Molecular Devices

IMAP™ Substrate Finder for Tyrosine Kinases
with **Progressive Binding System**

Product #R8134
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 be used to profile a kinase's substrate specificity. This IMAP Substrate Finder enables the researcher to screen a kinase of interest against 57 kinase substrates within a few hours. To improve the chances of success for finding a new or alternative substrate for a specific kinase, all substrates included on the IMAP Substrate Finder are documented as being substrate sequences for a particular part of the Kinome. This IMAP Substrate Finder focuses on the tyrosine kinase portion of the Human Kinome as described by Manning et al. [1]

Principle of the Assay

For this kit, the fluorescein (5FAM) labeled peptide sequences are dried down 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 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).

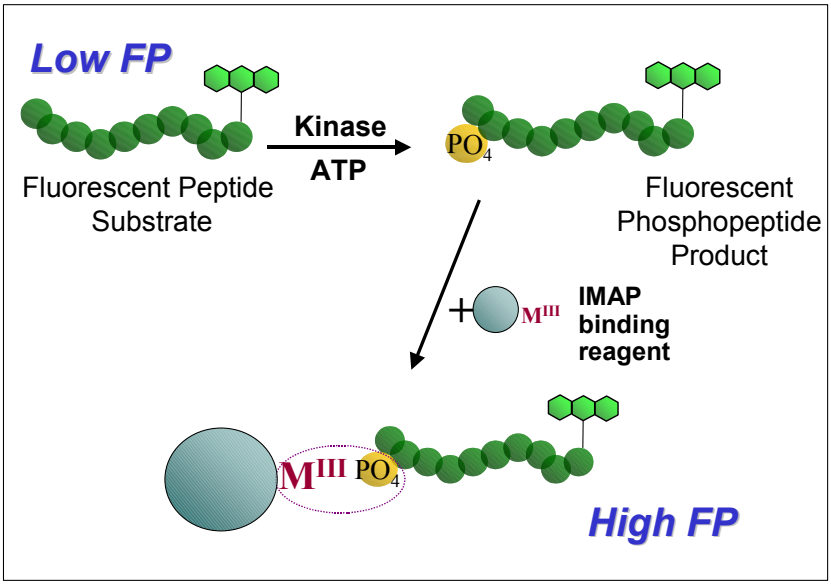

Figure 1. Principle of the IMAP system

[1] Manning et al. Science **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. Some of the substrate background values may be lowered 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 tyrosine kinase peptide substrates. Additional applications include testing enzyme preparations for purity, finding pairs of substrates for multiplexing, and characterization of kinases.

Materials and Equipment

Table 1: The IMAP™ Substrate Finder for Tyrosine Kinases Kit with Progressive Binding System (R8134) 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 Tyrosine Kinases plates each containing 57 substrates and 5 calibrators (phospho peptides) as positive controls	2	Two black 384 well plates, each containing 62 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 file for easy manipulation of all the substrate's information 3) Substrate Finder Data Analysis Macro, an 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	Upstate Biotechnology or other source
0.1 N HCl	Major Laboratory Supplier
1 M $MnCl_2$	Major Laboratory Supplier

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 for Tyrosine Kinases 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 7 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 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).

The kinase concentration to use for this Finder kit is very much at the users discretion and depends on the kinase itself, its specific activity and purity.
We recommend using the highest practical concentration of kinase to aid identifying potential substrates. In our experience, when using kinases from Upstate Biotechnologies, 1U/mL where 1U = 1nmole phosphate transferred/min is a good start. Follow-up experiments can be run using enzyme dilutions to choose the best of the potential substrates.

Table 4: Screening protocol for a kinase substrate using the IMAP Substrate Finder for Tyrosine kinases

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. Retain some 5x Buffer B to make the 120% Buffer B Binding Solution. To yield Complete Reaction Buffer, add DTT to the IMAP Reaction Buffer to make a final concentration of 1 mM and $MnCl_2$ to 2 mM to the 1x IMAP Reaction Buffer. The reaction buffer supplied already contains 10 mM $MgCl_2$. For one plate make 25 mL complete reaction buffer. The Complete 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. That is why this kit, unlike most MDC IMAP kits, contains Reaction Buffer with Tween-20 as carrier instead of BSA. Once a substrate is identified for a screen, assay optimization should therefore 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 **(for one plate make 4 mL 2x ATP solution).** 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) A7, B7, A8, B8 (controls for fl-interference, 60%A, 40%B condition) and A13, B13, A14, B14 (controls for fl-interference, 40%A, 60% B condition). A17, B17, A18, B18 (controls for fl-interference, 100% B condition). A21, B21, A22, B22 (controls for fl-interference, 120% B condition). These wells will serve as controls for any intensity-quenching components 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 100% B condition). I1, J1, I2, J2, (background for 120% 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 **(for one plate make 2 mL).** Add the kinase of interest in 10µl/well Complete Reaction Buffer at 2x its final concentration to the other 2 wells of each quadruplicate **(for one plate make 2 mL 2x enzyme solution).** 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 Make sure to add Binding Reagent slowly with rapid stirring **8 mL 85% A, 15% B, 1:400 Binding reagent**: Mix 6.8 mL of Buffer A with 1.2 mL Buffer B. Add 20 μL undiluted Binding Reagent to this **8 mL 60% A, 40% B, 1:1200 Binding Reagent:** Mix 4.8 mL of Buffer A with 3.2 mL Buffer B. Add 67 μL 1:10 pre-diluted Binding Reagent to this. **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 to this. **6 mL 10% A, 90% B 1:1500 Binding Reagent:** Mix 0.6 mL of Buffer A with 5.4 mL Buffer B. Add 40 μL 1:10 pre-diluted Binding Reagent to this. **6 mL 110% B, 1:1750 Binding Reagent:** dilute 5x Buffer B 1:4.55 (1.3 mL 5x Buffer B plus 4.7 mL water) Add 34 μL 1:10 pre-diluted Binding Reagent to this. 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® or equivalent material to avoid evaporation).**
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 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 Tyrosine 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. 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 after o/n to allow the substrates using high proportion Buffer B to equilibrate completely.

Example:

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

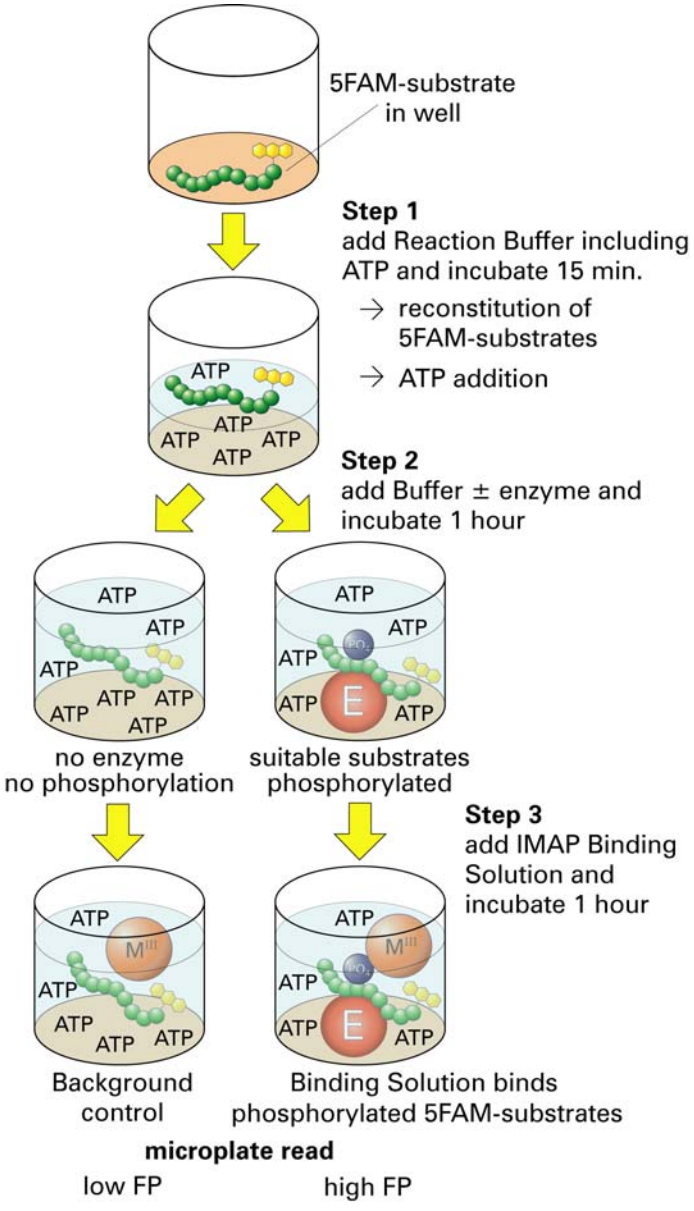

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Figure 2: Plate Layout overview for 384 well plate including numbering locator for peptide inside the wells.

	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	I3-J6	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: 10% A, 90% B, 1:1500 Binding Reagent
	Substrate peptide	
	Calibrator control	

	Buffer only Control	Binding Buffer: 110% B, 1:1750 Binding Reagent
	Substrate peptide	
	Calibrator control	

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

IMAP Substrate MAP Substrate Finder for Tyrosine Kinases							
Plate Position	**Sequence**	**Initial Target**	**Part number 8,000 tp**	**Part number 50,000 tp**	**Descriptive**	**Lit K_m (µM)**	**Literature Source**
A1-B2	Buffer only Background				subtract intensities from 85%A/15%B/1:400 wells		
C1-D2	Buffer only Background				subtract intensities from 60%A/40%B/1:1200 wells		
E1-F2	Buffer only Background				subtract intensities from 40%A/60%B/1:1500 wells		
G1-H2	Buffer only Background				subtract intensities from 10%A/90%B/1:1500 wells		
I1-J2	Buffer only Background				subtract intensities from 110%B/1:1750 wells		
A3-B4	Buffer Background						
C3-D4	5FAM-KKGEAIYAAPFA-NH2	Abl/Arg/Flt3	R7253	R7258	Abl/Arg synthetic substrate	15	Hantschel et al. Cell. 2003 Mar 21;112(6):845-57
E3-F4	5FAM-KKVVALYDYMPMN-NH2	BTK	RP7053	RP7553	BTK autophosphorylation site	59	Yamadori et al PNAS (1999) Vol. 96 6341-6346 Lowry et al: J. Biol. Chem (276) 45276-45281
G3-H4	5FAM-KKKSPGEYVNIGFG-NH2	IGF-1 receptor	RP7100	RP7600	IRS-1 derived peptide (Y895)		Favelyukis et al. Nat Struct Biol. 2001 Dec;8(12):1058-63 (modified)
I3-J4	5FAM-KKSRGDYMTMQIG-NH2	Insulin receptor/ IGF-1 receptor	R7267	R7268	IRS-1 derived (Y987)	30 (IR)	Xu et al. J Biol Chem. 1995 Dec 15;270(50):29825-30
K3-L4	5FAM-KKLATGDYMNMSP-NH2	Insulin Receptor	RP7073	RP7573	rat IRS-1derived (Y727)		Stein et al FEBS Lett. 2001 Mar 30;493(2-3):106-11
M3-N4	5FAM-AKAADGYVKPQIKQVV-NH2	JAK2	RP7076	RP7576	Stat5 derived		Saharinen et al. Mol Biol Cell (2003) 14:1448-1459
O3-P4	5FAM-GPKGTGYIKTELISVS-NH2	Jak2	RP7077	RP7577	Stat1 derived		Saharinen et al. Mol Cell Biol. 2000 May;20(10):3387-95
A5-B6	5FAM-AANITYAARRG-OH	PDGF-R	RP7085	RP7585	random peptide library derived	59.0	Chan et al. FEBS Lett. 1996 Sep 30;394(2):121-5
C5-D6	5FAM-GYIYGSFKK-OH	Src family	RP7089	RP7589	random combinatorial peptide library derived	55.0	Lam et al, Int. J. Peptide Protein Res.(1995) 545, 87-592

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Plate Position	Sequence	Initial Target	Part number 8,000 tp	Part number 50,000 tp	Descriptive	Lit K_m (µM)	Literature Source
E5-F6	5FAM-KKGEAIpYAAPFA-NH2	calibrator (C3-D4)	RP7106	RP7606	Abl/Arg synthetic substrate calibrator		Hantschel et al. Cell. 2003 Mar 21;112(6):845-57
A7-B8	Buffer Background	Buffer Background			Buffer Background		
C7-D8	5FAM-KKKKEEIYFFFG-NH2	CSK	R7269	R7270	CSKtide		Sondhi et al. Biochemistry. 1998 Jan 6;37(1):165-72.
E7-F8	5FAM-KHKKLAEGSAYEEV-NH2	EGF-R	RP7055	RP7555	PLC gamma derived (Y472)	37	Fry & McMichael Peptides. 1994;15(6):951-7
G7-H8	5FAM-TAENAEYLRVAPQ-NH2	EGF-R	RP7056	RP7556	EGF-R autophosph site derived (Y1173)	160	Beebe et al J Biol Chem. 2003 Jul 18;278(29):26810-6 Knighton et al. Proc Natl Acad Sci U S A. 1993 Jun 1;90(11):5001-5.
I7-J8	5FAM-KGSTAENAEYLRV-NH2	EGF-R	RP7057	RP7557	EGF-R autophosph site derived (Y1197)	120	Honegger et al., EMBO J. 1988 Oct;7(10):3053-60
K7-L8	5FAM-GMKVFIDPFTYEDPN-NH2	EphB3	RP7061	RP7561	ephrin receptor EphB3 potential autophosphorylation site derived		Gaudet et al., J Biomol Screen. 2003 Apr;8(2):164-75.
M7-N8	5FAM-AEEIYGVLAKKK-NH2	Fyn, Lyn Lck	RP7065	RP7565	synthetic peptide		Rapecki and Allen, J Pharmacol Exp Ther. 2002 Dec;303(3):1325-33.
O7-P8	5FAM-AAEEIYAARRG-NH2	HER2/Neu	RP7068	RP7568	soluble peptide libtrary derived		Chan et al. Cancer Lett. 2000 Nov 28;160(2):159-69
A9-B10	5FAM-KKKSPGEYVNIEFG-NH2	IGF-1 receptor	RP7069	RP7569	IRS-1 derived (Y895)	128 (IR), 26 (IGF-1R)	Favelyukis et al. Nat Struct Biol. 2001 Dec;8(12):1058-63 Xu et al. J Biol Chem. 1995 Dec 15;270(50):29825-30
C9-D10	5FAM-GHTDDGYMPMSPGV-NH2	Insulin Receptor	RP7072	RP7572	IRS-1 derived (Y612)		Boge & Roth, Anal. Biochem. (1995) 231 , 323-332
E9-F10	5FAM-KKKLPATGDYMNMSPVGD-NH2	Insulin Receptor, Src	RP7074	RP7574	IRS-1 derived (Y733)	24	Shoelson et al.Proc. Natl Acad. Sci. USA, 89, 2027-2031(1992)
G9-H10	5FAM-IYSGDYYR-NH2	Nyk/MER (Axl family)	RP7082	RP7582	Nyk autophosphorylation sites containing peptide (Y749, Y753, Y754)		Ling et al., J Biol Chem. 1996 Aug 2;271(31):18355-62
I9-J10	5FAM-DIMRDSNYISKG-NH2	PDGF R beta	RP7083	RP7583	PDGF-R derived peptide (aa 849-861)		Severinsson et al. Mol Cell Biol. 1990 Feb;10(2):801-9

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Plate Position	Sequence	Initial Target	Part number 8,000 tp	Part number 50,000 tp	Descriptive	Lit K_m (µM)	Literature Source
K9-L10	5FAM-KVEKIGEGTYGVV-NH2	Src family	R7157	R7172	p34cdc2 derived peptide (aa 6-18)		Cheng et al JBC (1992) 267:9248-56.
M9-N10	5FAM-KSAESIYGVLFSG-NH2	Src family	RP7090	RP7590	pOpt		Ellis et al. Biochem. J. (1998) 335, 277-284
O9-P10	5FAM-KVEKIGEGTYGVVYK-NH2	src family	RP7092	RP7592	p34cdc2 derived peptide (aa 6-19)		Cheng et al JBC (1991) 266:17919-17925
A11-B12	5FAM-GEEPLYWSFPAKKK-NH2	src's	RP7095	RP7595	phage display peptide library derived		Schmitz et al. J Mol Biol. 1996 Aug 2;260(5):664-77
C11-D12	5FAM-LEARLVAYEGWVAGK-NH2	Tie-2 receptor kinase	RP7098	RP7598	Phage Display library derived peptide		Deng et al. Comb Chem High Throughput Screen. 2001 Sep;4(6):525-33 Huang et al., J Biol Chem. 1999 Dec 31;274(53):38183-8
E11-F12	5FAM-ALQKDYENVGV-NH2	ZAP-70 Syk	R7189	R7234	modified optimal Zap 70 peptide		Isakov et al, J Biol Chem. 1996 Jun 28;271(26):15753-61
G11-H12	5FAM-KVEKIGEGTpYGVV-NH2	Calibrator	R7271	R7272	Calibrator		Cheng et al JBC (1992) 267:9248-56.
A13-B14	Buffer Background						
C13-D14	5FAM-EFPIYDFLPAKKK-NH2	BLK/Lyn/Bmx	R7188	R7233	phage display peptide library derived		Schmitz et al. J Mol Biol. 1996 Aug 2;260(5):664-77
E13-F14	5FAM-RRKGSTEEEAEYMNMAPQSS-NH2	EGF-R	RP7058	RP7558	EGF-R autophosphorylation site (Y1173)	15	Klingbeil et al. Arch Biochem Biophys. 1995 Feb 1;316(2):745-50
G13-H14	5FAM-GEEIYGEFD-NH2	EphB2	RP7060	RP7560	developed for Src kinases, chosen based on kinetic similarities between EphB and Src's		Binns et al. Mol Cell Biol. 2000 Jul;20(13):4791-805
I13-J14	5FAM-AEEEIYGEFEAKKK-NH2	Hck, Src, Brk	RP7066	RP7566	also called the "Src-optimal peptide"	33 (Src), 140 (Hck), 105 (Brk)	Sicilia et al., J Biol Chem. 1998 Jul 3;273(27):16756-63 Brk: Qui & Miller, J Biol Chem. 2002 Sep 13;277(37):34634-41
K13-L14	5FAM-KKEEEEYMMMMG-NH2	IGF-1 receptor/ Insulin receptor	RP7070	RP7570	synthetic peptide	150 (IR)	Favelyukis et al. Nat Struct Biol. 2001 Dec;8(12):1058-63 Yokoyama & Miller J Biol Chem. 2003 ;278(48):47713-23.
M13-N14	5FAM-REETGSEEYMNMDLG-NH2	Insulin Receptor	RP7071	RP7571	IRS1 derived (Y939)	40 (IR), 73 (IGF-1R)	Ablogu et al:J Biol Chem. 2000 Sep 29;275(39):30394-8 Xu et al. J Biol Chem. 1995 Dec 15;270(50):29825-30

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Plate Position	Sequence	Initial Target	Part number 8,000 tp	Part number 50,000 tp	Descriptive	Lit K_m (µM)	Literature Source
O13-P14	5FAM-VLPQDKEYYKVKEPGES-NH2	JAK2	RP7075	RP7575	JAK2 derived peptide		Saharinen et al. Mol Biol Cell (2003) 14:1448-1459
A15-B16	5FAM-KAIETDKEYYTVKD-NH2	JAK3	RP7078	RP7578	JAK1 activation loop derived peptide		Wang et al. Arch Biochem Biophys. 2003 Feb 1;410(1):7-15
C15-D16	5FAM-AEEEIYGEFEAKKKK-NH2	Src specific	RP7093	RP7593			Songyang Z ET AL, Nature. 1995 Feb 9;373(6514):536-9
E15-F16	5FAM-GEDPDYEWPSAKKK-NH2	syk	RP7096	RP7596	phage display peptide library derived		Schmitz et al. J Mol Biol. 1996 Aug 2;260(5):664-77
G15-H16	5FAM-ADEAYDYAA-NH2	general tyrosine kinase	RP7102	RP7602	Glu-Ala-Tyr random peptide		MDC development
I15-J16	5FAM-ALEKDYEEVGV-NH2	ZAP70	RP7104	RP7604	acidic original ZAP70 peptide		Isakov et al, J Biol Chem. 1996 Jun 28;271(26):15753-61
K15-L16	5FAM-EFPIpYDFLPAKKK-NH2	Calibrator	R7354	R7355	phage display peptide library derived		Schmitz et al. J Mol Biol. 1996 Aug 2;260(5):664-77
A17-B18	Buffer Background						
C17-D18	5FAM-AEEEEYFELVAKKKG-NH2	ACK1, EGF-R	RP7059	RP7559	artificial substrate	507 (ACK1)	Yokoyama &Miller J Biol Chem. 2003 Nov 28;278(48):47713-23. Epub 2003 Sep 22
E17-F18	5FAM-GEAEIYEAIE-NH2	Fps	RP7064	RP7564	artificial substrate		Saylor et al.Biochemistry. 1998 Dec 22;37(51):17875-81
G17-H18	5FAM-DEEIYEELK-NH2	Lyn	RP7079	RP7579	oriented peptide library derived peptide		Ruzzene et al., Eur J Biochem. 1997 Jun 1;246(2):433-9
I17-J18	5FAM-GEDNEYTAEDNEYTA-NH2	Lyn	RP7080	RP7580	main autophosphorylation site of most of the src enzymnes	20	Ruzza et al. J Pept Sci. 1998 Feb;4(1):33-45
K17-L18	5FAM-QEEEYVFIE-NH2	PDGF-R	RP7084	RP7584	peptide library derived peptide		Songyang Z ET AL, Nature. 1995 Feb 9;373(6514):536-9
M17-N18	5FAM-GEFLYGVFD-NH2	Src	RP7086	RP7586		100	Ramdas et al. J Pept Res. 1999 May;53(5):569-77
O17-P18	5FAM-GIYWHHY-NH2	Src	RP7087	RP7587	peptide library derived peptide	20	Lou et al. Bioorg Med Chem. 1996 May;4(5):677-82
A19-B20	5FAM-KKEEEEEEYMPMEDL-NH2	Src, Abl	RP7094	RP7594	Polyoma middle T derived	97	Garcia et al J Biol Chem. 1993 Nov 25;268(33):25146-51.
C19-D20	5FAM-KEDPDYEWPS-NH2	Syk	RP7097	RP7597	variant of the optimal p72Syk phosphorylation motif		Ellis et al. Biochem. J. (1998) 335, 277-284
E19-F20	5FAM-GEYAAEYADDAYAADYYAA-NH2	Tyrosine Kinase	RP7101	RP7601	Glu/Asp-Ala-Tyr random peptide		MDC development

Molecular Devices

Plate Position	Sequence	Initial Target	Part number 8,000 tp	Part number 50,000 tp	Descriptive	Lit K_m (µM)	Literature Source
G19-H20	5FAM-GEFLpYGVFD-NH2	Calibrator	RP7108	RP7608			Ramdas et al. J Pept Res. 1999 May;53(5):569-77
A21-B22	Buffer Background						
C21-D22	5FAM-GEEEPQFEEIPIYLELLP-NH2	CSK	RP7054	RP7554	polyoma-virus-middle-T-antigen-(mT)-derived peptide	63	Ruzzene et al., Eur J Biochem. 1997 Jun 1;246(2):433-9
E21-F22	5FAM-MEEIYGIFF-NH2	FGFR	RP7062	RP7562	peptide library derived		Ruzzene et al., Eur J Biochem. 1997 Jun 1;246(2):433-9
G21-H22	5FAM-AEEEYFFLF-NH2	FGFR	RP7063	RP7563	peptide library derived		Al-Obeidi et al. Biopolymers (1998) 47, 197-223.
I21-J22	5FAM-GGMEDIYFEFMGGK-NH2	HER2	RP7067	RP7567	peptide Library derived		Jan et al. Biochemistry. 2000 Aug 15;39(32):9786-803.
K21-L22	5FAM-GEFGEFGEYGEFGEF-NH2	Src	RP7088	RP7588	synthetic optimized peptide	80	Edison et al. J Biol Chem. 1995 Nov 10;270(45):27112-5.
M21-N22	5FAM-KEAYEAYEAYEAY-NH2	Src family	RP7091	RP7591	synthetic peptide sequences (EAY) 4		Ellis et al. Biochem. J. (1998) 335, 277-284
O21-P22	5FAM-GPWLEEEEEAYGWMDFK-NH2	Tyrosine Kinase	RP7103	RP7603	Gastrin derived peptide		Insogna et al. J Clin Invest. 1997 Nov 15;100(10):2476-85
A23-B24	5FAM-GEELQDDYEDMMEENLK-NH2	Zap70	RP7099	RP7599	pB3 (2-16 of cytoplasmic fragment of erythrocyte band 3)		Watts et al, FEBS Lett. 1996 Dec 2;398(2-3):217-22. Ellis et al. Biochem. J. (1998) 335, 277-284
C23-D24	5FAM-GEFGEFGEpYGEFGEF-NH2	Calibrator	RP7107	RP7607	synthetic peptide sequences (EAY) 4		Edison et al. J Biol Chem. 1995 Nov 10;270(45):27112-5.

Molecular Devices

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