Molecular Devices

IMAP™ Substrate Finder for Ser/Thr 1 (CAMK/AGC) Kinases with Progressive Binding System

Product #R8131
patents pending

Introduction

About the IMAP™ Substrate Finder Kit

The IMAP Substrate Finder Kit is an IMAP assay development tool that can significantly accelerate the identification of substrates for kinases or can be used to profile a kinase's substrate specificity. The IMAP Substrate Finder enables the researcher to screen a kinase of interest against 56 kinase substrates within a few of hours. To improve the chances of success for finding a new or alternative substrate for a specific kinase, all substrates included on the IMAP Ser/Thr Substrate Finder are documented as being substrate sequences for a particular part of the Kinome. This IMAP Substrate Finder focuses on the CAMK and AGC portions of the Human Kinome as described by Manning et al. [1]

Principle of the Assay

For this kit, the 5Fam labeled peptide sequences are dried down into a 384 well plate. The researcher just has to reconstitute the substrates by adding reaction buffer and ATP, add Kinase and incubate. Addition of the IMAP binding system stops the reaction and yields a robust Fluorescence Polarization change. The IMAP technology is based on the high affinity binding of phosphate by immobilized metal (M^{III}) coordination complexes on nanoparticles. This IMAP "binding reagent" complexes with phosphate groups on phosphopeptides generated in a kinase reaction. Such binding causes a change in the rate of the molecular motion of the peptide, and results in an increase in the fluorescence polarization value observed for the fluorescein label attached at the end of the peptide (Figure 1).

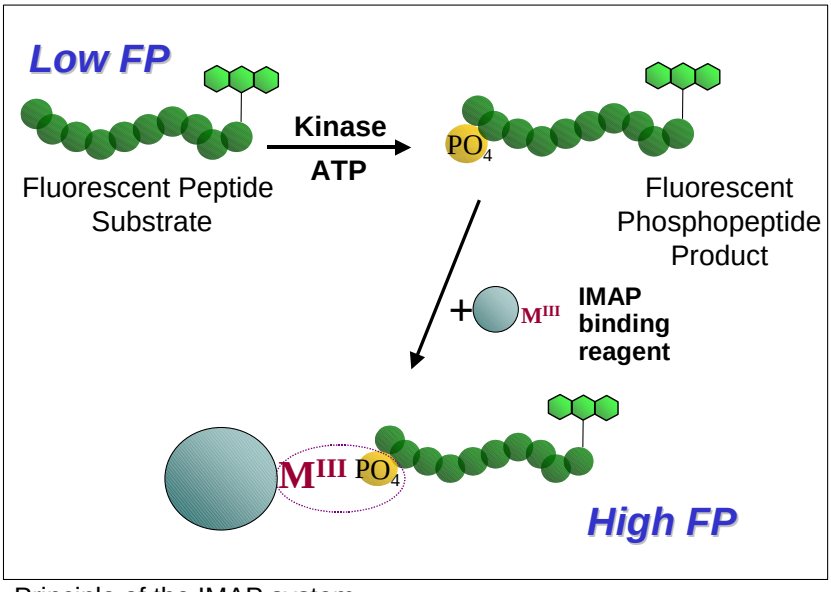

Figure 1. Principle of the IMAP system

[1] Manning et al. Science 6.Dec. 2002

About the Progressive Buffer System

To minimize the manipulations required to run a screen with 56 substrates, the ratios of Progressive Buffers A and B provided in this protocol were designed to cover a maximum number of sequences yet still obtain a good signal with a acceptable background. Some of the substrate background values may be lower with further optimization. Thus, upon discovery of a potential substrate, many factors should be examined including the ratio of IMAP Progressive Buffers A and B, ATP concentration, IMAP Progressive Binding Reagent concentration, and enzyme concentration. Please refer to the Progressive Buffer System Application Note for a more detailed explanation of the flexibility of this buffer system. Assayconditions used in the progressive Bindingsystem may not be appropriate for the Original Binding System.

Applications

The IMAP Substrate Finder Kit is an IMAP assay development tool that accelerates the identification of suitable substrates for performing IMAP kinase assays. Additionally, if a suitable substrate is already known, the IMAP Substrate Finder can help determine a kinase's substrate specificity by screening it's activity against this collection of Ser/Thr peptide substrates. Additional applications include testing enzyme preparations for purity, finding pairs of substrates for multiplexing, kinase characterization, etc.

Materials and Equipment

Table 1: The IMAP™ Substrate Finder Kit with Progressive Binding System (R8131) contains:

Reagent	Quantity	Description
IMAP Progressive Binding Reagent	0.150 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 (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.
Two IMAP Substrate Finder plates each with 56 Kinase substrates and 3 phosphopeptides as positive controls.	2	Two black 384 well plates, each containing 59 different, dried 5-FAM labeled peptides in quadruplicate. Reconstitution into a 20 µL kinase reaction will result into a 100 nM end 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 excel file for easy manipulation of all the substrate's information 3) Substrate Finder Data Analysis Macro, an excel 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 $MgCl_2$, 0.1% BSA, 0.05% NaN_3, pH 7.2. Other components that can be added without affecting the IMAP system are Mn^{2+}, Ca^{2+}, DTT, 2-mercaptoethanol, certain 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. BSA may bind to and interfere with the action of some inhibitory compounds. If this is the case, phosphate-free

bovine gamma globulin (BGG) or detergents such as Tween-20 or Triton X-100 may be substituted for the BSA in the above reaction buffer formulation.

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, aliquots at -70°C recommended	Major Laboratory Supplier
DL-Dithiothreitol (DTT), 100 mM stock in purified water, aliquots at -20°C recommended	Major Laboratory Supplier
Kinase enzyme	Upstate Biotechnology or other source

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

Equipment Item	**Source**
Analyst™ System with Release 2.0 Software	
One of the following: - Analyst AD - Analyst HT - Analyst GT	 MDC P/N 0200-6042 MDC P/N 0200-6044 MDC P/N 0200-6004

IMAP Substrate Finder protocol

Preparing the Reagents

This protocol is to be used as an initial screening guide only. This protocol can be done at room temperature. However, we recommend keeping the enzyme(s) on ice prior to plate addition. 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 7 or on 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 to screen for a substrate in a /+enzyme set-up in the presence of 100 µM ATP to help drive enzyme activity. After a potential substrate (or more) is 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 on page 8 for part numbers).

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).

Table 4: Protocol for screening for a Kinase substrate using the IMAP Sustrate Finder

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 one plate 6 mL in 30 mL) Add DTT to the IMAP Reaction Buffer to a final concentration of 1 mM to yield Complete Reaction Buffer.
2	Dilute your ATP stock to 200 µM (2x final concentration) with Complete Reaction Buffer. 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, 100% A condition) A17, B17, A18, B18 (controls for fl-interference, 75%A, 25%B condition) and A23, B23, A24, B24 (controls for fl-interference, 60%A, 40% B condition). These wells will serve as controls for intensity quenching components in the enzyme preparation provided by the Customer **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 100% Progressive Binding Buffer A) C1, D1, C2, D2 (background for 75%A, 25% B Progressive Binding Buffer) E1, F1, E2, F2 (background for 60%A, 40% B Progressive Binding Buffer) 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. Add the kinase of interest in 10µl/well Complete Reaction Buffer at 2x its final concentration to the other 2 wells of each quadruplicate. 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): **15 mL of 100%A, 1:400 Binding reagent**: Add 37.5 µL Binding reagent to 15 ml of a 100 1X Buffer A%. **10 mL 75%A, 25%B, 1:600 Binding reagent:** Mix 7.5 mL of Buffer A with 2.5 mL Buffer B. Add 16.7 µL Binding reagent to this. **10 mL 60%A, 40%B, 1:600 Binding reagent:** Mix 6.0 mL of Buffer A with 4.0 mL Buffer B. Add 16.7 µL Binding reagent to this. 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

7	Read the Fluorescence Polarization (FP) using the 505 dichroic, 485 excitation, and 530 emission. Measure the **fluorescence polarization** (FP). Settings for the MDC Analyst AD, HT or GT include: • Continuous lamp • Excitation fluorescein 485nm-20fwhm • Emission 530nm-25fwhm • Fluorescein 505 nm dichroic • Z-height 3mm • Attenuator out • SmartRead or Comparator • Sensitivity 0 • Integration time of 100,000 μsec
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 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, all subsequent orders of that exact peptide should be available within our standard 24 hour shipping policy (initial bulk orders may require extra time).

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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-N6	M7-N8	M9-N10	M11-N12	M13-N14	M15-N16	M17-N18	M19-N20	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

	empty wells for additional reactions or controls

	Buffer Control	**Binding Solution: 100%A, 1:400 Binding reagent**
	Substrate peptide	
	Calibrator control	

	Buffer Control	**Binding Solution: 75%A, 25%B, 1:600 Binding reagent**
	Substrate peptide	
	Calibrator control	

	Buffer Control	**Binding Solution: 60%A, 40%B, 1:600 Binding reagent**
	Substrate peptide	

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

IMAP Substrate Map							
Plate Position	**Sequence**	**Initial Target**	**part number 8,000 tp**	**part number 50,000 tp**	**Descriptive**	**Lit. Km (µM)**	**Literature Source**
A1-B2					Buffer Background (used in FP calculation)		
C1-D2					Buffer Background (used in FP calculation)		
E1-F2					Buffer Background (used in FP calculation)		
A3-B4					Buffer Background		
C3-D4	5FAM-LKKLTRRP**S**FSAQ-OH	AMP kinase	RP7001	RP7501	ADR1 (222-234) derived	4	Michell et al. JBC 271 28445-28450 (1996)
E3-F4	5FAM-LKKLTRRASFSGQ-OH	CaM KI	RP7004	RP7504	ADR1 (222-234) derived	17	Chin et al JBC 272, 31235-31240 (1997)
G3-H4	5FAM-LKKLRRRLSDANF-NH2	CaM KI	RP7005	RP7505	ADR/Synapsin Chimera	7	Hook et al JBC 274, 20215-20222 (1999)
I3-J4	5FAM-LRRRLSDANF-NH2	CaM KI, Cam KIV	R7273	R7274	synapsin-site1, IMAP validated	103	Chin et al JBC 272, 31235-31240 (1997)
K3-L4	5FAM-PLRRTLSVAA-OH	CaM KII	RP7006	RP7506	similar to syntide-2	4	Wu et al Novartis Web presentation at Lab-robotics.org
M3-N4	5FAM-PLSRTLSVSSLPGL-NH2	Cam KII , CASEIN K1	RP7045	RP7545	Glycogen synthase der.		Kemp et al Methods in Enzymology (1991) 200, 121-134
O3-P4	5FAM-PLARTLSVAGLPGKK-OH	CaM KII,	RP7007	RP7507	Syntide-2		Hashimoto & Sonderling, Arch Biochem Biophys. 1987 Feb 1;252(2):418-25
A5-B6	5FAM-ALKLVRYPSFVITAK-NH2	CHK1	R7185	R7230	CHK1tide, IMAP validated		O'Neill T et al. JBC 277:16102-16115, 2002
C5-D6	5FAM-KKRPQRRYSNVF-OH	Death accociated protein kinase (DAPK)	RP7008	RP7508	derived from homology modeling of a bound PHK peptide	9	Velentza et al: J. Biol. Chem., Oct 2001; 276: 38956 - 38965
E5-F6	5FAM-KKLNRTLSVA-OH	MAPKAPK2/1	R7127	R7169	Glycogen Synthase derived peptide, IMAP validated	0.17	Leighton et al FEBS (1995) 375 289-29 Stokoe et al. (1993) Biochem. J., 296, 843-849
G5-H6	5FAM-NVKSKIGSTENLK-NH2	MARK2	RP7009	RP7509	tau repeat-1 peptide		Biernat et al, Mol. Biol. Cell, Vol 13, 4013-4028 (2002)
I5-J6	5FAM-PKKAKRRAAEGSSNVFS-NH2	MLCK	RP7010	RP7510	MYOSIN LIGHT CHAIN derived	3	Michnoff et al, JBC (1986) 261, 8320-8326
K5-L6	5FAM-DTATKSGSTTKNRFV-NH2	MNK1/2	RP7012	RP7512	eIF4E (202-216) derived		Knauf et al. Mol Cell Biol. 2001 Aug;21(16):5500-11
M5-N6	5FAM-GEILSRRPSYRK-NH2	MSK1	RP7046	RP7546	CREBtide		Deak et al., EMBO J. 17, pp4426-4441 (1998)

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Plate Position	Sequence	Initial Target	part number 8,000 tp	part number 50,000 tp	Descriptive	Lit. Km (µM)	Literature Source
O5-P6	5FAM-KKRAARATSNVFA-OH	smMLCK	RP7015	RP7515	MYOSIN LIGHT CHAIN derived	8	Davies et al Biochem J (2000) 351, 95-105 S. Osada et al. J. Biol. Chem. (1990) 265, 22434 Wu et al Novartis Web presentation at Lab-robotics.org
A7-B8	5FAM-RPRTSSF-OH	Akt, S6 Kinase	RP7016	RP7516	glycogen synthase kinase 3 alpha derived	5	Wu et al Novartis Web presentation at Lab-robotics.org
C7-D8	5FAM-GGLRRASLG-OH	PKA , Aurora, S6 Kinase	RP7017	RP7517	Kemptide plus Gly spacer		Cox and Taylor, Biochemistry. 1995 Dec 12;34(49):16203-9.
E7-F8	5FAM-KKRNRTLTK-OH	p70S6K, Rsk2	RP7018	RP7518	artificial peptide sequence		Crawley et al. JBC 271, 16357-16362 (1996)
G7-H8	5FAM-AMARAASAAALARRR-OH	PASK, AMPK	RP7019	RP7519	Amara peptide	200	Rutter et al PNAS (2001)Vol 98, 8991-8996
I7-J8	5FAM-RKRRQTSM-OH	Pim	RP7020	RP7520	p21 (Cip1/WAF,114-147) derived		Wang et al. Biochim Biophys Acta. 2002,1593(1):45-55
K7-L8	5FAM-GRTGRRNSI-NH2	PKA	R7250	R7255	derived from PKI(14-22), IMAP validated	0.1	Glass et al.: (1989) J. Biol. Chem. 264:8802-8810
M7-N8	5FAM-ERRKSKSGA-NH2	PKA	RP7021	RP7521	cyclic AMP-regulated phosphoprotein ARPP-21 derived	0.8	Hemmings et al. (1989) J. Biol. Chem. 264:7726-7733.
O7-P8	5FAM-ARTKRSGSV-OH	PKA	RP7022	RP7522	phosphorylase b kinase beta regulatory chain derived	10	Zetterqvist O., and Ragnarsson U. (1982) FEBS Lett. 139:287-290
A9-B10	5FAM-GGGGGGRRASLG-OH	PKA, Aurora, S6 Kinase	RP7023	RP7523	bovine liver pyruvate kinase derived	2.4	Foster et al. (1984) J. Biol. Chem. 259:13049-13055.
C9-D10	5FAM-RTKRSGSVYEPLKI-NH2	PKA, PKC	RP7024	RP7524	Malantide	18 (PKC)	Murray, K.J., et al., Biochem J., 267, 703 (1990)., Zhao Z.H.et al. (1991) Biochem Biophys. Res. Commun. 176:1454-1461.
E9-F10	5FAM-KRREILSRRPSYR-NH2	PKA, cAMP dependent Kinase	RP7025	RP7525	similar to CREBtide	4	Colbran et al. Biochem Cell Biol. (1992) 70, 1277-82, Meredith et al.:Nature Biotechnology Vol 18, (2000) 309-312
G9-H10	5FAM-RKRSRAEA-NH2	PKA, PKG	RP7026	RP7526	Histone H2B derived	16	Colbran J.L.et al. (1992) J. Biol. Chem.267:9589-9594
I9-J10	5FAM-AAKIQASFRGHMARKK-OH	PKC	RP7027	RP7527	Neurogranin derived	0.15	Meredith et al.:Nature Biotechnology Vol 18, (2000) 309-312 Chen et al. (1993) Biochemistry 32:1032-1039.
K9-L10	5FAM-KKISGRLSPIMTEQ-NH2	PKC, DYRK	R7315	R7316	eIF2B sequence around Ser539 derived, IMAP validated		Campbell & Proud FEBS (2002) 510, 1-2, pp. 31-36
M9-N10	5FAM-KRTLRR-OH	PKC	RP7028	RP7528	EGF r derived	300	deBont et al, Int J. Prot Res 33, 115 (1989)
O9-P10	5FAM-KRAKRKTAKKR-OH	PKC	RP7029	RP7529	artificial peptide sequence	0.5	Toomik, R at al. 1997 Biochem. J. 322:455–460
A11-B12	5FAM-RKRQGSVRRRVH-OH	PKC	RP7030	RP7530	PKCε pseudosubstrate (A to S mutated) derived		Sando et al. 1998 JBC 273:34022

Plate Position	Sequence	Initial Target	part number 8,000 tp	part number 50,000 tp	Descriptive	Lit. Km (µM)	Literature Source
C11-D12	5FAM-RRGRTGRGRRGIYR-OH	PKC	RP7031	RP7531	protein 3 of hepatitis C virus (1487-1500) derived	11	Borowski et al. Biol Chem. 2000 Jan;381(1):19-27.
E11-F12	5FAM-RFARKGSLRQKNV-OH	PKC	RP7032	RP7532	PKC pseudosubstrate domain derived (25Ala-Ser mutated)	0.2	House & Kemp, Science 238, 1726-1728 (1987) Meredith et al.:Nature Biotechnology Vol 18, (2000) 309-312 S. Majumdar et al. Biochim. Biophys. Acta 1993 1176 276-286,
G11-H12	5FAM-PLSRTLSVAAKK-OH	PKC, DMPK	RP7033	RP7533	Glycogen Synthase (1-12) derived	4	Davies et al Biochem J (2000) 351, 95-105 S. Osada et al. J. Biol. Chem. (1990) 265, 22434 Wu et al Novartis Web presentation at Lab-robotics.org
I11-J12	5FAM-VRKRTLRRL-OH	PKC, DMPK	RP7034	RP7534	EGF-R derived		Meredith et al.:Nature Biotechnology Vol 18, (2000) 309-312 Heasley and Johnson Mol Pharmacol 35, 331-338 (1989) Mounsey et al, JBC 275, 23362-23367.(2000) (DMPK)
K11-L12	5FAM-ERMRPRKRQGSVRRRV-NH2	PKC, Mst2	RP7048	RP7548	PKC epsilon pseudo substrate (A->S) derived, IMAP validated	68	Schaap D., & Parker P.J. (1990) J. Biol. Chem. 265:7301-7307.
M11-N12	5FAM-AKRRRLSS**L**RA-OH	ROCK II , PAK1/PRK2	R7184	R7229	Ribosomal Protein S6 derived, IMAP validated		Yu et al. JBC 272 (15): 10030.
O11-P12	5FAM-DRKKRYTVGNPY-NH2	ROCK-II	RP7036	RP7536	LIMK derived peptide	42	Turner et al. Arch Biochem Biophys 2002 405:13-20
A13-B14	5FAM-RRRITSAARR-OH	ROCK-II	RP7037	RP7537	GFAP (glial fibrillary acidic protein)-derived	7	Turner et al. Arch Biochem Biophys 2002 405:13-20
C13-D14	5FAM-KKKKKRFSFKKAFKLAGFAFKKNKK-OH	PKC	RP7038	RP7538	myristolated alanine-rich C-kinase substrate (MARCKS) derived	0.05	Graff et al. (1991) J. Biol. Chem. 266:14390-14398
E13-F14	5FAM-QKRPSQRSKYL-OH	PKC	RP7039	RP7539	myelin basic protein derived	7	Yasuda I.et al. (1990) Biochem. Biophys. Res. Commun. 166:1220-1227
G13-H14	5FAM-KKASFKAKK-OH	PKC	RP7040	RP7540	Histone H1 derived	9	Buday L et al. (1992) Int. J. Biochem. 24:777-782
I13-J14	5FAM-GRTGRRN**pS**I-OH	PKAtide Calibrator	R7303	R7304	Calibrator derived from PKI(14-22) IMAP validated		Glass et al. (1989) J. Biol. Chem. 264:8802-8810
K13-L14	5FAM-KKLNRTL**pS**VA-OH	GS-der. Calibrator	R7317	R7318	Calibrator for Glycogen Synthase derived peptide, IMAP validated		Leighton et al FEBS (1995) 375 289-29 Stokoe et al. (1993) Biochem. J., 296, 843-849

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Plate Position	Sequence	Initial Target	part number 8,000 tp	part number 50,000 tp	Descriptive	Lit. Km (µM)	Literature Source
A17-B18					Binding Buffer Background		
C17-D18	5FAM-HMRSAMSGLHLVK-OH	AMP kinase	RP7002	RP7502	Sams peptide, rat acetyl-CoA carboxylase (73-87)A77R86-87 derived	33	Michell et al. JBC 271 28445-28450 (1996)
E17-F18	5FAM-RRFRFTANLSKRYEA-NH2	CDPK (calcium dependent Kinase)	RP7003	RP7503	ER-located ACA2 calcium pump (36-50) derived		Huang at al, Arch Biochem Biophys. 2001 Sep 1;393(1):61-6
G17-H18	5FAM-QNPSRCSVSLSNVEA-NH2	MNK1/2	RP7011	RP7511	cPLA2 derived (721-736)		Hefner et al J Biol Chem. 2000 Dec 1;275(48):37542-51
I17-J18	5FAM-RRQLFRGFSFVAK-OH	RSK2-CTD	RP7013	RP7513	CTDtide	140	Chrestensen & Sturgill JBC 277, 27733-27741(2002)
K17-L18	5FAM-HQHLRLSTSSGRLLY-OH	SIK	RP7014	RP7514	hIRS1 derived		Horike et al (2003) J Biol Chem. May 16;278(20):18440-7
M17-N18	5FAM-GRRESLTSFG-NH2	PKG, PKA	RP7035	RP7535	IP3 receptor derived	35	Komalavilas and Lincoln, JBC 269, 8701-8797 (1994)
O17-P18	5FAM-VSRSGLYRSPSMPENLNRPR-OH	PRK2	R7275	R7276	Dual specificity phosphatase CDC25C (133-152) derived, IMAP validated		Sanchez,Y., et al., Science 277: 1497-1501, 1997 Furnari, B., et al., Science 277: 1495-1497, 1997
A19-B20	5FAM-KKALRRQETVDAL-NH2	CaM KII	RP7041	RP7541	Tre 286 autophosph. Site CamKII derived		Meredith et al.:Nature Biotechnology Vol 18, (2000) 309-312
C19-D20	5FAM-AMRLERQDSIFYPK-NH2	CHK2	R7186	R7231	CHK2tide, IMAP validated		O'Neill T JBC 277:16102-16115, 2002
E19-F20	5FAM-RLISASEFDRPLR-NH2	PKG	RP7042	RP7542	Bovine lung cGMP specific phosphodiesterase derived	68	Colbran J.L.et al. (1992) J. Biol. Chem.267:9589-9594
G19-H20	5FAM-GNNIEGMILLSELSRRRIR-NH2	PKR	RP7049	RP7549	eIF-2-alpha (40-57) derived		
I19-J20	5FAM-GRPRTSSFAEG-OH	SGK/Akt	R7110	R7136	Crosstide, IMAP validated	4	Wu et al Novartis Web presentation at Lab-robotics.org.
K19-L20	5FAM-GRPRTS**pS**FAEG-OH	Crosstide Calibrator	R7159	R7171	Phospho Crosstide, IMAP validated		Wu et al Novartis Web presentation at Lab-robotics.org.
A23-B24					Binding Buffer Background		
C23-D24	5FAM-MHRQETVDC-NH2	Cam KII	RP7043	RP7543	CAM-K II alpha chain derived	10	Colbran R.J., et al.. (1988) J. Biol. Chem. 263:18145-18151

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R3505 Rev A 3/26/04