Molecular Devices

IMAP™ Substrate Finder for Ser/Thr kinases 2 (TKL/STE/CK1/CMGC)

with **Progressive Binding System**

Product #R8140
patents pending

Introduction

About the IMAP™ Substrate Finder Kit

The IMAP Substrate Finder Kits are IMAP assay development tools that can significantly accelerate the identification of substrates for kinases or that may be used to profile a kinase's substrate specificity.The IMAP Substrate Finder for Serine/Threonine Kinases 2 enables the researcher to screen a kinase of interest against 61 kinase substrates within a few hours. The substrates are documented as known substrates for kinases of the TKL, STE, CK1 and CMGC families of the Human Kinome as described by Manning et al.[1]

Principle of the Assay

The IMAP technology is based on the high affinity binding of phosphate by immobilized metal (M^{III}) coordination complexes on nanoparticles. Fluorescein (5FAM) labeled peptide sequences are lyophilized into a 384 well plate. The researcher simply reconstitutes the substrates by adding reaction buffer and ATP, then the kinase of interest is added. Following the incubation period for reaction, addition of the IMAP binding system stops the reaction. The binding of the phosphorylated peptide to the binding entities results in a change in the rate of the molecular motion of the peptide which is measured as a robust fluorescence polarization change (Figure 1).

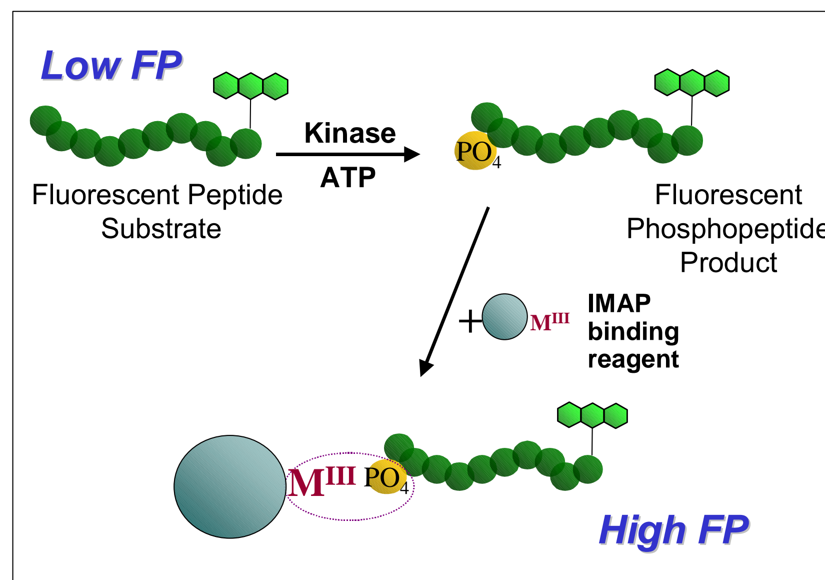


Figure 1. Principle of the IMAP system

[1] Manning et al. Science **298**:1912-34, (2002)

About the Progressive Buffer System

The ratios of Progressive Buffers A and B, recommended in this protocol, were designed to provide conditions that give a good signal with an acceptable background. However, the delta mP may be increased with further optimization. Thus, upon discovery of a potential substrate, many factors should still be examined including the ratio of IMAP Progressive Buffers A and B, ATP concentration, IMAP Progressive Binding Reagent concentration, and enzyme concentration. Due to the diverse nature of the substrates, five different Binding Solutions are needed for this Substrate Finder. For each Binding Solution condition a calibrator (phosphopeptide control) is provided. Please refer to the Progressive Buffer System Application Note for a more detailed explanation of the flexibility of this buffer system. Assay conditions used in the Progressive Binding System may not be appropriate for the Original Binding System.

Applications

The IMAP Substrate Finder Kits are IMAP assay development tools that accelerate the identification of suitable substrates for performing IMAP kinase assays. Additionally, if a suitable substrate is already known, IMAP Substrate Finders can help determine a kinase's substrate specificity, allowing the researcher to screen a diverse collection of Serine/Threonine kinase peptide substrates. Additional applications include testing enzyme preparations for purity, identifying pairs of substrates suitable for multiplexing, and characterization of kinases.

Materials and Equipment

Table 1: The IMAP™ Substrate Finder for Serine/Threonine Kinases 2 Kit with Progressive Binding System (R8140) contains:

Reagent	Quantity	Description
IMAP Progressive Binding Reagent	0.15 mL	One vial, store 4°C. ****Do Not Freeze the Binding Reagent**
IMAP Progressive Binding Buffer A (5x)	12 mL	One bottle, store 4°C.
IMAP Progressive Binding Buffer B (5x)	12 mL	One bottle, store 4°C.
IMAP Reaction Buffer including 0.01% Tween-20 as carrier (5x)##	12 mL	One bottle, store 4°C. The Complete Reaction Buffer consists of a 1x stock with added DTT (1mM final reaction concentration). This may vary depending on the enzyme.
IMAP™ Substrate Finder for Ser/Thr kinases 2 (TKL/STE/CK1/CMGC): 2 plates each containing 61 substrates and 6 calibrators (phospho peptides) as positive controls	2	Two black 384 well plates, each containing 67 different, dried 5FAM labeled peptides in quadruplicate. Reconstitution into a 20 µL kinase reaction will result in a 100 nM concentration.
Instructions for file downloads on enclosed postcard in kit.	1	Access to: 1) An electronic Product Insert (links to literature on WorldWideWeb) 2) IMAP Substrate Mapper, an interactive spreadsheet file for easy manipulation of all the substrate's information 3) Substrate Finder Data Analysis Macro, a spreadsheet macro that simplifies data manipulation and evaluation.

##The 1x Reaction Buffer (made from the supplied 5x concentrated stock) contains 10 mM Tris-HCl, 10 mM $MgCl_2$, 0.01% Tween-20, 0.05% NaN_3, pH 7.2. Other components that can be added without affecting the IMAP system are, Ca^{2+}, DTT, 2-mercaptoethanol, other detergents, and NaCl. Some components may compete with phosphopeptide binding sites on the Binding Reagent and therefore require Binding Solution conditions different from those starting conditions listed in Table 6 and Appendix B (Table 8). These include EDTA and EGTA as well as phosphate and structurally related molecules.

Storage and Handling

All kit components are to be stored at 4°C

IMPORTANT: **Do Not Freeze the Binding Reagent or Buffers**

When stored properly, the kit components are stable for six months from the date of receipt.

Materials Required but not Provided

The following tables list the materials that may be required but are not supplied in this kit.

Table 2: Reagents and Supplies

Reagent Item	Source
Adenosine 5' Triphosphate (ATP), 10 mM stock in purified water, storage of aliquots at -70°C recommended	Major Laboratory Supplier
DL-Dithiothreitol (DTT), 100 mM stock in purified water, storage of aliquots at -20°C recommended	Major Laboratory Supplier
Kinase enzyme	Carna Biosciences, Cell SignalingTechnologies, Upstate Biotechnology or other source
0.1 N HCL	Major Laboratory Supplier

Table 3: Compatible Instruments available from Molecular Devices (MDC):

Equipment Item	Source
One of the following: Analyst® System with Release 2.0 Software - Analyst AD - Analyst HT w/o or with stacker - Analyst GT w/o or with stacker SpectraMax® M5	 MDC P/N 0200-6042 MDC P/N 0200-6044, 0200-6043 (with stacker) MDC P/N 0200-6004, 0200-6003 (with stacker) MDC P/N M5

IMAP Substrate Finder for Serine/Threonine Kinases 2 protocol

Preparing the Reagents

This protocol is only to be used as an initial screening guide. This protocol can be done at room temperature. However, we recommend keeping the enzyme(s) on ice prior to use.

The IMAP Substrate Finder contains only one peptide per well. However, some wells are empty and are available for use as additional reactions or controls (please see the IMAP Substrate Finder layout (Figure 2) on page 9 or the IMAP Substrate Mapper file). Each peptide substrate has four replicates on each plate. All four replicates of each substrate are arranged together in a square formation. We recommend screening for a substrate in a **minus/plus** enzyme set-up in the presence of 100 µM ATP to help drive enzyme activity. After one or more potential substrates are found, optimal assay conditions for final HTS requirements may need to be determined. Each substrate is individually available for follow-up analysis (see Table 5 starting on page 10 for part numbers and source information).

When following the protocol below, the four replicates on a single plate will be used for the substrate identification of one kinase by performing **a minus/plus enzyme** experiment, in duplicate. In order to properly use the Substrate Finder Data Analysis Macro, the layout and purpose of each well must be retained as described in detail in Table 4 Step 3.

As directed below, the kinase reaction will contain final concentrations of **100 nM substrate** and **100 µM ATP** (although this may be modified according to specific enzymatic requirements).

The kinase concentration to use for the Substrate Finder kit is at the users discretion and depends on the kinase, its specific activity and purity.

We recommend using the highest practical concentration of kinase to aid identifying potential substrates. When using kinases commercially available at high specific activity, 1U/mL (where 1U = 1 nmol phosphate transferred/min) is a good starting point. Follow-up experiments can be run using enzyme dilutions to choose the most optimal substrates.

Table 4: Screening protocol for a kinase substrate using the IMAP Substrate Finder for Ser/Thr kinases 2 (TKL, STE, CK1, CMGC).

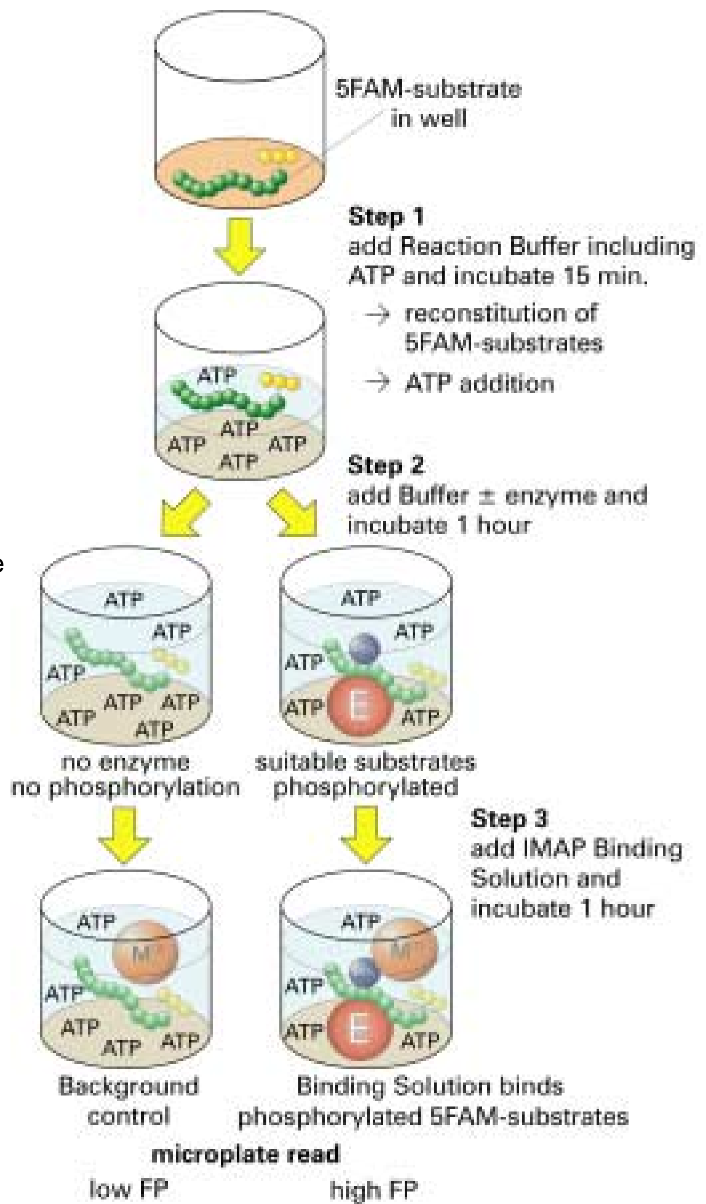
Step	Action
1	Dilute the 5x buffers (Reaction Buffer, Binding Buffer A and B) 1:5 with high quality distilled water to yield the 1x buffers used in this assay, for example, for one plate use 5 mL in 25 mL. Add DTT to the diluted IMAP Reaction Buffer to make a final concentration of 1 mM to yield Complete Reaction Buffer. The reaction buffer supplied already contains 10 mM $MgCl_2$ after dilution. For one plate make 25 mL complete reaction buffer. The Reaction Buffer in this kit also serves as a peptide reconstitution buffer. For several of the peptides on this plate, reconstitution is more complete using Tween-20 as a carrier. Once a substrate is identified for a screen, assay optimization should include comparison of Tween-20 vs. BSA as a reaction buffer additive.

2	Dilute your ATP stock to 200 µM (2x final concentration) with Complete Reaction Buffer **(4 mL 2x ATP solution per plate).** Add 10 µL of the 2x ATP solution (200 µM) to each well containing substrates or phospho-substrates (positive controls) Also add 10 µL of the 2x ATP solution (200 µM) to each of the following wells: A3, B3, A4, B4 (controls for fluorescence (fl) interference, 85%A, 15%B condition) A15, B15, A16, B16 (controls for fl-interference, 60%A, 40%B condition) and A19, B19, A20, B20 (controls for fl-interference, 40%A, 60% B condition) A21, B21, A22, B22 (controls for fl-interference, 25%A, 75% B) A23, B23, A24, B24 (controls for fl-interference, 100% B condition). These wells will serve as controls for any background fluorescence in your enzyme preparation. **Note:** *For optimal reconstitution of the peptide substrate:* *Spin the plate for 2 min. at about 1000 rpm to ensure complete coverage of the plate bottom, then allow the peptides to reconstitute for 15 min at RT.* *Alternatively, place on orbital shaker for 15 min (ensure no splashing occurs as it will lead to cross-contamination).*
3	Add 20 µL Complete Reaction Buffer to the following Buffer Background control wells. A1, B1, A2, B2 (background for 85%A, 15%B condition) C1, D1, C2, D2 (background for 60%A, 40%B condition) E1, F1, E2, F2 (background for 40%A, 60% B condition). G1, H1, G2, H2 (background for 25%A, 75% B). I1, J1, I2, J2, (background for 100% B condition). The intensities of these wells will be used to calculate the “Background Subtracted” parallel and perpendicular intensity values for the different Binding Buffer Conditions) Add 10 µL Complete Reaction Buffer to two of each substrate’s quadruplicate wells. These will serve as a no-enzyme control **(2 mL per plate)**. Add the kinase of interest in 10µl/well Complete Reaction Buffer at 2x its final concentration to the other 2 wells of each quadruplicate **(2 mL 2x enzyme solution per plate).** Add any other controls you would like to include. **Note:** If you want to use the IMAP Substrate Finder Analysis Macro, for each substrate quadruplicate, use the two left wells for “minus enzyme” and the two right wells for “plus enzyme”. For example: wells A3, B3 are “minus enzyme” while the wells A4, B4 are “plus enzyme”. Spin plate or shake on orbital shaker again as described in step 2. See Figure 2 for graphic illustration of the plate set-up.
4	Cover the plate to protect from light and let kinase reaction proceed at RT. The recommended incubation time is 1h.

5		Prepare the Progressive Binding Solutions (amounts are for a single plate): Make an intermediate dilution (pre-dilution) of the Binding Reagent at 1:10 in 0.1N HCl **(for each plate dilute 20 µL of Binding reagent in 200µL 0.1N HCl).** Make sure to add Binding Reagent slowly with rapid stirring **15 mL 85% A, 15% B, 1:400 Binding reagent**: Mix 12.8 mL of Buffer A with 2.3 mL Buffer B. Add 37.5 µL undiluted Binding Reagent. **6 mL 60% A, 40% B, 1:1200 Binding Reagent:** Mix 3.6 mL of Buffer A with 2.4 mL Buffer B. Add 50 µL 1:10 pre-diluted Binding Reagent. **6 mL 40% A, 60% B, 1:1500 Binding Reagent:** Mix 2.4 mL of Buffer A with 3.6 mL Buffer B. Add 40 µL 1:10 pre-diluted Binding Reagent. **6 mL 25% A, 75% B, 1:1500 Binding Reagent:** Mix 1.5 mL of Buffer A with 4.5 mL Buffer B. Add 40 µL 1:10 pre-diluted Binding Reagent. **6 mL 100% B, 1:1750 Binding Reagent:** 6 mL 1x Buffer B Add 34 µL 1:10 pre-diluted Binding Reagent. Mix all solutions vigorously for this step Add 60µl of the appropriate Binding Solution to the corresponding wells of the Substrate Finder plate as indicated in Figure 2.
6		Cover plate and incubate approx.1h RT for the first read. **For final results, especially for the Binding Solution conditions with a high proportion of Buffer B incubate 6h or over night at RT (seal plate with Parafilm® to avoid evaporation).**

7	**Read in Fluorescence Polarization** (FP) mode: Settings for the MDC Analyst AD, HT or GT include: • Continuous lamp • Excitation filter: 485nm-20fwhm • Emission filter: 530nm-25fwhm • Dichroic mirror: 505 nm • Z-height: 3mm • Attenuator: out • SmartRead or Comparator for AD or HT • Sensitivity: 0 for AD or HT • Integration time: 100,000 µsec for AD or HT, 20,000 µsec for GT Settings for the MDC SpectraMax M5 • Endpoint Mode • Excitation: 485 nm • Emission: 530 nm • Auto cut-off: 515 nm • Readings per well: 30 • PMT sensitivity: medium • Autocalibrate: On • Automix: Off • Settling Time: Off
8	Data analysis: Calculate the FP difference between minus enzyme wells and plus enzyme wells. Data calculations and analysis can be facilitated by use of the Substrate Finder Data Analysis Macro. This file can be downloaded from Molecular Devices' website using the instructions on the enclosed postcard. This file uses a simple cut-and-paste format to yield both graphic and tabular results.
9	To order a peptide off of the IMAP Substrate Finder for Ser/Thr Kinases 2 plate for further evaluation, please refer to the RPXXXX part number listed on Table 5. Also, please allow 2 weeks for an initial peptide order. Initial bulk orders may require extra time.

Quick Start Guide:



In **Step 1** the substrates are reconstituted by addition of 10 µL 200 µM ATP in complete reaction buffer to all four wells of each quadruplicate
(for example for the quadruplicate A3-B4: into A3, B3, A4 and B4)
For complete reconstitution spin down or shake, and incubate 15 min.

In **Step 2** add to the left two wells in each quadruplicate (for example for the quadruplicate A3-B4 into A3 and B3)10 µL Complete Reaction Buffer and to the right two wells (for example for the quadruplicate A3-B4 into A4 and B4)
10 µL kinase enzyme diluted in complete reaction buffer. Spin or shake carefully again.
Let reaction proceed 1h at room temperature.

In **Step 3:** add 60 µL of the appropriate Binding Solution to all wells. Incubate and read after 1h, 3h and overnight to allow the substrates using a high proportion of Buffer B to equilibrate completely.

Example:

A3	A4
Minus Enzyme	Plus Enzyme
Minus Enzyme	Plus Enzyme
B3	B4

Figure 2: Plate Layout overview for 384 well plate including numbering locator for peptide inside the wells.

	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24
A B	A1-B2	A3-B4	A5-B6	A7-B8	A9-B10	A11-B12	A13-B14	A15-B16	A17-B18	A19-B20	A21-B22	A23-B24
C D	C1-D2	C3-D4	C5-D6	C7-D8	C9-D10	C11-D12	C13-D14	C15-D16	C17-D18	C19-D20	C21-D22	C23-D24
E F	E1-F2	E3-F4	E5-F6	E7-F8	E9-F10	E11-F12	E13-F14	E15-F16	E17-F18	E19-F20	E21-F22	E23-F24
G H	G1-H2	G3-H4	G5-H6	G7-H8	G9-H10	G11-H12	G13-H14	G15-H16	G17-H18	G19-H20	G21-H22	G23-H24
I J	I1-J2	I3-J4	I5-J6	I7-J8	I9-J10	I11-J12	I13-J14	I15-J16	I17-J18	I19-J20	I21-J22	I23-J24
K L	K1-L2	K3-L4	K5-L6	K7-L8	K9-L10	K11-L12	K13-L14	K15-L16	K17-L18	K19-L20	K21-L22	K23-L24
M N	M1-N2	M3-N4	M5-P6	M7-N8	M9-N10	M11-N12	M13-N14	M15-N16	M17-N18	M19-P20	M21-N22	M23-N24
O P	O1-P2	O3-P4	O5-P6	O7-P8	O9-P10	O11-P12	O13-P14	O15-P16	O17-P18	O19-P20	O21-P22	O23-P24

IMAP Peptide Plate Mapping

	Empty wells for additional controls.

	Buffer only Control	Binding Buffer: 85%A, 15%B, 1:400 Binding reagent
	Substrate peptide	
	Calibrator control	

	Buffer only Control	Binding Buffer: 60%A, 40%B, 1:1200 Binding reagent
	Substrate peptide	
	Calibrator control	

	Buffer only Control	Binding Buffer: 40%A, 60%B, 1:1500 Binding reagent
	Substrate peptide	
	Calibrator control	

	Buffer only Control	Binding Buffer: 25% A, 75% B, 1:1500 Binding reagent
	Substrate peptide	
	Calibrator control	

	Buffer only Control	Binding Buffer: 100% B, 1:1750 Binding reagent
	Substrate peptide	
	Calibrator control	

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Table 5: IMAP SF Ser/Thr 2 Substrate Map: Plate position is equivalent to position written inside the wells on plate scheme in Figure 2

Plate Position	Sequence	Initial Target	MDC part number 8000 dp	MDC part number 50000 dp	Descriptive	Lit Km (µM)	Literature source
A1-B2					subtract intensities from 85%A/15%B/1:400 wells		
C1-D2					subtract intensities from 60%A/40%B/1:1200 wells		
E1-F2					subtract intensities from 40%A/60%B/1:1500 wells		
G1-H2					subtract intensities from 25%A/75%B/1:1500 wells		
I1-J2					subtract intensities from 100%B/1:1750 wells		
A3-B4					Buffer Background		
C3-D4	5FAM-KKRKSSLRRWSPLTPRQMSFDC-NH2	"generic"	RP7109	RP7609	Multide contains consensus sequences for various Ser/Thr protein kinases including PKA, PKC, Cam Kinase and MAP kinases in a single peptide		Kameshita I et al: J Biochem (Tokyo). 1999 Dec;126(6):991-5.
E3-F4	5FAM-AQMQLSQEVFNGGKK-NH2	ATM kinase	RP7111	RP7611	Attide, oriented peptide library derived	23	O'Neill et al, J Biol Chem. 2000 Jul 28;275(30):22rp719-27
G3-H4	5FAM-GRJKKIASVEJJKK-NH2	BRI1 (plant kinase, family of th leucine-rich-repeat containing kinases))	RP7112	RP7612	analogous to CSK and Lyn kinase domain	83	Oh et al.:Plant Physiol. 2000 Oct;124(2):751-66.
I3-J4	5FAM-GGGRPPTLSPIPHIPR-NH2	CDK	RP7134	RP7634	G1 peptide		Ikuta et al. J Biol Chem. 2001 Jul 20;276(29):27548-54.
K3-L4	5FAM-GGGPATPKKAKKL-OH	CDK1, CDK2, CDK5	R7252	R7257	Histone H1 derived peptide, IMAP validated		Clare et al. J Biol Chem. 2001 Dec 21;276(51):48292-9.

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Plate Position	Sequence	Initial Target	MDC part number 8000 dp	MDC part number 50000 dp	Descriptive	Lit Km (µM)	Literature source
M3-N4	5FAM-GGGRSPGRRRRK-OH	CDK1/ cdc2(+Cyclin B)	RP7114	RP7614	consensus sequence from several proteins	1.5	Srinivasan et al: Biochem J. 309 927-931 (1995)
O3-P4	5FAM-KHHKSPKHR-OH	CDK5	RP7115	RP7615	oriented degenerate library derived		Songyang et al. Mol Cell Biol. 1996 Nov;16(11):6486-93.
A5-B6	5FAM-YSPTSPSYSPTSPSYSPTSPS-OH	CDK7	RP7116	RP7616	CDK7tide, modified CTD4 (carboxy-terminal domain (CTD) of the largest subunit of the RNA polymerase II (RNA pol II))		Rossignol et al.EMBO J. Vol. 16 No. 7 pp. 1628-1637, 1997;
C5-D6	5FAM-RGSPRYSPRHS-OH	Clk/Sty	RP7118	RP7618	SPRY peptide (ASF/SF2 RS domain derived)	150	Colwill et al. J Biol Chem. 1996 Oct 4;2rp71(40):24569-75
E5-F6	5FAM-RRRFRPASPLRGPPK-OH	Dyrk1A	RP7120	RP7620	DYRKtide	35	Himpel et al.: J Biol Chem. 2000 Jan 28;275(4):2431-8.
G5-H6	5FAM-KKISGRLSPIMTEQ-NH2	DYRKIA	R7315	R7316	Woodtide, aa324–334 of FKHR, IMAP validated		Wood et al. Biochem J. 2001 May 1;355(Pt 3):597-607
I5-J6	5FAM-ILLSELSRRRIRAI-NH2	eIF-2alpha kinases (HRI, PKR)	RP7121	RP7621	eIF-2 derived synthetic peptide (Ser-51)		Martin de la Vega et al.Cell Signal. 1999 Jun;11(6):399-404
K5-L6	5FAM-TGPLSPGPF-OH	Erk1	RP7122	RP7622	oriented degenerate library derived		Songyang et al. Mol Cell Biol. 1996 Nov;16(11):6486-93.
M5-N6	5FAM-ATGPLSPGPFGRR-OH	Erk2	RP7123	RP7623	MBP derived peptide		Prowse, C. N. et al. Biochemistry 39, 6258 (2000).
O5-P6	5FAM-KQAEAVTSPR-NH2	Erks	RP7124	RP7624	rat tyrosine hydroxylase (24-33) derived		Lee et al: J Biol Chem. 1996 Nov 1;2rp71(44):27299-303.
A7-B8	5FAM-APRTPGGRR-OH	Erks Map Kianse	RP7125	RP7625	MBP der (94-101)		Lee et al: J Biol Chem. 1996 Nov 1;2rp71(44):27299-303.
C7-D8	5FAM-KQTARKSTGGKAPRK-OH	Histone Kinase H3	RP7126	RP7626	Histone H3 derived (N-terminus)		Murnion 2001 J. Biol. Chem. 276:26656
E7-F8	5FAM-GSLAREWHKTTQMSAAGTYA-NH2	HPK1	RP7127	RP7627	MLK3 activation loop derived		Leung and Lassam Biol Chem. 2001 Jan 19;276(3):1961-7.
G7-H8	5FAM-ARFSRFAGSSPSQSSMVAR-OH	IRAK1, IRAK4	RP7129	RP7629	IRAK1 autophosphorylation loop derived		Li et al.: Proc Natl Acad Sci U S A. 2002 Apr 16;99(8):5567-72.
I7-J8	5FAM-RSGSPMIR-OH	KIS	RP7130	RP7630	modified MBP sequence surounding Ser 164		Maucuer A et al: Eur J Biochem. 2000 Jul;267(14):4456-64
K7-L8	5FAM-QKSQRSQAENPV-NH2	MAPK	RP7132	RP7632	MBP derived peptide (aa 74-85)		
M7-N8	5FAM-FFKNIVTPRTPPPSQGK-OH	MAPK	RP7133	RP7633	MBP derived peptide		Clark Lewis et al J Biol Chem. 1991 Aug 15;266(23):15180-4.
O7-P8	5FAM-QKRPSQRSKYL-OH	MAPK	RP7039	RP7539	MBP derived peptide (4-14)	7	Yasuda I.et al. (1990) Biochem. Biophys. Res. Commun. 166:1220-1227

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Plate Position	Sequence	Initial Target	MDC part number 8000 dp	MDC part number 50000 dp	Descriptive	Lit Km (µM)	Literature source
A9-B10	5FAM-ERMRPRKRQGSVRRRV-NH2	MST2	RP7048	RP7548	PKC-epsilontide		Van Der Hoeven et al: Biochem J. 2000 May 1;347 Pt 3:781-5
C9-D10	5FAM-KKRNRRLSVA-OH	NDR	RP7138	RP7638	identified from small peptide library		Millward et al. EMBO J. 1998 Oct 15;17(20):5913-22.
E9-F10	5FAM-IRRLSTRRR-OH	Nek2	RP7139	RP7639	PLMtide, IMAP validated		Sportsman et al. Assay Drug Dev Technol. 2004 Apr;2(2):205-14.
G9-H10	5FAM-GTFRSSIRRLSTRRR-OH	Nek6	RP7140	RP7640	PLM derive (58-72)	40	Lu et al. J Biol Chem. 1994 Mar 4;269(9):6603-7
I9-J10	5FAM-FLAKSFGSPNRAYKK-OH	NEK6/NEK7	RP7141	RP7641	CDK7 derived (aa: 158-169)		Larochelle et al EMBO J. 2001 Jul 16;20(14):3749-59, Upstate Biotechnologies substrate
K9-L10	5FAM-PKTPKKAKKL-OH	p34-cdc2 (CDK1)	RP7144	RP7644	Histone H1 derived (aa 9-18)		Sharma et al.: J Biol Chem. 1999 Apr 2;274(14):9600-6.
M9-N10	5FAM-RPRPASVPPS-OH	PAK Kinase (autokinase)	RP7145	RP7645	Synthide3		Yang & Huang: J Biol Chem. 1994 Nov 25;269(47):29855-9
O9-P10	5FAM-KKRPQRRYSNVF-OH	PAK2	RP7008	RP7508	derived from homology modeling of a bound PHK peptide, PAK2 results from Substrate Finder Ser/Thr 1		DAPK: Velentza et al: J. Biol. Chem., Oct 2001; 276: 38956 - 38965
A11-B12	5FAM-RQMSFRL-OH	Phosphorylase Kinase	RP7148	RP7648	degenerate peptide library derived	350	Lowe et al. EMBO J. (1997) 16, 6646–6658
C11-D12	5FAM-RAGSPMAR-OH	PITALRE	RP7149	RP7649	MBP derived peptide, modified (aa159-166, S160 to A160)		Garriga et al. Biochem J. 1996 Dec 15;320 (Pt 3):983-9.
E11-F12	5FAM-PLARTLSVAGLPGKK-OH	Raf kinase	RP7007	RP7507	Synthide2		Lee et al: J Biol Chem. 1996 Nov 1;2rp71(44):27299-303.
G11-H12	5FAM-ALARAASAAALARRR-OH	SOS2 (salt overly sensitive 2)	RP7151	RP7651	peptide derived from the recognition sequences of PKC or SNF1/AMPK	138	Gong et al. J Biol Chem. 2002 Aug 2;277(31):28340-50. Epub 2002 May 23.
I11-J12	5FAM-MAGGRRRHSRRRAK-OH	SRPK	RP7152	RP7652	directed peptide library		Wang et al.: J Cell Biol. 1998 Feb 23;140(4):737-50.
K11-L12	5FAM-GRSRSRSRSR-OH	SRPK1, Clk/Sty	RP7153	RP7653	RSRS peptide, (ASF/SF2 RS domain derived)	55	Colwill et al. J Biol Chem. 1996 Oct 4;2rp71(40):24569-75
M11-N12	5FAM-Histone H1 protein, recombinant		R7439	R7440	Histone H1	3	Brizuela et al. Proc Natl Acad Sci U S A. 1989 Jun;86(12):4362-6.
O11-P12	5FAM-ARKRERAYSFGHHA-NH2	Akt	RP7110	RP7610	AKTtide	8.8	Obata et al. J Biol Chem. 2000 Nov 17;275(46):36108-15.
A13-B14	5FAM-KRASVVGTTYWM-OH	PAK1	RP7146	RP7646	MIHCK derived peptide		Brzeska et al. Proc Natl Acad Sci U S A. 1997 Feb 18;94(4):1092-5.
C13-D14	5FAM-GGGPA-pT-PKKAKKL-COOH	Calibrator	R7372	R7373	Phospho Histone H1-derived peptide, IMAP validated		Clare et al. J Biol Chem. 2001 Dec 21;276(51):48292-9.

Plate Position	Sequence	Initial Target	MDC part number 8000 dp	MDC part number 50000 dp	Descriptive	Lit Km (µM)	Literature source
A15-B16					Buffer Background		
C15-D16	5FAM-GRPRTSSFAEG-OH	PLK3	R7110	R7136	Crosstide		Davies et al: Biochem J. 2000 Oct 1;351(Pt 1):95-105
E15-F16	5FAM-KEHQVLMKTVCGTPGY-NH2	Cam Kinase Kinase alpha	RP7113	RP7613	Cam Kinase IV (Thr 196) derived	0.5	Okuno et al. J. Biochem, (Japan) 130, 503-513, (2001)
G15-H16	5FAM-VSRSGLYRSPSMPENLNRPR-OH	cTak, PAR1b	R7275	R7276	CDC25C derived peptide		Sanchez,Y., et al., Science 277: 1497-1501, 1997 Furnari, B., et al., Science 277: 1495-1497, 1997
I15-J16	5FAM-IPTTPITTTYFFFK-NH2	Erk2, p38	R7434	R7435	p38-tide		Chen et al: Biochemistry. 2000 Feb 29;39(8):2079-87.
K15-L16	5FAM-AKELDQGSLCTSFVGTLQ-NH2	IKKs , TBK-1	RP7128	RP7628	IKKtide for IKKepsilon	IKKε:34, IKKβ:43 TBK-1: 24,	Kishore et al. J Biol Chem. 2002 Apr 19;277(16):13840-7 Huynh at al. J Biol Chem. 2002 Apr 12;277(15):12550-8
M15-N16	5FAM-FLTEYVATRWYRAPEIMLN-NH2	MAP Kinase Kinase	RP7131	RP7631	p44 derived peptide (aa 200-218)		Watabe et al: J Biol Chem. 1996 Jun 14;2rp71(24):14067-72.
O15-P16	5FAM-SGYLVDSVAKTIDA-NH2	MEKK3	RP7136	RP7636	MKK3 derived (aa 183-196)		Jeon et al: J Biochem (Tokyo). 2002 May;131(5):693-9
A17-B18	5FAM-FLPVPEYINQSVP-NH2	MKK6	RP7137	RP7637	EGF-R derived (aa1086-1098)		
C17-D18	5FAM-ADAQHATPPKKKRKVEDPKD -NH2	p34 $^{cdc2\ Kinase}$	RP7143	RP7643	Protein kinase p34 substrate		Lee et al: J Biol Chem. 1996 Nov 1;2rp71(44):27299-303.
E17-F18	LVEPLTPSGEAPNQ(K-5FAM)-COOH	p38, MAP kinase	R7129	R7170	EGF-R erived peptide, IMAP validated, shortened Stevetide		Sakata et al. J Biol Chem. 1995 Dec 22;270(51):30823-8
G17-H18	5FAM-TESQSLTLTDVENLHLPLPLLQ-NH2	TIP kinase	RP7154	RP7654	Beta casein derived (Ser 122)	6	Guesdon et al. J Biol Chem. 1997 Nov 28;272(48):30017-24.
I17-J18	5FAM-SKRSTMVGTPYWM-OH	PAK1	RP7147	RP7647	PAK derived peptide		Brzeska et al. Proc Natl Acad Sci U S A. 1997 Feb 18;94(4):1092-5.
K17-L18	5FAM-GNNIEGMILLSELSRRRIR-NH2	PKR	RP7049	RP7549	eIF-2-alpha (40-57) derived, IMAP validated		IMAP results, Molecular Devices
M17-N18	5FAM-GRPRTS-pS-FAEG-COOH	Calibrator	R7159	R7171	Phospho Crosstide, IMAP validated		Davies et al: Biochem J. 2000 Oct 1;351(Pt 1):95-105
O17-P18	LVEPL-pT-PSGEAPNQ(K-5FAM)-COOH	Calibrator	R7319	R7320	EGFR-derived phosphopeptide, IMAP validated, shortened Stevetide		Sakata et al. J Biol Chem. 1995 Dec 22;270(51):30823-8

Molecular Devices

Plate Position	Sequence	Initial Target	MDC part number 8000 dp	MDC part number 50000 dp	Descriptive	Lit Km (µM)	Literature source
A19-B20					Buffer Background		
C19-D20	5FAM-EPPQSQEAFADLWK-NH2	dsDNA activated Kinase	RP7119	RP7619	p53 derived peptide sequence derived 11-24	290	Lees-Miller SP: Mol Cell Biol. 1992 Nov;12(11):5041-9.
E19-F20	5FAM-GRHDSGLDSMK-NH2	IKK beta	R7254	R7259	NF kappa B inhibitor alpha derived peptide, IMAP validated		Hideshima et al: J Biol Chem. 2002 May 10;277(19):16639-47
G19-H20	5FAM-RHLPPLLLQSWMHQPHQ-OH	Nek8	RP7142	RP7642	Beta casein derived peptide	300	Holland et al, JBC 277, 16229-16240, (2002)
I19-J20	5FAM-DELMEFSLADAK-NH2	PLK1	RP7150	RP7650	Cdc 25 derived (mutated)		Nakajima et al. J Biol Chem. 2003 Jul 11;278(28):25277-80.
K19-L20	5FAM-GRHDSGLD-pS-MK-NH2	Calibrator	R7301	R7302	IkBa-derived phosphopeptide, Calibrator, IMAP validated		Hideshima et al: J Biol Chem. 2002 May 10;277(19):16639-47
A21-B22					Buffer Background		
C21-D22	5FAM-HAAIGDDDDAYSITA-NH2	casein kinase 1	R7311	R7312	CK1tide, IMAP validated		Pulgar et al, Eur J Biochem. 1999 Mar;260(2):520-6. Marin et al. Proc Natl Acad Sci U S A. 2003 Sep 2;100(18):10193-200.
E21-F22	5FAM-RRREEEEEESAAA-NH2	GRK-1	RP7052	RP7552	synthetic peptide		Yu et al. J Neurochem. 1999 Sep;73(3):1222-7.
G21-H22	5FAM-HAAIGDDDDAY-pS-ITA-NH2	Calibrator	RP7155	RP7655	Phospho-CK1tide		Pulgar et al, Eur J Biochem. 1999 Mar;260(2):520-6. Marin et al. Proc Natl Acad Sci U S A. 2003 Sep 2;100(18):10193-200.
A23-B24					Buffer Background		
C23-D24	5FAM-RRRADDSDDDDD-NH2	Casein Kinase 2	R7294	R7295	CK2tide, IMAP validated		Chaillot et al. Protein Eng. 2000 Apr;13(4):291-8.
E23-F24	5FAM-ADPDHDHTGFLTEYVATRWR -NH2	MEK kinase	RP7135	RP7635	ERK1/2 derived (172-190) (regulatory sequence common to ERK 1 and ERK 2 MAP kinases)		Lee et al: J Biol Chem. 1996 Nov 1;2rp71(44):27299-303.
G23-H24	5FAM-RRRADD-pS-DDDDD-NH2	Calibrator	R7347	R7348	Phospho CK2tide, IMAP validated		Chaillot et al. Protein Eng. 2000 Apr;13(4):291-8.

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