IMAP Evaluation Demo Kit

Available Kit

	Data Points	Kit
IMAP® Evaluation Demo Kit	800, 20 µL reactions (80 µL final volumes)	R8166

Contents

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MOLECULAR DEVICES

Patents: http://www.moleculardevices.com/patents

Product manufactured by Molecular Devices, LLC.
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Chapter 1: Assay Principle

IMAP technology is based on the specific, covalent-coordinate, high-affinity interaction of trivalent metal that contains nanoparticles with phosphogroups. These phosphogroups can be free, or linked to serines, threonines, tyrosines, or other molecules, making IMAP a generic platform to assess kinase, phosphatase and phosphodiesterase activity. This basic principle is used in the IMAP Binding System with both fluorescence polarization (FP) and time-resolved fluorescence resonance energy transfer (TR-FRET) as a read-out. In a microwell assay format, fluorescently labeled peptides are phosphorylated in a kinase reaction. Addition of the IMAP Binding System stops the kinase reaction and specifically binds the phosphorylated substrates. Phosphorylation and subsequent binding of the substrate to the beads can be detected either by FP (Figure 1) or TR-FRET (Figure 2).

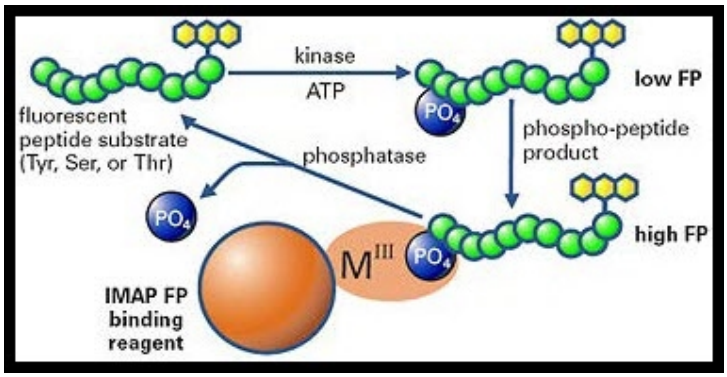

Figure 1: IMAP FP Diagram

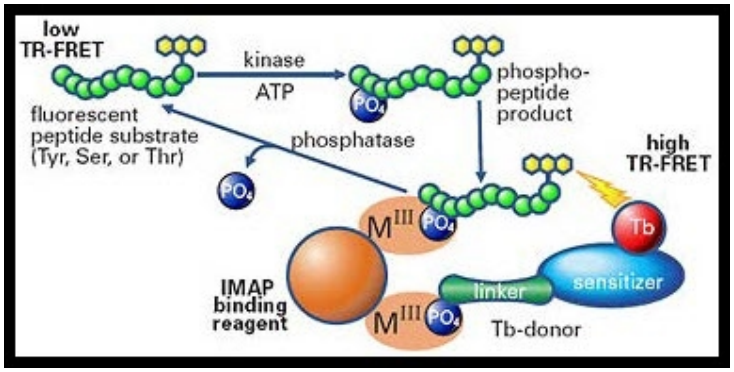

Figure 2: IMAP TR-FRET diagram. Components are the same as for FP, except that a Terbium (Tb) donor is added to the IMAP Binding System.

To set up IMAP FP and TR-FRET calibration curves, no enzyme reaction is necessary. Mix fluorescently labeled peptide and phospho-peptide in varying proportions to obtain calibration standards ranging from 0 to 100 percent phosphorylated, then add IMAP Binding Solution, incubate for the recommended time, and read. Use the resulting calibration curves in conjunction with kinase, phosphatase and phosphodiesterase assays to determine percent phosphorylation in experimental samples, or they can serve as a demonstration of microplate reader performance for FP and TR-FRET modes. See Optimization Protocol on page 7.

Applications

The IMAP Evaluation Demo Kit allows you to set up calibration to use in conjunction with kinase, phosphatase and phosphodiesterase assays to determine percent phosphorylation in experimental samples. The calibration curves can also demonstrate microplate reader performance for FP and TR-FRET modes.

Chapter 2: Materials and Equipment

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Kit Components

Components of the Assay Kit

Reagent	Quantity	Description
5x IMAP Progressive Binding Buffer A	12 mL	One bottle, store 4°C
5x IMAP Progressive Binding Buffer B	12 mL	One bottle, store 4°C
5x IMAP Reaction Buffer with 0.1% BSA	12 mL	One bottle, store 4°C
5x IMAP Reaction Buffer with 0.01% Tween-20	12 mL	One bottle, store 4°C
IMAP Progressive Binding Reagent	0.15 mL	One vial, store 4°C **Do not freeze**
Tb-Donor, 880 data points	lyophilized	One bottle, store -20°C After reconstitution store 4°C
5FAM-PKAtide, 10 nmoles	lyophilized	One vial, store -20°C
5FAM-PhosphoPKAtide, 10 nmoles	lyophilized	One vial, store -20°C

Materials Required But Not Provided

Item	Suggested Vendor
Black 384-well polystyrene microplate (Corning catalog #3573 is recommended)	Major laboratory suppliers (MLS)
White 384-well polystyrene microplate (Corning catalog #3572 is recommended)	MLS

Storage and Handling

All reagents are stable for six months from the date of receipt when stored at 4°C, except for the lyophilized FAM-PKAtide and FAM-phospho-PKAtide, which are stored at -20°C. Upon reconstitution these may be stored at 4°C for up to two weeks, or at -20°C for up to six months.

WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site: support.moleculardevices.com/

Supported Instruments

The IMAP Evaluation Demo Kit is designed for use with the following instruments from Molecular Devices:

- FlexStation® 3 Multi-Mode Microplate Reader
- SpectraMax® i3x Multi-Mode Microplate Reader
- SpectraMax® iD3 Multi-Mode Microplate Reader
- SpectraMax® M5 Multi-Mode Microplate Reader
- SpectraMax® M5e Multi-Mode Microplate Reader
- SpectraMax® Paradigm Multi-Mode Microplate Reader

Each microplate reader has a unique set of settings to run the protocols for this reagent kit. See the *Configuring SpectraMax and FlexStation Multi-Mode Microplate Readers for IMAP Assays Setup Guide* on the Molecular Devices Knowledge Base for details.

Chapter 3: Optimization Protocol

Use this protocol as an initial guide only to set up calibration curves for FP and TR-FRET.

The protocol is for 384-well plates. Adjust the volumes for 96-well or 1536-well plates.

To run the optimization protocol:

1. Prepare calibration curves:
   Reconstitute reagents and make working solutions.
   a. Make a 1x working solution of Reaction Buffer that contains 0.01% Tween by diluting the 5x stock solution 1:5 in water. For IMAP FP assays that use certain peptide substrates, the Reaction Buffer that contains BSA may yield improved results.

      Note: You do not need to add DTT to Reaction Buffer when you set up IMAP calibration standard curves. However, for enzyme assays, you should add DTT to Complete Reaction Buffer for a final assay concentration of 1 mM.

   b. Reconstitute 5FAM-PKAtide and 5FAM-PhosphoPKAtide by adding 0.5 mL of 1x Reaction Buffer to each vial. Invert gently to mix. Store the 20 µM stock solutions at 4°C for up to two weeks or at -20°C for up to six months.
   c. Make 1x working solutions of Progressive Binding Buffer A and Buffer B by diluting each 5x stock solution 1:5 with water. Store at 4°C for up to 6 months.
   d. Reconstitute Tb-Donor with 0.15 mL water. Invert gently to mix.
   (continued next page)

2. Make calibration standards.
   a. Make 100 nM solutions of 5FAM-PKAtide and 5FAM-PhosphoPKAtide by diluting the 20 µM stocks 1:200 in Reaction Buffer, for example, 10 µL of 20 µM stock plus 1990 µL Reaction Buffer.
   b. Combine 5FAM-PKAtide and 5FAM-PhosphoPKAtide 100 nM solutions (see the following table) to yield calibration standards ranging from 0% to 100% phosphorylation. Use the same set of calibration standards for both FP and TR-FRET.

Note: For TR-FRET, higher concentrations, you can use up to at least 1 µM (see Figure 4 on page 11, sample IMAP TR-FRET calibration standard curves).

Calibration Standards

Calibration Standard	% phosphorylated	Preparation
Standard 1	100	400 µL 5FAM-PhosphoPKAtide (100 nM)
Standard 2	50	200 µL Standard 1 + 200 µL 5FAM-PKAtide (100 nM)
Standard 3	25	200 µL Standard 2 + 200 µL 5FAM-PKAtide (100 nM)
Standard 4	12.5	200 µL Standard 3 + 200 µL 5FAM-PKAtide (100 nM)
Standard 5	6.2	200 µL Standard 4 + 200 µL 5FAM-PKAtide (100 nM)
Standard 6	3.1	200 µL Standard 5 + 200 µL 5FAM-PKAtide (100 nM)
Standard 7	1.6	200 µL Standard 6 + 200 µL 5FAM-PKAtide (100 nM)
Standard 8	0	200 µL 5FAM-PKAtide (100 nM)

   c. Set up the FP plate: Dispense 20 µL of each calibration standard into quadruplicate wells of a black 384-well plate. Include a quadruplicate set of buffer controls that contain 20 µL of Reaction Buffer only.

Plate Layout for IMAP FP (use black plate)

	Columns 1-2	Columns 3-4
Rows A-B	100%	Buffer
Rows C-D	50%	
Rows E-F	25%	
Rows G-H	12.5%	
Rows I-J	6.2%	
Rows K-L	3.1%	
Rows M-N	1.6%	
Rows O-P	0%	

d. Set up the TR-FRET plate: Dispense 20 µL of each calibration standard into quadruplicate wells of a white 384-well plate. Include two quadruplicate sets of controls that contain 20 µL of Reaction Buffer only. One set is for the buffer control, and the other set is the Tb only control.

Plate Layout for IMAP FP (use white plate)

	Columns 1-2	Columns 3-4
Rows A-B	100%	Buffer
Rows C-D	50%	Tb only
Rows E-F	25%	
Rows G-H	12.5%	
Rows I-J	6.2%	
Rows K-L	3.1%	
Rows M-N	1.6%	
Rows O-P	0%	

3. Dilute the Progressive Binding Reagent.
   To avoid pipetting volumes of Progressive Binding Reagent below 20 uL, you can prepare an intermediate dilution. Dilute Progressive Binding Reagent 1:10 in 0.1 N HCl, for exampl, 20 µL Progressive Binding Reagent + 180 µL 0.1 N HCl. You should make this intermediate dilution fresh for each assay and you can use it for both FP and TR-FRET.
4. Make Binding Solution for FP*
   a. Combine 62 µL of the intermediate dilution of Progressive Binding Reagent from step 3 with 2.44 mL Buffer A to make 2.5 mL of Binding Solution that contains a final dilution of 1:400 Progressive Binding Reagent.
   b. Pipet 60 µL of Binding Solution into each well of the FP plate, including the buffer control.
   c. Incubate for 30 minutes and read the plate in FP mode using the *IMAP FP_FAM* protocol in the SoftMax® Pro Data Acquisition and Analysis Software or use the settings indicated in the *Configuring SpectraMax and FlexStation Multi-Mode Microplate Readers for IMAP Assays Setup Guide* document.

5. Make Binding Solution for TR-FRET.*
   a. Make a solution that contains 80% Buffer A and 20% Buffer B. Make enough volume to be able to add 60 µL to each well, for example, 2.4 mL Buffer A + 0.6 mL Buffer B.
   b. Combine 37 µL of the intermediate dilution of Progressive Binding Reagent from step 4 with 2.96 mL of 80% Buffer A/20% Buffer B to make 3 mL of Binding Solution that contains a final dilution of 1:800 Progressive Binding Reagent.
   c. Set aside enough volume of Binding Solution for buffer-only controls, for example, 300 µL. Do not add Tb-Donor to this Binding Solution.
   d. Add 1:400 Tb-Donor to the remaining Binding Solution, for example, 6.7 µL Tb-Donor + 2.7 mL Binding Solution.
   e. Pipet 60 µL of Binding Solution with Tb-Donor into calibration standard and Tb only control wells.
   f. Pipet 60 µL Binding Solution without Tb-Donor into buffer control wells.
   g. Incubate for 1 hour and read in TRF mode using the *IMAP TR-FRET_FAM* protocol in the SoftMax Pro Software or use the settings indicated in the *Configuring SpectraMax and FlexStation Multi-Mode Microplate Readers for IMAP Assays Setup Guide* document.

Note: * The Binding Solution compositions apply to PKAtide. Other peptides may require different ratios of Buffer A and Buffer B. See the *IMAP Substrates* document. All reagents are stable for six months from the date of receipt when stored at 4°C, except for the lyophilized.

Chapter 4: Sample IMAP FP and TR-FRET Calibration Curves

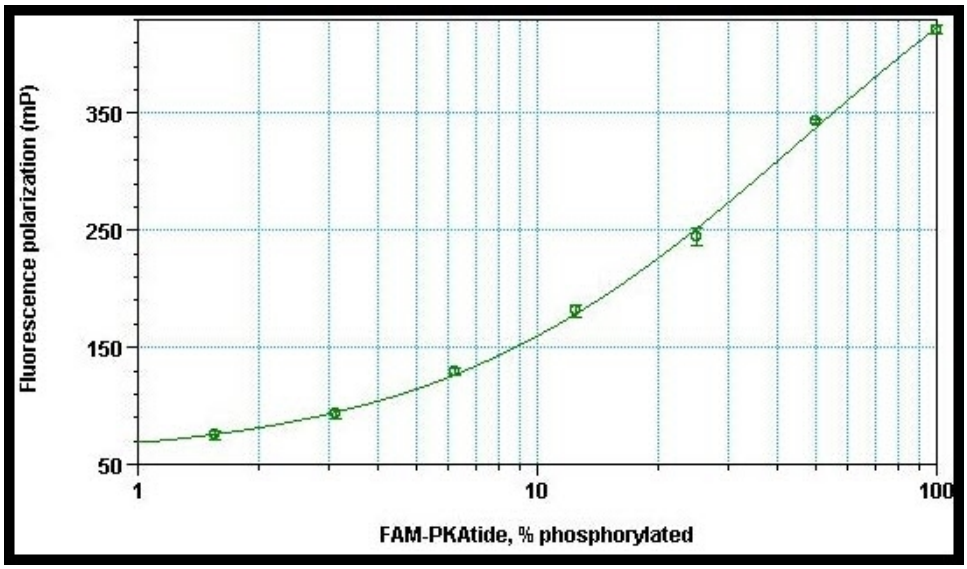

Figure 3. IMAP FP calibration curve with 5FAM-PKAtide and 5FAM-PhosphoPKAtide.

When you use a 4-parameter curve fit, omit the 0% phosphorylated standard from the plot.

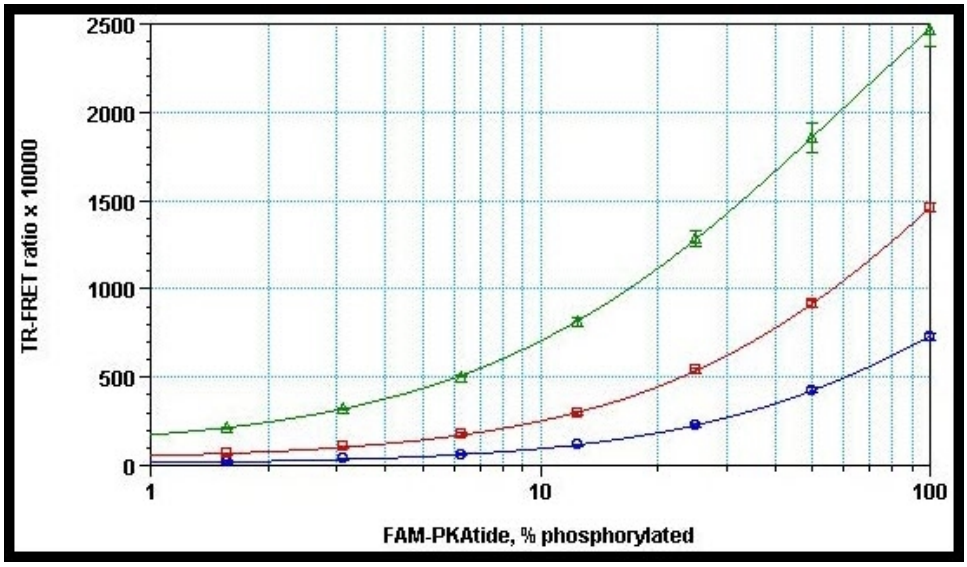

Figure 4

IMAP TR-FRET calibration curves with 5FAM-PKAtide and 5FAM-PhosphoPKAtide. Blue circles, 0.3 µM peptide; red squares, 1.0 µM peptide; green triangles, 3.0 µM peptide. When you use a 4-parameter curve fit, omit the 0% phosphorylated standard from the plot.

For information to generate and work with calibration curves, see the Molecular Devices IMAP application note #4, *Developing Calibration Curves for IMAP*.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—support.moleculardevices.com/—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Please have the instrument serial number (on the rear of the instrument), or reagent kit lot number, and any related sample data files available when you call.

Contact Us

Phone: +1-800-635-5577
Web: moleculardevices.com
Email: info@moldev.com

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