Store at -20°C

#7400

Serine/Threonine Kinase Substrate Screening Kit

☑2 assays

(After initial use, store plates at appropriate temperature)

Cell Signaling TECHNOLOGY®

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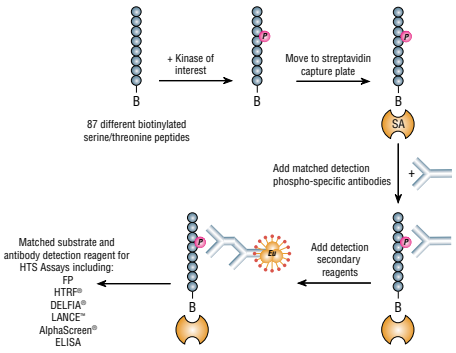
Products Included	Products #	Kit Quantity
Antibody Plates: 96-well plates		6
87 Phospho-Specific Antibodies	See table	2 X 100 µl each
Peptide plates: 96-well plates	9804	3
87 Phospho-Peptides (Biotinylated)		50 µl, 12.5 µM each
87 X 2 Nonphospho-Peptides (Biotinylated)		50 µl, 12.5 µM each
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
CD Rom; data, overview		1
Kinase of interest (not included)		

Background: In order to expedite the isolation of substrates for novel kinases, Cell Signaling Technology has developed a kinase/substrate development kit. Numerous serine/threonine kinases have become pharmaceutically important, including GSK, Akt and p38. With the cloning of hundreds of uncharacterized serine/threonine kinases, drug discovery researchers are seeking to quickly find substrates for high throughput screening programs.

With a total of 87 different peptide/phospho-specific antibody pairs on the array, a very diverse group of substrates is represented. Signal-to-noise ratios have been optimized and range from 10 to over 1000. The phospho-specific antibody and peptide substrate pair can be used for subsequent high throughput peptide based kinase screens (e.g., FP, TRF, AlphaScreen).



Kinase of interest is incubated with biotinylated substrate peptides. Phosphorylated products are captured on streptavidin-coated plates. Cell Signaling Technology antibodies serve as primary detection reagents; DELFIA® Europium (Eu) anti-rabbit or anti-mouse chelates are used as secondary antibodies. Positive hits from Serine/Threonine Kinase Substrate Screening Kit (#7400) can be used in any subsequent peptide based kinase assay.

Description: The Serine/Threonine Kinase Substrate Screening Kit provides 87 biotinylated nonphospho-peptides as kinase substrates. Also included are biotinylated phospho and nonphospho-peptides of identical sequence as positive and negative controls respectively. Potential substrate phosphorylation is detected by the corresponding phospho-specific antibodies.

The paired phospho-specific antibody and peptide substrate can be used for high throughput peptide based kinase screens (e.g. FP, TRF, AlphaScreen).

Specificity/Sensitivity: 10 to 1000+ fold signal-to-noise ratio of phospho- versus nonphospho-peptides measured by DELFIA® assay.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with synthetic phospho-peptides (KLH-coupled) and are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are derived from mice immunized with synthetic phospho-peptides coupled to KLH.

Quality Control: The quality of the peptides was evaluated by reverse-phase HPLC and by mass spectometry.

Storage: Antiboidies are supplied in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl (TBS), 0.05% Tween-20, 1.0 µg/ml BSA. Store at 4°C. Do not aliquot the antibodies.

Peptides are supplied at 12.5 µM in 1X kinase buffer (25 mM Tris (pH 7.5), 5 mM beta-glycerolphosphate, 2 mM DTT, 0.1 mM Na_3V0_4, 5 mM $MgCl_2$). Store peptides at -20°C.

KEY:
Plate, Row, and Column designation refer to positions on Kinase Substrate Screening Kit
P = CST phospho-peptide number (postive control found in wells 1, 4, 7, 10)
NP = CST nonphospho-peptide number (negative control wells found in wells 2, 5, 8, 11 and kinase substrates found in wells 3, 6, 9, 12)
SNR = Signal-to-noise ratio (P DELFIA® units/NP DELFIA® units)

Plate	Row	Column	P#	NP#	CST Ab #	Target/Antibody Description	S/N
1	a	1,2,3	872	870	2974	Phospho-mTOR (Ser2481) Antibody	1343
1	a	4,5,6	910	907	2301	Phospho-DARPP-32 (Thr75) Antibody	394
1	a	7,8,9	826	821	3081	Phospho-C/EBPβ (Ser105) Antibody (Rat Specific)	160
1	a	10,11,12	848	844	9291	Phospho-Bad (Ser112) Antibody	78
1	b	1,2,3	1132	1131	3361	Phospho-CaMKII (Thr286) Antibody	948
1	b	4,5,6	911	908	3141	Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody	340
1	b	7,8,9	1166	1133	9336	Phospho-GSK-3β (Ser9) Antibody	143
1	b	10,11,12	850	846	2341	Phospho-Chk1 (Ser345) Antibody	76
1	c	1,2,3	842	836	9121	Phospho-MEK1/2 (Ser217/221) Antibody	840
1	c	4,5,6	871	869	9571	Phospho-eNOS (Ser1177) Antibody	290
1	c	7,8,9	489	485	9601	Phospho-(Ser) 14-3-3 Binding Motif Antibody	119
1	c	10,11,12	1152	1151	3114	Phospho-VASP (Ser239) Antibody	74
1	d	1,2,3	1168	1167	9376	Phospho-PKCδ/θ (Ser643/676) Antibody	698
1	d	4,5,6	489	485	9528	Phospho-cdc25C (Ser216) Antibody	243
1	d	7,8,9	503	505	9611	Phospho-(Ser/Thr) Akt Substrate Antibody	117
1	d	10,11,12	829	824	9461	Phospho-FoxO1 (Ser256) Antibody	72
1	e	1,2,3	1148	1147	1932	Phospho-PLK (Ser137) Antibody	561
1	e	4,5,6	888	890	9631	Phospho-(Ser/Thr) Phe Antibody	228
1	e	7,8,9	1128	1127	9591	Phospho-MSK1 (Ser376) Antibody	97
1	e	10,11,12	1150	1149	9151	Phospho-SEK1/MKK4 (Thr261) Antibody	71
1	f	1,2,3	861	865	9297	Phospho-Bad (Ser155) Antibody	533
1	f	4,5,6					
1	f	7,8,9	922	924	9287	Phospho-p53 (Ser20) Antibody	95
1	f	10,11,12	493	494	9131	Phospho-Stat3 (Tyr705) Antibody	69
1	g	1,2,3	945	940	9455	Phospho-4E-BP1 (Thr70) Antibody	512
1	g	4,5,6	1126	1125	9181	Phospho-Elk-1 (Ser383) Antibody	194
1	g	7,8,9	828	823	2441	Phospho-eIF4G (Ser1108) Antibody	91
1	g	10,11,12	851	847	9231	Phospho-MKK3/MKK6 (Ser189/207) Antibody	65
1	h	1,2,3	1130	1129	2864	Phospho-c-Abl (Thr735) Antibody	463
1	h	4,5,6	923	915	9307	Phospho-Rb (Ser780) Antibody	170
1	h	7,8,9	946	943	9114	Phospho-cdc2 (Thr161) Antibody	84
1	h	10,11,12	862	858	9331	Phospho-GSK-3α/β (Ser21/9) Antibody	61
2	a	1,2,3	1215	1136	9204	Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody	56
2	a	4,5,6	1128	1127	9631	Phospho-(Ser/Thr) Phe Antibody	43
2	a	7,8,9	909	905	2531	Phospho-AMPK-α (Thr172) Antibody	28
2	a	10,11,12	488	484	2291	Phospho-(Thr) PDK1 Substrate Antibody	16

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SNR = Signal-to-noise ratio (P DELFIA® units/NP DELFIA® units)

Plate	Row	Column	P#	NP#	CST Ab #	Target/Antibody Description	S/N
2	b	1,2,3	1144	1143	3068	Phospho-Aurora A (Thr288)/Aurora B (Thr232)/Aurora C (Thr198) Antibody	156
2	b	4,5,6	1148	1147	2261	Phospho-(Ser) PKC Substrate Antibody	40
2	b	7,8,9	825	820	9295	Phospho-Bad (Ser136) Antibody	26
2	b	10,11,12	863	859	2401	Phospho-HSP27 (Ser82) Antibody	58
2	c	1,2,3	503	505	9621	Phospho-(Ser/Thr) PKA Substrate Antibody	20
2	c	4,5,6	849	845	9164	Phospho-c-Jun (Ser73) Antibody	36
2	c	7,8,9	1182	1181	9101	Phospho-p44/42 MAP Kinase (Thr202/Tyr204) Antibody	25
2	c	10,11,12	1396	1395	2981	Phospho-(Ser) Arg-X-Tyr/Phe-X-pSer Motif Antibody	13
2	d	1,2,3	490	498	9621	Phospho-(Ser/Thr) PKA Substrate Antibody	53
2	d	4,5,6	827	822	3311	Phospho-Cofilin (Ser3) Antibody	34
2	d	7,8,9	873	877	9551	Phospho-PTEN (Ser380) Antibody	22
2	d	10,11,12	1178	1177	9464	Phospho-FoxO1 (Thr24)/FoxO3a (Thr32) Antibody	9
2	e	1,2,3	888	890	9341	Phospho-p90RSK (Ser380) Antibody	50
2	e	4,5,6	1146	1145	2661	Phospho-Chk2 (Thr68) Antibody	30
2	e	7,8,9	1180	1179	9211	Phospho-p38 MAP Kinase (Thr180/Tyr182) Antibody	22
2	e	10,11,12	1032	1031	9221	Phospho-ATF-2 (Thr71) Antibody	6
2	f	1,2,3	491	500	2261	Phospho-(Ser) PKC Substrate Antibody	49
2	f	4,5,6	1124	1123	9261	Phospho-c-Jun (Ser63) II Antibody	28
2	f	7,8,9	1138	1137	9346	Phospho-p90RSK (Thr573) Antibody	10
2	f	10,11,12	884	880	9741	Phospho-eIF4E (Ser209) Antibody	33
2	g	1,2,3	1140	1139	9621	Phospho-(Ser/Thr) PKA Substrate Antibody	45
2	g	4,5,6	1184	1183	9251	Phospho-SAPK/JNK (Thr183/Tyr185) Antibody	28
2	g	7,8,9	1132	1131	9621	Phospho-(Ser/Thr) PKA Substrate Antibody	267
2	g	10,11,12	491	500	9621	Phospho-(Ser/Thr) PKA Substrate Antibody	20
2	h	1,2,3	1214	1213	2851	Phospho-(Ser/Thr) ATM/ATR Substrate Antibody	203
2	h	4,5,6	853	855	9631	Phospho-(Ser/Thr) Phe Antibody	20
2	h	7,8,9	864	876	2851	Phospho-(Ser/Thr) ATM/ATR Substrate Antibody	24
2	h	10,11,12	874	878	9621	Phospho-(Ser/Thr) PKA Substrate Antibody	32
3	a	1,2,3	875	879	9621	Phospho-(Ser/Thr) PKA Substrate Antibody	76
3	a	4,5,6	1170	1169	2851	Phospho-(Ser/Thr) ATM/ATR Substrate Antibody	140
3	a	7,8,9	1176	1175	9631	Phospho-(Ser/Thr) Phe Antibody	20
3	a	10,11,12				intentionally left blank	
3	b	1,2,3				intentionally left blank	
3	b	4,5,6				intentionally left blank	
3	b	7,8,9				intentionally left blank	
3	b	10,11,12				intentionally left blank	
3	c	1,2,3				intentionally left blank	

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SNR = Signal-to-noise ratio (P DELFIA® units/NP DELFIA® units)

Plate	Row	Column	P#	NP#	CST Ab #	Target/Antibody Description	S/N
3	c	4,5,6				intentionally left blank	
3	c	7,8,9				intentionally left blank	
3	c	10,11,12				intentionally left blank	
3	d	1,2,3	864	876	9286	Phospho-p53 (Ser15) (16G8) mAb	107
3	d	4,5,6	1184	1183	9255	Phospho-SAPK/JNK (Thr183/Tyr185) (G9) mAb	59
3	d	7,8,9	1130	1129	9386	Phospho-Threonine (42H4) mAb	25
3	d	10,11,12	487	483	9391	Phospho-Threonine-Proline mAb (P-Thr-Pro-101)	47
3	e	1,2,3	839	833	9246	Phospho-IκB-α (Ser32/36) (5A5) mAb	83
3	e	4,5,6	1180	1179	9216	Phospho-p38 MAPK (Thr180/Tyr182) (28B10) mAb	36
3	e	7,8,9	1135	1134	9386	Phospho-Threonine (42H4) mAb	20
3	e	10,11,12	825	820	9606	Phospho-(Ser) 14-3-3 Binding Motif (4E2) mAb	11
3	f	1,2,3	838	832	9706	Phospho-Histone H3 (Ser10) (6G3) mAb	83
3	f	4,5,6	912	913	9634	Phospho-(Ser/Thr) PDK1 Docking Motif (18A2) mAb	17
3	f	7,8,9	1032	1031	9391	Phospho-Threonine-Proline mAb (P-Thr-Pro-101)	17
3	f	10,11,12	1132	1131	9391	Phospho-Threonine-Proline mAb (P-Thr-Pro-101)	24
3	g	1,2,3	489	485	9606	Phospho-(Ser) 14-3-3 Binding Motif (4E2) mAb	62
3	g	4,5,6	488	484	9386	Phospho-Threonine (42H4) mAb	31
3	g	7,8,9	1184	1183	9391	Phospho-Threonine-Proline mAb (P-Thr-Pro-101)	16
3	g	10,11,12	888	890	9634	Phospho-(Ser/Thr) PDK1 Docking Motif (18A2) mAb	8
3	h	1,2,3	1182	1181	9106	Phospho-p44/42 MAPK (Thr202/Tyr204) (E10) mAb	60
3	h	4,5,6	1146	1145	9386	Phospho-Threonine (42H4) mAb	31
3	h	7,8,9	1174	1173	9136	Phospho-Stat3 (Ser727) (6E4) mAb	15
3	h	10,11,12	1135	1134	9391	Phospho-Threonine-Proline Mouse mAb (P-Thr-Pro-101)	25

Protocol for Serine/Threonine Kinase Substrate Screening Kit

Suggested Kinase Assay Conditions:
Combine 10 µM peptide substrate, 200 µM ATP and 10-100 nM Kinase in a 25 µl reaction. Incubate at 30°C for 30 minutes.

Peptide substrate is supplied in 1X kinase buffer, which was diluted from 10X Kinase Buffer #9802.

Note: Optimal incubatioin times and enzyme concentrations must be determined empirically for each particular kinase.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X TBS, 0.05% Tween-20 (TBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** tBST, 10% BSA, 100 mM EDTA
4. DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
5. DELFIA® Europium-labeled Anti-rmouse IgG (PerkinElmer Life Sciences #AD0124)
6. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
7. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

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B Protocol

1. Set up Reaction cocktails (Mock/No Kinase and Plus Kinase) in tubes on ice.

   For each 25 µl reaction, add the following to the Reaction cocktails:
   0.5 µl ATP (10 mM)
   10-100 nM Kinase (Plus Kinase Cocktail Only)

   Each 96-well plate will require Plus Kinase Reaction cocktail for 32 x 5 µl reactions and Mock/No Kinase Reaction cocktail for 64 x 5 µl. It is recommended that sufficient Reaction cocktail solution be prepared for 33 and 65 reactions respectively.
2. *Prepare substrate lates on ice. Spin peptide plates 5 minutes at 1000 rpm.

   For kinase reaction with 10 µM peptide substrate, transfer 20 µl peptide solution (12.5 µM) to two 96-well plates on ice. Maintain primary plate configuration with all transfers.
3. Add 5 µl Reaction cocktails to peptide substrate plates. Keep on ice. Add Mock/No Kinase reaction cocktail to plate columns 1-2, 4-5, 7-8, 10-11. Add Plus Kinase Reaction coccktail to plate columns 3, 6, 9, 12. Mix with gentle agitation or pipetting.
4. Incubate reaction plate at 30°C for 30 minutes.
5. *Spin plates 5 minutes at 1000 rpm.
6. Add 100 µl Stop Buffer to each well and mix by pipetting.
7. Transfer 5-100 µl of each reaction to 96-well streptavidin-coated plate and incubate at room temperature for 30 minutes.

Indicated DELFIA® signal-to-noise values were obtained by capturing 5 pmoles peptide on streptavidin. DELFIA® signal-to-noise ratio was calculated as the ratio between phospho-peptide and nonphospho-peptide DELFIA® signals.

8. *Wash three times with 100 µl/well TBS/T.
9. Add 100 µl primary antibody from antibody plate to matched well of streptavidin-coated plate.
10. Mix and incubate at room temperature with rocking for 60 minutes.
11. *Wash three times with 100 µl/well TBS/T.
12. Add 100 µl/well diluted Europium-labeled anti-rabbit or anti-mouse IgG antibody. Dilute the anti-rabbit IgG antibody 1:1000 and the anti-mouse IgG antibody 1:500 in DELFIA® Assay buffer.
13. Incubate at room temperature for 30 minutes.
14. *Wash five times with 100 µl/well TBS/T.
15. Add 100 µl/well DELFIA® Enhancement Solution.
16. Incubate at room temperature for 5 minutes.
17. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

***IMPORTANT: Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.**

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com