experiments

						Phosphopept	ide Assignments
Experiment	Cell Type	Treatment	Antibody	CS	Protease	Redundant	Non-Redundant
9	HeLa	Control	S/T Kinase	11953	trypsin	949	616
10	HeLa	Control	S/T Kinase	11954	trypsin	917	641
11	HeLa	UV	S/T Kinase	11955	trypsin	1353	812
12	HeLa	UV	S/T Kinase	11956	trypsin	1394	811

A total of **592** redundant peptide assignments (Table 3 Details Tab) to **186** non-redundant peptides (Table 3 Summary Tab) using the PTMScan<sup>®</sup> Direct: Ser/Thr Kinases Reagent.

A 5% default false positive rate was used to filter the SORCERER results.

### Table Overview

PTMScan® Direct Report • Ser/Thr Kinases

#### Tables consist of 5 different sheets:

Column Definitions, Details (qualitative), Summary (quantitative), Fold Change Ascending Order, and Fold Change by Protein Type

# Each sheet is accessed by clicking on the tabs located at the bottom of table window.

Column Definitions Details Summary Fold Change Descending Order Fold Change by Protein Type

(More examples can be seen highlighted in **blue boxes** on the following slides).

Tables open to the Fold Change Descending Order Sheet.



### **Column Definitions Sheet**

#### PTMScan® Direct Report • Ser/Thr Kinases

Open Save Print Import Copy Paste	AutoSum Sort A-Z Sort Z-A Gallery Toolbox Zoom Help Paste S	peci Down 👘 🗰 🤇	Iolumns Rows
	Sheets Charts SmartArt Graphics WordArt		
A	8	С	D
Column Name	Definition	In Details	In Summar
Accession	is the UniProt accession number for the assigned protein	Y	Y
Calculated m / 7	is the calculated, accurate mass-to-charge ratio of the identified peptide from the primary amino acid sequence and associated modifications	v	v
Charge	is the observed charge state for the identified pentide	Ý	Ý
Count in Details	is the number of times the designated modification site is represented from other peptide assignments in the Details tab. These redundant assignments can be as a result of multiple charge states, presence of oxidized methionine and overlapping sequence.		Y
Delta Cn	is the delta Xcorr value. In general, a Delta Cn of 0.1 or greater is good	Ŷ	
Description	is the FASTA database description of the protein entry	Y	Y
Fold Change	is the relative fold-change between the peak intensity of the experimental (numerator) and control (denominator) conditions. A negative value indicates the peptide is more abundant in the control condition		Y
Gene Name	is the gene name for the assigned protein. In some cases, the Gene Name is hyperlinked (when in blue text) when there are protein- specific antibodies available at CST	Y	Y
kD	is the translated molecular weight of the assigned protein	Y	
Mass Accuracy (ppm)	is the mass measurement error obtained between the observed and theoretical mass of the assigned peptide	Y	
MS2 Spectrum Number	is the spectrum number of the MS2 channel of the raw LC-MS/MS data file	Y	
Peak Apex MS Spectrum Number	is the spectrum number that is referenced for the corresponding apex peak intensity measurement of the parent ion from the MS channel of the raw LC-MS/MS data file	Y	
Pentide	is the amino acid sequence for the pentide assignment	Y	Y

Click tab to access sheets.

Column Definitions Sheets contain description of column headers in data tables.

#### PTMScan<sup>®</sup> Direct Report • Ser/Thr Kinases

ew Open Sa	e Print Import Copy Paste	AutoSum Sort A-Z Sort Z-A G	allery Toolbox Zoom He	) 🔯 🖬 🛛	Columns Rows	
	She	ets Charts Smart	tArt Graphics WordArt			
PTMS	CAN DIRECT RESULTS	C	D	E	F	
Table	#3: Hala Calle Trunsin Dia	act DTMScan Diro	t Sor/Thr Vinaco	Popagont		
Table	#3: HeLa Cells, Hypsin Dig	est, Prinstan Direc	L Sel/III Killase	Reagen		
Samp	les: Control = CS 11953 & 1	1954; + UV Treat	ment =CS 11955	& 11956		
Lener	d: * - phosphorylation # -	ovidized methioni	ne <b>S</b> - nublished	site Rlue Text	- CST antihody	
Leger		onaized meenon	ne, <b>s</b> published	Site, Dide Text	cor anabody	
)				1		
Row In	lex Protein Type	Gene Name	Protein Name	Site	Descripti	
Protein	inase, Ser/Thr (non-receptor)			1. State		
8	Protein kinase, Ser/Thr (non-recepto	or) AKT1	Akt1	§473	RAC-alpha serine/thre	
9	Protein kinase, Ser/Thr (non-recepto	or) AKT3	Akt3	§472	RAC-gamma serine/th	
10	Protein kinase, Ser/Thr (non-recepto	or) AKT3	Akt3	\$472, 474	RAC-gamma serine/th	
11	Protein kinase, Ser/Thr (non-recepto	or) AKT3	Akt3	§472, 474	RAC-gamma serine/th	
12	Protein kinase, Ser/Thr (non-recepto	or) AKT3	Akt3	§472, §476	RAC-gamma serine/th	
13	Protein kinase, Ser/Thr (non-recepto	or) AKT3	Akt3	473, 474	RAC-gamma serine/th	
14	Protein kinase, Ser/Thr (non-recepto	or) AKT3	Akt3	473, §476	RAC-gamma serine/th	
15	Protein kinase, Ser/Thr (non-recepto	or) AKT3	Akt3	474	RAC-gamma serine/th	
16	Protein kinase, Ser/Thr (non-recepto	or) AKT3	Akt3	§476	RAC-gamma serine/th	
17	Protein kinase, Ser/Thr (non-recepto	or) ATM; LOC651610	ATM; LOC651610	§1981; 1859	serine-protein kinase	
18	Protein kinase, Ser/Thr (non-recepto	or) ATM; LOC651610	ATM; LOC651610	§1981; 1859	serine-protein kinase	
19	Protein kinase, Ser/Thr (non-receptor	or) ATM; LOC651610	ATM; LOC651610	1983; 1861	serine-protein kinase	
20	Protein kinase, Ser/Thr (non-receptor	or) AURKA	AurA	§288	serine/threonine-prot	
21	Protein kinase, Ser/Thr (non-recepto	) AURKB; AURKC; AURKC	AurB; AurC; AurC iso2	§232; §198; §164	serine/threonine-prote	
22	Protein kinase, Ser/Thr (non-recepto	) AURKB; AURKC; AURKC	AurB; AurC; AurC iso2	§232; §198; §164	serine/threonine-prote	
23	Protein kinase, Ser/Thr (non-receptor	r) AURKB; AURKC; AURKC	AurB; AurC; AurC iso2	§232; §198; §164	serine/threonine-prote	

**Details Sheets (qualitative)** contain a redundant list of all peptides identified in the study with their accompanying Sorcerer metrics. The Details Sheet includes all information from the Summary Sheet with additional data for each peptide.

11

PTMScan® Direct Report • Ser/Thr Kinases

	0	00				1	Table3_ST_Kinase_Hela_	UV_FINAL.xIs			$\bigcirc$		
	0			🖹 🖬 🛱	570	- 2	• 🏡 🚮	100% -	0		â 🛲 ,		
	Nos	u Onen Sa	up Print In	moort Conv Pacta		AutoS	um Sort A-7 Sort 7-A Calleou	Toolhoy Zoom I	-lalo Pacta	Snaci Down Co	dumns Rows		
	INCI	Autosum son A-2 son 2-A Ganery Tobloox 200m Help Faste specific Down Columns Rows											
	Sneets Charts SmartArt Graphics WordArt												
>	~	DTMO	CAN			L .	U	. E.		к.			
ble Header	1	PIMS	CAN	DIRECT RES	50115								
	3	Table	<b>Fable #3:</b> HeLa Cells, Trypsin Digest, PTMScan Direct Ser/Thr Kinase Reagent										
niain information		-		, , , , ,	11050	0.440				0.54			
pout the study	5	Samp	oles: (	Control = CS	11953	& 119	54; + UV Treatme	nt =CS 11955	8 11	956			
nd legend text.	7	Leae	nd: *	- nhosnhorv	lation :	# - 0)	idized methionine	S - nuhlisher	d site	Blue Text - C	ST antihody a		
		Lege		phosphory	acion, i	, 0,	dazed meenomie,	3 published	1 5/1007	DILLE TEAL C	or anabody a		
					_ 007								
	10		-		Fold	Change		1					
	11	Index	Ind	lex in Detail	UV : (	Control	Gene Name	Protein Nam	ne	Site	Desc		
	17	6	6 Protein kinase, Ser/Thr (non-receptor)										
	18	7		8		1.8	AKT1	Akt1		§473	RAC-alpha serine/I		
	19	8		9		1.0	AKT3	Akt3		§472	RAC-gamma serine		
	20	9		11		1.3	AKT3	Akt3		§472, 474	RAC-gamma serine		
	21	10		12		1.3	AKT3	Akt3		§472, §476	RAC-gamma serine		
	22	11		13		1.7	AKT3	Akt3		473, 474	RAC-gamma serine		
	23	12		14		1.7	AKT3	Akt3		473, §476	RAC-gamma serine		
	24	13		15		1.0	AKT3	Akt3		474	RAC-gamma serine		
	25	14		16		1.0	AKT3	Akt3		§476	RAC-gamma serine		
	26	15		17		7.6	ATM; LOC651610	ATM; LOC651610		§1981; 1859	serine-protein kina		
	27	16		19		1.6	ATM; LOC651610	ATM; LOC651610		1983; 1861	serine-protein kina		
	28	17		20		1.0	AURKA	AurA		§288	serine/threonine-p		
	29	18		22		1.0	AURKB: AURKC: AURK	C AurB: AurC: AurC	iso2	\$232; \$198; \$164	serine/threonine-p		
	30	19		28		1.1	BCR	Bcr		\$177	breakpoint cluster		
	31	20		29		2.1	BRAF	B-Raf		\$446	serine/threonine-n		
	32	21		30		1.7	BRAF	B-Raf		\$447	serine/threonine-n		
	33	22		32		5.6	BRD2	BRD2		256	bromodomain-con		
	G		4-1-11	Column Definitions	Details S	ummary ]	Fold Change Descending Order	Fold Change by Protein	Type +	0	) 4 +		
		No	rmal View	Ready					Cour	u-1 0	SCRL @ CAPS @ NUM		
							lick tab to access sheets						

Summary Sheets (quantitative) contain a non-redundant list of sites quantified in the study.

### Fold Change Descending Order Sheet

PTMScan<sup>®</sup> Direct Report • Ser/Thr Kinases

0	00		Ľ	Table3_ST_Kinase_Hela_U	V_FINAL.xls		2
P New	v Open Save	Print Import Copy Paste	AutoSur	n Sort A-Z Sort Z-A Gallery	100% • 🕐	Paste Speci Down Col	imns Rows
			Sheets	Charts SmartArt Gr	aphics WordArt		
$\diamond$	A	В	C	D	E	F	
1	PTMS	CAN DIRECT RE	SULTS				
3	Table	#3: HeLa Cells, 7	Trypsin Digest,	PTMScan Direct Se	er/Thr Kinase Re	agent	
5	Sampl	es: Control = CS	11953 & 1195	54; + UV Treatmen	t =CS 11955 & .	11956	
-							
7	Legen	a: * - phosphory	iation, # - oxi	aized methionine,	<b>g</b> - published sit	e, Blue Text - CS	i antibody
-	-						
10			Fold Change				
11	Index	Index in Detail	UV : Control	Gene Name	Protein Name	Site	De
12	1	135	55.9	MAPK10; MAPK10; MAPH	JNK3; JNK3 iso2; JNK1	; §221, §223; §183, §185	mitogen-activate
13	2	117	53.4	MAPK10; MAPK10; MAPH	JNK3; JNK3 iso2; JNK1	; 1; 217, §223; 179, §185;	mitogen-activat
14	3	242	.44.9	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2	is§185; §183, §185; §183	mitogen-activat
15	4	580	28.4	MAP2K4	MKK4	§257, §261	dual specificity
16	5	575	24.6	MAP2K4	MKK4	§257	dual specificity
17	6	460	23.3	RPS6KA5	MSK1	§376	ribosomal prote
18	7	582	11.6	MAP2K4	MKK4	§261	dual specificity
19	8	253	10.0	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2	is3, 188; §183, 188; §183,	mitogen-activat
20	9	539	8.3	MAP2K3	МККЗ	212, §222	dual specificity
21	10	511	8.1	VRK1	VRK1	§378	serine/threonine
22	11	512	7.9	ZAK	ZAK	§648, §649	mitogen-activate
23	12	588	7.9	MAP2K6; MAP2K6	MKK6; MKK6 iso2	§207, §211; §151, §155	dual specificity r
24	13	269	7.7	МАРКАРКЗ	MAPKAPK3	§313	MAP kinase-acti
25	14	17	7.6	ATM; LOC651610	ATM; LOC651610	§1981; 1859	serine-protein k
26	15	457	7.6	RPS6KA4; RPS6KA4	MSK2; MSK2 iso2	§360; §360	ribosomal protei
27	16	2	7.3	DCK	dCK	§13	deoxycytidine ki
28	17	264	6.5	MAPKAPK2	MAPKAPK2	§334	MAP kinase-activ
		-> >: Column Definitions	Details Summary Fo	ld Change Descending Order 🦉	old Change by Protein Type	$\mathbf{\mathbf{D}}$	)4

Table Header contain informatio about the study and legend text.

Click tab to access sheets.

Fold Change Descending Order Sheets contain a non-redundant list of sites quantified in the study organized by maximum fold change across samples.

13

#### Fold Change by Protein Type Sheet

PTMScan<sup>®</sup> Direct Report • Ser/Thr Kinases

	0	00				Table3_ST_Kinase_Hela	LUV_FINAL.xls		C
	2 Nev	Open Sa	ve Print Im	port Copy Paste	Auto	sum Sort A-Z Sort Z-A Galle	ry Toolbox Zoom Help P	Paste Speci Down	Columns Rows
					Sheets	Charts SmartArt	Graphics WordArt		
	0	A	-	В	C	D	E	F	1
>	1	PTMS	CAN D	IRECT RES	SULTS				
e Header	-						~ /		
ninformation	3	Table	2 #3: H	eLa Cells, T	rypsin Diges	st, PTMScan Direct	Ser/Thr Kinase Re	agent	
the study	5	Samp	oles: Co	ontrol = CS	11953 & 11	954; + UV Treatme	ent =CS 11955 & .	11956	
gend text.	7	Lege	nd: * -	phosphory	lation, <b>#</b> - c	oxidized methionine	e, <b>§</b> - published sit	e, Blue Text - (	CST antibody
	10				Fold Change				
	11	Index	Inde	x in Detail	UV : Control	Gene Name	Protein Name	Site	Des
1	15	4	Protein	kinase, Ser/Th	r (non-receptor)		li od kriste Statisticki se i destri		
	16	5		17	7.6	ATM; LOC651610	ATM; LOC651610	§1981; 1859	serine-protein ki
	17	6		19	4.6	ATM; LOC651610	ATM; LOC651610	1983; 1861	serine-protein ki
	18	7		32	5.6	BRD2	BRD2	256	bromodomain-co
	19	8		74	2.8	CDK2; CDK3	CDK2; CDK3	§14, §15; §14, §15	cell division prot
	20	9		78	4.8	CDKL3	CdkL3	311	cyclin-dependent
	21	10		87	2.9	CHEK1	Chk1	§317	serine/threonine
	22	11		89	2.6	CHEK1	Chk1	343	serine/threonine
	23	12		91	2.5	CHEK1	Chk1	§345, 362	serine/threonine
	24	13		92	2.7	CHEK1	Chk1	348, 357	serine/threonine
	25	14		93	4.2	EIF2AK4	GCN2	254, 264	eukaryotic transl
	26	15		94	4.2	EIF2AK4	GCN2	254, 274	eukaryotic transl
	27	16		117	53.4	MAPK10; MAPK10; M	APK JNK3; JNK3 iso2; JNK1	; 1; 217, §223; 179, §18	<ol><li>5; mitogen-activate</li></ol>
	28	17		135	55.9	MAPK10; MAPK10; M.	APK JNK3; JNK3 iso2; JNK1	; .§221, §223; §183, §1	85 mitogen-activate
	29	18		190	3.3	MAPK14; MAPK14	p38-alpha; p38-alpha i	sa §180, §182; §180, §1	82 mitogen-activate
	30	19		212	-5.4	MAPK7; MAPK7	ERK5; ERK5 iso2	§218, §220; §219, §2	21 mitogen-activate
	31	20		242	44.9	MAPK9; MAPK9; MAP	K9 JNK2; JNK2 iso2; JNK2	is§185; §183, §185; §1	83 mitogen-activate
	(m	1	4	Column Definitions	Details Summary	Fold Change Descending Order	Fold Change by Protein Type	+	3411
			LA AND		C DOLCOMMAN COMMAND				

Fold Change by Protein Type Sheets provide the overall quantitative results sorted by protein type containing only those sites that increase above 2.5-fold (GREEN) and those sites that decrease below 2.5-fold (RED).

PTMScan® Direct Report • Ser/Thr Kinases

			Fold Change			
>	Index	Index in Detail	UV : Control	Gene Name	Protein Name	Site
Index	6	Protein kinase, Ser/Th	r (non-receptor)			
The table is indexed	7	8	1.8	AKT1	Akt1	§473
for easy re-sorting	8	9	1.0	AKT3	Akt3	§472
	9	11	1.3	AKT3	Akt3	§472, 474
to original oraer.	10	12	1.3	AKT3	Akt3	§472, §476
	11	13	1.7	AKT3	Akt3	473, 474
	12	14	1.7	AKT3	Akt3	473, §476
	13	15	1.0	AKT3	Akt3	474
	14	16	1.0	AKT3	Akt3	§476
	15	17	7.6	ATM; LOC651610	ATM; LOC651610	§1981; 1859
	16	19	4.6	ATM; LOC651610	ATM; LOC651610	1983; 1861
	17	20	1.0	AURKA	AurA	§288
	18	22	-1.0	AURKB; AURKC; AURKC	AurB; AurC; AurC iso2	§232; §198; §164
	19	28	-1.1	BCR	Bcr	§177
	20	29	2.1	BRAF	B-Raf	§446
	21	30	1.7	BRAF	B-Raf	§447
	22	32	5.6	BRD2	BRD2	256
	23	35	2.1	CDK1; CDK2; CDK3	CDK1; CDK2; CDK3	4, §15; §14, §15; §14, §
	24	42	1.0	CDK1; CDK2; CDK3	CDK1; CDK2; CDK3	§15; §15; §15
	25	51	2.1	CDK1; CDK2; CDK3	CDK1; CDK2; CDK3	5, §19; §15, §19; §15, §
	26	53	1.0	CDK1; CDK2; CDK3	CDK1; CDK2; CDK3	§19; §19; §19
	27	58	2.4	CDK12	CRK7 iso2	§1244
	28	61	1.7	CDK12	CRK7	§1246
	29	64	-1.2	CDK13; CDK13	CHED; CHED iso2	1225, 1226; 1165, 1166

#### Index in Detail

Λ

Index numbers from the Details Sheet is included for easy lookup of peptides between tabs.

٨

**Fold Change** Redundancy is eliminated from the Details Table to provide a summary of the quantitative results. Fold changes are expressed as the ratio of UV treated to Control peptide ion abundance in each cell line.

Green signifies sites that increase at least 2.5-fold. Red signifies sites that decrease at least 2.5-fold.

PTMScan® Direct Report • Ser/Thr Kinases

....

	-	Fold Change	products are available	e for Gene Names or Site	s (seen in blue text).	1
Index	Index in Detail	UV : Control	Gene Name	Protein Name	Site	
6	Protein kinase, Ser/Th	r (non-receptor)				
7	8	1.8	AKT1	Akt1	§473	
8	9	1.0	AKT3	Akt3	§472	
9	11	1.3	AKT3	Akt3	§472, 474	
10	12	1.3	AKT3	Akt3	§472, §476	
11	13	1.7	AKT3	Akt3	473, 474	
12	14	1.7	AKT3	Akt3	473, §476	
13	15	1.0	AKT3	Akt3	474	-
14	16	1.0	AKT3	Akt3	§476	
15	17	7.6	ATM; LOC651610	ATM; LOC651610	§1981; 1859	
16	19	4.6	ATM; LOC651610	ATM; LOC651610	1983; 1861	Noval sites of
17	20	1.0	AURKA	AurA	§288	phosphorylation
18	22	-1.0	AURKB; AURKC; AURKC	AurB; AurC; AurC iso2	§232; §198; §164	C. Duda liaka a daika
19	28	-1.1	BCR	Bcr	§177	<b>9</b> Published sile:
20	29	2.1	BRAF	B-Raf	§446	
21	30	1.7	BRAF	B-Raf	§447	-
22	32	5.6	BRD2	BRD2	256	
23	35	2.1	CDK1; CDK2; CDK3	CDK1; CDK2; CDK3	4, §15; §14, §15; §14, §	1
24	42	1.0	CDK1; CDK2; CDK3	CDK1; CDK2; CDK3	§15; §15; §15	Hypertext
25	51	2.1	CDK1; CDK2; CDK3	CDK1; CDK2; CDK3	5, §19; §15, §19; §15, §	product
26	53	1.0	CDK1; CDK2; CDK3	CDK1; CDK2; CDK3	§19; §19; §19	pages
27	58	2.4	CDK12	CRK7 iso2	§1244	(blue text).
28	61	1.7	CDK12	CRK7	§1246	
29	64	-1.2	CDK13; CDK13	CHED; CHED iso2	1225, 1226; 1165, 1166	

Summary Sheets contain Gene Name, Protein Name, and phosphorylation Site for each identified peptide.

PTMScan<sup>®</sup> Direct Report • Ser/Thr Kinases

#### URL

Links to PhosphoSitePlus® pages are given for each protein.

Ductoin Nouse	Cito	Description	Accession	V
Protein Name	Site	Description	Accession	URL
Akt1	§473	RAC-alpha serine/threonine-protein kinase	P31749	http://www.phosphosite.org/pr
Akt3	\$472	RAC-gamma serine/threonine-protein kinase is	s Q9Y243	http://www.phosphosite.org/pr
Akt3	\$472, 474	RAC-gamma serine/threonine-protein kinase is	Q9Y243	http://www.phosphosite.org/pr
Akt3	§472, §476	RAC-gamma serine/threonine-protein kinase is	Q9Y243	http://www.phosphosite.org/pr
Akt3	473, 474	RAC-gamma serine/threonine-protein kinase is	Q9Y243	http://www.phosphosite.org/pr
Akt3	473, §476	RAC-gamma serine/threonine-protein kinase is	Q9Y243	http://www.phosphosite.org/pr
Akt3	474	RAC-gamma serine/threonine-protein kinase is	Q9Y243	http://www.phosphosite.org/pr
Akt3	§476	RAC-gamma serine/threonine-protein kinase is	Q9Y243	http://www.phosphosite.org/pr
ATM; LOC651610	§1981; 1859	serine-protein kinase ATM	Q13315; XP_945884	http://www.phosphosite.org/pr
ATM; LOC651610	1983; 1861	serine-protein kinase ATM	Q13315; XP_945884	http://www.phosphosite.org/pr
AurA	§288	serine/threonine-protein kinase 6	014965	http://www.phosphosite.org/pr
AurB; AurC; AurC iso2	§232; §198; §164	serine/threonine-protein kinase 12	6GD4; Q9UQB9; Q9UQB	http://www.phosphosite.org/pr
Bcr	§177	breakpoint cluster region protein isoform 1	P11274	http://www.phosphosite.org/pr
B-Raf	§446	serine/threonine-protein kinase B-raf	P15056	http://www.phosphosite.org/pr
B-Raf	§447	serine/threonine-protein kinase B-raf	P15056	http://www.phosphosite.org/pr
BRD2	256	bromodomain-containing protein 2 isoform 1	P25440	http://www.phosphosite.org/pr
CDK1; CDK2; CDK3	4, §15; §14, §15; §14, §	cell division protein kinase 1 isoform 4	P06493; P24941; Q0052	Ehttp://www.phosphosite.org/pr
CDK1; CDK2; CDK3	§15; §15; §15	cell division protein kinase 1 isoform 4	P06493; P24941; Q0052	6http://www.phosphosite.org/pr
CDK1; CDK2; CDK3	5, §19; §15, §19; §15, §	cell division protein kinase 1 isoform 4	P06493; P24941; Q0052	Ehttp://www.phosphosite.org/pr
CDK1; CDK2; CDK3	§19; §19; §19	cell division protein kinase 1 isoform 4	P06493; P24941; Q0052	6 http://www.phosphosite.org/pr
CRK7 iso2	§1244	cell division protein kinase 12 isoform 2	Q9NYV4-2	http://www.phosphosite.org/pr
CRK7	§1246	cell division protein kinase 12 isoform 2	Q9NYV4	http://www.phosphosite.org/pr
CHED: CHED iso2	1225, 1226; 1165, 1166	cell division protein kinase 13 isoform 1	014004; 014004-2	http://www.phosphosite.org/pr

Multiple isoforms for a single peptide are separated by semicolon.

Summary Sheets include protein/peptide/site information (Description, Accession).



PTMScan® Direct Report • Ser/Thr Kinases

#### **Count in Details**

The Summary Sheet includes the Count in Details: number of peptides in the Details Tab for each site.

					V
Protein Name	Site	Peptide	Charge	Calc. m/z	<b>Count in Details</b>
Akt1	§473	RPHFPQFS*YSASGTA	2	866.8776	1
Akt3	§472	RPHFPQFS*YSASGRE	3	615.9405	1
Akt3	§472, 474	RPHFPQFS*YS*ASGRE	3	642.5959	2
Akt3	§472, §476	RPHFPQFS*YSAS*GRE	3	642.5959	1
Akt3	473, 474	RPHFPQFSY*S*ASGRE	2	963.3902	1
Akt3	473, §476	RPHFPQFSY*SAS*GRE	2	963.3902	1
Akt3	474	RPHFPQFSYS*ASGRE	3	615.9405	1
Akt3	§476	RPHFPQFSYSAS*GRE	3	615.9405	1
ATM; LOC651610	§1981; 1859	SLAFEEGS*QSTTISSLSEK	2	1040.9697	2
ATM; LOC651610	1983; 1861	SLAFEEGSQS*TTISSLSEK	3	694.3156	1
AurA	§288	RTT*LCGTLDYLPPEM#IEGR	2	1159.5315	1
AurB; AurC; AurC iso2	§232; §198; §164	RKT*M#CGTLDYLPPEM#IEGR	3	793.6896	3
Bcr	§177	KGHGQPGADAEKPFY*VNVEFHHER	5	566.6637	5
B-Raf	§446	RDS*SDDWEIPDGQITVGQR	2	1127.4922	1
B-Raf	§447	RDSS*DDWEIPDGQITVGQR	3	751.9973	2
BRD2	256	SLHS*AGPPLLAVTAAPPAQPLAK	3	763.0785	1
CDK1; CDK2; CDK3	4, §15; §14, §15; §14, §	IGEGT*Y*GVVYK	2	673.2775	6
CDK1; CDK2; CDK3	§15; §15; §15	IGEGTY*GVVYK	2	633.2943	12
CDK1; CDK2; CDK3	5, §19; §15, §19; §15, §	IGEGTY*GVVY*K	2	673.2775	2
CDK1; CDK2; CDK3	§19; §19; §19	IGEGTYGVVY*K	2	633.2943	1
CRK7 iso2	§1244	RT*PTMPQEEAAEK	2	784.3447	6
CRK7	§1246	RTPT*M#PQEEAAACPPHILPPEK	3	856.0658	4
CHED; CHED iso2	1225, 1226; 1165, 1166	ENGSGHEASLQLRPPPEPS*T*PVSGQDDLIQHQDM#R	4	1000.4398	2

\* Phosphorylation

#Oxidized methionine

Summary Sheets includes protein/peptide/site information (Peptide, Charge, Calc. m/z, Count in Details).

#### **Average Retention Time**

The Summary Sheet includes Average

PTMScan® Direct Report • Ser/Thr Kinases

Retention Tim	ne of intensity measureme	ents.	Raw Intensity					
Protein Name	Site	V Average RT	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)		
Akt1	§473	48.73	18,884	26,795	43,849	36,785		
Akt3	§472	42.92	980,450	1,231,127	1,126,554	1,135,765		
Akt3	§472, 474	46.51	3,972,424	4,095,174	5,532,008	5,131,506		
Akt3	§472, §476	46.51	3,972,424	4,095,174	5,532,008	5,131,506		
Akt3	473, 474	46.51	370,282	390,054	721,741	589,526		
Akt3	473, §476	46.51	370,282	390,054	721,741	589,526		
Akt3	474	42.92	980,450	1,231,127	1,126,554	1,135,765		
Akt3	§476	42.92	980,450	1,231,127	1,126,554	1,135,765		
ATM; LOC651610	§1981; 1859	55.31	1,124,418	1,172,466	8,760,573	8,688,443		
ATM; LOC651610	1983; 1861	55.31	490,120	846,045	3,045,710	3,081,149		
AurA	§288	56.90	15,680	12,001	12,600	15,383		
AurB; AurC; AurC iso2	§232; §198; §164	45.28	74,939	50,459	60,762	59,400		
Bcr	§177	36.17	654,108	702,180	653,579	545,408		
B-Raf	§446	54.46	3,017,528	2,712,298	5,927,468	5,908,738		
B-Raf	§447	54.46	16,247,832	16,765,891	29,239,026	28,518,806		
BRD2	256	58.44	223,277	263,740	1,407,789	1,296,523		
CDK1; CDK2; CDK3	4, §15; §14, §15; §14, §	40.58	10,649,900	9,804,520	21,271,400	21,491,700		
CDK1; CDK2; CDK3	§15; §15; §15	37.32	2,657,941,170	2,520,258,327	2,542,491,645	2,650,156,788		
CDK1; CDK2; CDK3	5, §19; §15, §19; §15, §	40.58	10,649,900	9,804,520	21,271,400	21,491,700		
CDK1; CDK2; CDK3	§19; §19; §19	37.32	2,657,941,170	2,520,258,327	2,542,491,645	2,650,156,788		
CRK7 iso2	§1244	24.99	314,282	272,122	725,684	708,192		
CRK7	§1246	39.04	958,193	984,045	1,577,773	1,677,072		
CHED; CHED iso2	1225, 1226; 1165, 1166	46.02	1,031,731	955,701	765,701	956,815		
			٨	Raw In	tensity	^		

Also included is the raw peak heights (Raw Intensity).

**Bold values** = intensities manually reviewed. **Red values** = multiple identifications for 1 m/z measurement due to ambiguous phosphorylation site localization. **Blue values** = peak area measurement. **Purple values** = Peak area and multiple identifications.

PTMScan® Direct Report • Ser/Thr Kinases

		Average Raw Intensity Control UV		Ratio	Fold Change	
Protein Name	Site			UV : Control	UV : Control	Species
Akt1	§473	22,840	40,317	1.77	1.77	human
Akt3	§472	1,105,789	1,131,160	1.02	1.02	human
Akt3	§472, 474	4,033,799	5,331,757	1.32	1.32	human
Akt3	§472, §476	4,033,799	5,331,757	1.32	1.32	human
Akt3	473, 474	380,168	655,634	1.72	1.72	human
Akt3	473, §476	380,168	655,634	1.72	1.72	human
Akt3	474	1,105,789	1,131,160	1.02	1.02	human
Akt3	§476	1,105,789	1,131,160	1.02	1.02	human
ATM; LOC651610	§1981; 1859	1,148,442	8,724,508	7.60	7.60	human
ATM; LOC651610	1983; 1861	668,083	3,063,430	4.59	4.59	human
AurA	\$288	13,841	13,992	1.01	1.01	human
AurB; AurC; AurC iso2	§232; §198; §164	62,699	60,081	0.96	-1.04	human
Bcr	§177	678,144	599,494	0.88	-1.13	human
B-Raf	\$446	2,864,913	5,918,103	2.07	2.07	human
B-Raf	\$447	16,506,862	28,878,916	1.75	1.75	human
BRD2	256	243,509	1,352,156	5.55	5.55	human
CDK1; CDK2; CDK3	4, §15; §14, §15; §14, §	10,227,210	21,381,550	2.09	2.09	human
CDK1; CDK2; CDK3	§15; §15; §15	2,589,099,749	2,596,324,217	1.00	1.00	human
CDK1; CDK2; CDK3	5, §19; §15, §19; §15, §	10,227,210	21,381,550	2.09	2.09	human
CDK1; CDK2; CDK3	§19; §19; §19	2,589,099,749	2,596,324,217	1.00	1.00	human
CRK7 iso2	\$1244	293,202	716,938	2.45	2.45	human
CRK7	\$1246	971,119	1,627,423	1.68	1.68	human
CHED; CHED iso2	1225, 1226; 1165, 1166	993,716	861,258	0.87	-1.15	human

**Summary Sheets** include Average Raw Intensity, Ratio, Fold Change, and species of samples profiled. When necessary, ratios are normalized based on the median Log2 Ratio (not shown).

PTMScan<sup>®</sup> Direct Report • Ser/Thr Kinases

		7	7 Amino Acids	Upstream/Downstream			
	Peptide	e sequence 7 amin	o acids to each	Includes any know	n upstream effectors		
	side of t	he modified residue	e also included.	and downstream substrates.			
			V	V	V		
Protein Name	Site	kD	-7/+7 peptide	Upstream Kinase	Downstream Target		
ALt1	5473	56	PDHEDOES*VSASGTA	ATM ALTI DNA-DK TIK	TIK PAKI POKI PIKIPI		
Akt1	5473	56	PDHEDOES*VSASCRE	PHI PP PHI PP2	, ILK, PARI, PORI, PIRJRI		
Akt3	5472 474	56	DDUEDOES*VSASCDE HEDOESVS				
ALLA	5472,474	56	PDHEDOEC*VCACCDE HEDOECVC				
AKL3	5472 5476	56	PDHEDOES*VSASCRE DOESVSAS	*( DHI DD DHI DD)			
Akt3	473 474	56	DHEDOESV*SASCRE, HEDOESVS	*			
Akta	473, 474	56	PHEPOESY*SASCRE_ DOESYSAS	*0			
ALT2	475, 9470	56	HEDDESVS*ASCRE-, PQF313A3	· c			
AKC3	4/4	50	POESVEAS*CRE				
ATM: LOCEE1610	9470 51091, 1950	251, 226	CLAFFECCE OCTTICE: CLAFFECC	CATM ATE DOMID DODO	ATM COCAD MOCI NES		
ATM: LOC651610	\$1901, 1059	251, 226	SLAFEEGS QSTTISS, SLAFEEGS	*CATM ATE DEMID DEP2	CATM, CDCA2, MDC1, NBS		
ATM: LOC651610	1092: 1961	251, 330	AFEECSOC *TTISSIS: AFEECSOC	AIM, AIK, PPMID, PPP2	CATH, CDCA2, MDC1, NB3		
AURA	5200	351, 350	APEEDSQ3 TTISSLS, AFEEDSQ3	Aura DAKI DKACa DDD	a		
AurR: AurC: AurC ico?	5222 5108: 5164	30: 36: 32	APSI REVT*MCCTLDV: TESI REVT	AUTA, FARI, FRACE, FFF			
AurB: AurC: AurC iso2	5232, 5108, 5164	39, 30, 32	APSI PRET MCGTLDY, TPSI PRET	*NAUER MST1 DDD2CA: DD			
AurB: AurC: AurC iso2	5222, 5198, 5164	39, 30, 32	APSI REVT*MCGTLDY: TESI DEVT	*NAUR MST1 DDD2CA: DD	INCENE		
Rer	5177	142	ADAEVDEV*//N//EEUU	Abl BCD/ABL Even Hele	Gab2 Grb2 Hek SOS1		
Bor	91// 5177	145		Abi, BCR/ABI, Fyn, Hck,	Gab2, Gib2, Hck, SOS1		
Ber	g1//	145		Abi, BCR/ABI, Fyn, Hck,	Gab2, Gib2, Hck, SOS1		
BCF	91//	145		ADI, BCR/ABL, Fyn, Hck,	Gab2, Grb2, Hck, SOS1		
BCF	91//	143		ADI, BCR/ABL, Fyn, Hck,	Gab2, Grb2, Hck, SOS1		
BCF	91//	143	ADAEKPFY*VIVEFHH	ADI, BCR/ABL, FYN, HCK,	Gab2, Grb2, Hck, SOS1		
B-Kar	\$446	84	KILGRRDS*SDDWEIP	PAK1, PKACa	B-Kat, HRas		
B-Rar	5447	84	ILGRRDSS*DDWEIPD				

**kD** Included is the molecular weight of the protein from which the peptide is derived.



PTMScan® Direct Report • Ser/Thr Kinases

#### **Spectrum Numbers**

The Details Sheet includes the MS/MS (or MS2) and MS scan numbers for each peptide assignment.

			MS2 Spectr	um Number		Peak Apex MS Spectrum Number			
Protein Name	Site	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)
Akt1	\$473			10329				10331	
Akt3	\$472	9012		1		9000		1	
Akt3	\$472, 474	al 55555	9860						
Akt3	\$472, 474		9813	9653			9862	9691	
Akt3	\$472, \$476			1	9685				9724
Akt3	473, 474	9985							
Akt3	473, \$476			9676	9730				
Akt3	474		8925						
Akt3	§476			8727				8697	
ATM; LOC651610	§1981; 1859	12598	12443	12305	12315	12622	12457	12337	12365
ATM; LOC651610	§1981; 1859				12329				12365
ATM; LOC651610	1983; 1861		12463	12320				12317	
AurA	§288				12864				
AurB; AurC; AurC iso2	§232; §198; §164				12040				12025
AurB; AurC; AurC iso2	§232; §198; §164	9781	9437	9289	9315	9715	9524	9336	9364
AurB; AurC; AurC iso2	§232; §198; §164		9609	9274	9594			9336	9364
Bcr	§177	8080	7996	7710	7792	8089	8011	7793	7799
Bcr	§177			7724			10110210200	7793	
Bcr	§177			6889				6878	
Bcr	§177			6913				6878	
Bcr	§177	7185	7078			7163	7072		
B-Raf	§446		12161	12020	12088		12217	12077	12105
B-Raf	§447	12326	12145	12018	12066	12382	12217	12077	12105

V

Experimental details that are mainly used as a reference to raw data.



PTMScan® Direct Report • Ser/Thr Kinases

#### Retention Time

The Details Sheet includes the Retention Time.

Xcorr values (or Sorcerer cross-correlation value) for each peptide assignment.

**Xcorr Values** 

			Retenti	on Time		Xcorr			
Protein Name	Site	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)
Akt1	5473	48.75	48 84	48.68	48.65			2 945	
Akt3	\$472	10110	10101	10100	10100	2,723		2.0.10	
Akt3	5472, 474					2.725	1,759		
Akt3	\$472, 474	46.51	46.50	46.49	46.55		2.241	2,610	
Akt3	\$472, \$476								2.475
Akt3	473, 474					2.096			
Akt3	473, §476	46.51	46.50	46.49	46.55			2.416	1.920
Akt3	474	42.88	43.01	42.90	42.87		2.342		
Akt3	\$476							2.386	
ATM; LOC651610	§1981; 1859	55.34	55.22	55.30	55.36	4.470	4.043	4.954	5.206
ATM: LOC651610	g1901; 1059 1092: 1961	EE 24	55.22	FF 20	EE 26		3,335	2.010	3.095
ATM; LOC651610	1983; 1801	55.34	55.22	55.30	55.30		2.225	3.010	2.245
AurA	9288 5333, 5109, 5164	50.85	50.88	50.90	50.91				2.245
AurB; AurC; AurC iso2	9232; 9198; 9104	54.40	34.29 45.21	54.11 4E 17	24.25	2 102	2 262	2 560	2.028
AurB; AurC; AurC iso2	9232; 9198; 9104 5323; 5109; 5164	45.49	45.21	45.17	45.25	3.105	3.202	3.500	3.337
Auro; Auro; Auro Isoz	9232; 9198; 9104	45.49	45.21	45.17	45.23	2 655	2.510	2.040	3,342
Ber	g1//	39.59	39.71	39.70	39.55	3.055	3.574	3,450	2.0/1
BCF	91//	39.59	39.71	39.70	39.53			2.399	
BCL	91//	36.20	30.25	30.32	35.97	-		3.908	
BCF	§1//	36.20	36.25	36.32	35.97	2.225		2.968	
Bcr	91//	36.13	36.25	36.40	35.91	3.236	3.101	1112-22	1. 5224
B-Raf	§446	54.52	54.43	54.42	54.48		4.489	4.548	4.499
B-Raf	§447	54.52	54.43	54.42	54.48	3.522	2.970	4.075	3.575

V

Experimental details that are used as a reference and measure of confidence to the peptide assignment.

PTMScan® Direct Report • Ser/Thr Kinases

V

#### **Mass Accuracy**

1

#### DeltaCN

Details Sheet includes the measured peptide mass accuracy.

The Details Sheet also includes the DeltaCN value for each peptide assignment.

			Mass Accu	racy (ppm)		DeltaCn			
Protein Name	Site	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)
Akt1	8473			-0.2430				0.2150	
Akt3	\$472	-0.6327		1		0.2110			
Akt3	\$472, 474		-0.5137			1 27.002.002.000	0.0100		
Akt3	\$472, 474		-0.3745	-0.3538			0.1640	0.2700	
Akt3	\$472, \$476		1.27.25.0.12.00		-0.2473				0.2710
Akt3	473, 474	-0.3855				0.1150			
Akt3	473, §476			0.0107	-0.1024			0.1020	0.1930
Akt3	474		-0.9578				0.0890		
Akt3	\$476			-0.9372				0.2060	
ATM; LOC651610	§1981; 1859	0.0369	-0.0390	-0.1356	-0.1226	0.3440	0.3890	0.4460	0.4300
ATM; LOC651610	§1981; 1859				0.5040				0.2760
ATM; LOC651610	1983; 1861		3.0721	1.1350			0.0710	0.1880	
AurA	§288				-3.6148				0.2120
AurB; AurC; AurC iso2	§232; §198; §164				-0.8550				0.1360
AurB; AurC; AurC iso2	§232; §198; §164	-0.6978	-0.5948	-0.9769	-0.3733	0.1240	0.2170	0.1050	0.1270
AurB; AurC; AurC iso2	§232; §198; §164		-0.5036	-0.7075	-0.0379		0.0730	0.0860	0.1390
Bcr	§177	-0.9322	-1.1151	-0.8226	-0.8171	0.3450	0.2150	0.2530	0.2440
Bcr	§177		100000000	-0.8226		For the Dec		0.0320	u tra cubicido
Bcr	§177			-0.6769				0.2400	
Bcr	§177			-0.4988				0.1570	
Bcr	§177	-0.6182	-0.9508			0.2690	0.0900		
B-Raf	§446		-0.0489	-0.1124	0.2550		0.4370	0.3840	0.4220
B-Raf	§447	-0.4061	-0.5254	-0.2965	-0.2295	0.2810	0.1660	0.2690	0.3340

Experimental details that are used as a reference and measure of confidence to the peptide assignment.

PTMScan® Direct Report • Ser/Thr Kinases

Include the Rsp value and the Peptide Prophet Probability for each peptide assignment.

			V	,				V				
Protein Name	Rsp				PP Probability							
	Site	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)	Species	Motif	Lax
Akt1	\$473			6				0.999		human	1	0
Akt3	\$472	1				0.999				human	1	0
Akt3	\$472, 474		389				0.249			human	1	0
Akt3	\$472, 474		20	1			0.976	0.999		human	1	0
Akt3	§472, §476				67				0.997	human	1	0
Akt3	473, 474	721				0.565				human	1	0
Akt3	473, §476			9	112			0.893	0.794	human	1	0
Akt3	474		103				0.899			human	1	0
Akt3	§476			6				0.994		human	1	0
ATM; LOC651610 ATM; LOC651610	§1981; 1859 §1981; 1859	1	1	1	1 2	1.000	1.000	1.000	1.000	human human	1 1	0
ATM; LOC651610	1983; 1861		13	12			0.843	0.999		human	1	0
AurA	\$288				263				0.737	human	1	0
AurB; AurC; AurC iso2	§232; §198; §164				2				0.988	human	1	0
AurB; AurC; AurC iso2	§232; §198; §164	31	2	14	4	0.982	0.999	0.989	0.995	human	1	0
AurB; AurC; AurC iso2	§232; §198; §164		18	1	2	20100-0-000	0.923	0.978	0.996	human	1	0
Bcr	§177	2	1	12	1	1.000	1.000	1.000	1.000	human	1	0
Bcr	§177			60				0.973		human	1	0
Bcr	§177			1				1.000		human	1	0
Bcr	§177			1				1.000		human	1	0
Bcr	§177	27	56			1.000	0.989			human	1	0
B-Raf	§446		1	1	1		1.000	1.000	1.000	human	1	0
B-Raf	§447	1	1	1	1	1.000	1.000	1.000	1.000	human	1	0

Λ

Includes whether the preptide is motif or lax.

Experimental details that are used as a reference and measure of confidence to the peptide assignment.

### Qualitative Table

PTMScan® Direct Report • Ser/Thr Kinases

#### MS2 Spectrum Number

V

The site, T222, is assigned 17 times in all curation sets (count number of MS2 entries).

Protein Name				MS2 Spectrum Number						
	Site	Peptide	Charge	Control (CS11953)	Control (CS11954)	UV (CS11955)	UV (CS11956)			
MAPKAPK2	§222	ETTSHNSLTT*PCYTPY	2				9667			
MAPKAPK2	§222	ETTSHNSLTT*PCYTPYYVAPEVLGPEK	3	14309	14175	14046	14027			
MAPKAPK2	§222	ETTSHNSLTT*PCYTPYYVAPEVLGPEK	3			14078	14019			
MAPKAPK2	§222	ETTSHNSLTT*PCYTPYYVAPEVLGPEKYDK	3	13579	13409	13303	13277			
MAPKAPK2	§222	ETTSHNSLTT*PCYTPYYVAPEVLGPEKYDK	3	13590	13442		13347			
MAPKAPK2	§222	ETTSHNSLTT*PCYTPYYVAPEVLGPEKYDK	3	13557	13470					
MAPKAPK2	§222	ETTSHNSLTT*PCYTPYYVAPEVLGPEKYDK	4		13380					

#### Peptide

Λ

The site is represented in 3 overlapping sequences (incomplete digestion).

#### ∧ Charge

The phosphorylated peptide assignments are seen in multiple charge states.



26

#### Informatics Table

PTMScan® Direct Report • Ser/Thr Kinases

#### PTMSCAN DIRECT INFORMATICS TABLE

Table #4: HeLa Cells, Trypsin Digest, PTMScan Direct Ser/Thr Kinase Reagent

Samples: Control = CS 11953 & 11954; + UV = CS 11955 & 11956

#### Legend: \* - phosphorylation, ^ - multiple protein identifications for one peptide

gene_name	species	protein_type	protein_function_description	entrez_geneid	NCBI_acc	NCBI_site	SWISSPROT_acc
Akt1	human	ec 2.7.11.1; kinase; protei	h kina an oncogenic AGC kinase that plays a critic	207	NP_005154	S473	P31749
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S476	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S474	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	Y473	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	Y473	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S476	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S474	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S472	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S472	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S472	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S476	Q9Y243
Akt3	human	ec 2.7.11.1; kinase; protei	h kina an AGC kinase that plays a critical role in co	10000	NP_005456	S474	Q9Y243
AMPKA1^	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	S184	Q13131
AMPKA1^	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	T183	Q13131
AMPKA1	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	T490	Q13131
AMPKA1	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	S494	Q13131
AMPKA1	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	S496	Q13131
AMPKA1	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	T488	Q13131
AMPKA1	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	S496	Q13131
AMPKA1	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	S486	Q13131
AMPKA1	human	autophagy; ec 2.7.1; ec 2	.7.11 a protein kinase of the CAMKL family that p	5562	NP_006242	T490	Q13131

The **Informatics Table** contains specific information for each identified peptide: Gene ontology protein type, accession numbers, site designation, PubMed references, ScanSite, Upstream and downstream genes. The Informatics Table also includes statistical data within the context of this study, peptide count in each curation set and in cell type.

### Summary of Available Services

PTMScan® Direct Report • Ser/Thr Kinases

#### Service Offerings from Cell Signaling Technology

**PTMScan® Discovery Services** - a discovery-mode proteomics technology for identifying and quantifying post-translational modifications.

**KinomeView<sup>™</sup> Services** - a western blotting service using Phospho-Motif Antibodies to provide a kinome-wide view of cellular phosphorylation.

PTMScan<sup>®</sup> Motif Antibody Kits and Proteomics System - product lines that enable the use of PTMScan Technology on your own.

AQUA<sup>™</sup> Peptide Synthesis - custom synthesized, isotope-labeled AQUA peptides for validating and quantifying protein markers and post-translational modifications.

**PTMScan® Direct Services** - a targeted-mode proteomics screening technology with defined assay endpoints.

