IMAP FP Phosphodiesterase Evaluation Assay Kit

With Progressive Binding System

The IMAP® FP Phosphodiesterase Assay Kit is designed to evaluate activity of phosphodiesterases (PDEs) that use cyclic-AMP (cAMP) or cyclic-GMP (cGMP) as substrate. This kit uses IMAP technology for non-antibody fluorescence polarization (FP) detection of enzyme activity and includes Fluorescein-labeled derivatives of cAMP and cGMP as substrates. This assay is a simple, homogeneous mix and read procedure that allows accurate determination of PDE activity. Molecular Devices also offers an IMAP TR-FRET (time resolved-fluorescence resonance energy transfer) assay for PDE's.

Available Kit

	Data Points	Kit
IMAP FP Phosphodiesterase Evaluation Assay Kit	800 (20 µL reactions)	R8175

Contents

R3565 B
January 2024

MOLECULAR DEVICES

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

Product manufactured by Molecular Devices, LLC.
3860 N. First Street, San Jose, California, 95134, United States of America.
Molecular Devices, LLC is ISO 9001 registered.

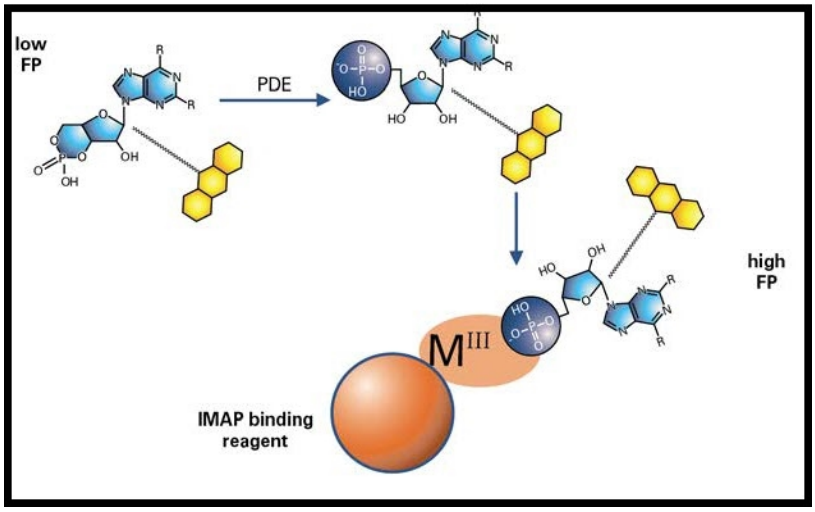
Chapter 1: About the Progressive Binding System

1

Use IMAP Buffer Kits that are based on IMAP's antibody-free detection of phosphorylation in conjunction with fluorescent labeled substrates and enzymes to evaluate enzyme activity of kinases, phosphatases, and phosphodiesterases. The assay is a simple, homogeneous mix and read procedure that allows accurate determination of enzyme activity. The Progressive Binding System is adjustable to allow you to determine maximum performance for each application. The IMAP Progressive Binding System kits described in this product Insert are designed for detection in Fluorescence Polarization Mode.

Assay Principle

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 or on nucleotide monophosphate generated from cyclic nucleotides (cAMP/cGMP) through phosphodiesterases. Such binding causes a change in the rate of the molecular motion of the phosphate bearing molecule, and results in an increase in the fluorescence polarization value observed for the fluorescent label attached to the substrate.



Principle of the IMAP FP PDE Assay System

Applications

The IMAP PDE Evaluation kit is designed for use in biochemical in-vitro assays of phosphodiesterase activity. It provides both Fluorescein labeled cAMP and cGMP as possible substrates to evaluate the optimal configuration for the assay. For technology suited for high throughput screening applications, you should use IMAP Binding System Component sizes 8000 data points Screening Express Buffer System (part number R8127), FAM-cAMP (part number R7505),
FAM-cGMP (part number R7507). For larger amounts or for screening purposes contact Molecular Devices.

Chapter 2: Materials and Equipment

2

Kit Components

Components of the IMAP FP Progressive Binding System

Reagent	Description
IMAP Progressive Binding Buffer A	Baseline binding buffer
IMAP Progressive Binding Buffer B	Affects FP background by reducing, or blocking, the non-phosphate-based binding of the fluorescent substrate to the Binding Reagent.
IMAP Progressive Binding Reagent	Introduces the phosphate binding entities. This Binding Reagent binds to phosphate residues via a coordinate covalent complex bond.

Components of the IMAP FP Phosphodiesterase Evaluation Kit

Reagent	Quantity	Description
IMAP Progressive Binding Reagent	150 µL	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 with 0.1% BSA (5x)*	12 mL	One bottle, store 4°C
IMAP Reaction Buffer with 0.01% Tween-20 (5x)**	12 mL	One bottle, store 4°C
Fluorescein- labeled cAMP*** Substrate	50 µL	One vial 100 µM cAMP Substrate in 0.1% Acetic Acid + 0.01% Tween-20 Store at -20°C
Fluorescein- labeled cGMP*** Substrate	50 µL	One vial 100 µM cGMP Substrate in 0.1% Acetic Acid + 0.01% Tween-20 Store at -20°C

* The 1X IMAP Reaction Buffer with BSA made from the supplied 5X concentrated stock contains 10 mM Tris-HCl, pH 7.2, 10 mM $MgCl_2$, 0.05% NaN_3, and 0.1% phosphate-free BSA as the carrier.
** The 1X IMAP Reaction Buffer with Tween-20 made from the supplied 5X concentrated stock contains 10 mM Tris HCl, pH 7.2, 10 mM $MgCl_2$, 0.05% NaN_3, and 0.01% Tween-20 as the carrier.
*** TAMRA-labeled versions of these substrates are also available separately.

Materials Required But Not Provided

Item	Suggested Vendor
Phosphodiesterase 1	SIGMA: P9529
Calmodulin	SIGMA: P2277
$CaCl_2$	Major laboratory suppliers
Black polystyrene 384-well microplate (Corning catalog #3573 is recommended)	Major laboratory suppliers

Storage and Handling

Store all kit components at 4°C except the cAMP and cGMP Substrates, which should be aliquoted to avoid repeated freeze-thaw cycles and stored at -20°C.

IMPORTANT: Do Not Freeze the Binding Reagent.

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

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 Screening Express Assay 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® iD5 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: Assay Protocols

Quick Start Protocol

To run the Quick Start protocol:

1. 20 µL Phosphodiesterase reaction/well:
   - Enzyme and activators, as needed.
   - 100 nM fluorescent labeled substrate.
2. 1 hour reaction time, or as desired.
3. Add 60 µL IMAP Binding Solution.
   - FAM-cAMP:85% A, 15% B, Binding Reagent 1:600.
   - FAM-cGMP:75% A, 25% B, Binding Reagent 1:600.
4. Let equilibrate according to Binding Buffer composition.
5. Read FP.

Adjust volumes for plates other than standard 384-well.

Optimization Protocol

Use this protocol as an initial guide only to detect phosphodiesterase 1 (PDE1) activity acting on the cGMP and/or cAMP substrate. Adjust reaction concentrations, activators, and other conditions to optimize the assay system and between different PDE's. You can do this protocol at room temperature. You should keep the enzyme on ice, if possible, prior to adding it to the assay plate.

Other components you can add 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 the starting conditions listed in the IMAP Substrate Product Insert Table 1. These include high concentrations of EDTA and EGTA as well as phosphate and structurally related molecules.

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

To run the optimization protocol:

1. Prepare the Complete Reaction Buffer:
   Concerning Reaction Buffer Choice:
   IMAP Reaction Buffer with BSA provides a more sensitive assay for some PDEs in terms of EC_{50}, than the IMAP Reaction Buffer with Tween. However, BSA may bind to certain test compounds, possibly resulting in a rightward shift of the compound IC_{50}. This is a generally observed phenomenon and not IMAP-specific. If a test compound in the IMAP assay binds to BSA, you should switch the assay buffer to IMAP Reaction Buffer with Tween to overcome these issues. Both Reaction Buffer with BSA and Reaction Buffer with Tween are included in this kit.
   a. Make a 1X solution of IMAP Reaction Buffer by adding 40 mL of purified water per 10 mL of the supplied 5X concentrated stock. When stored at 4°C, the 1X solution of Reaction Buffer is stable for six months.
   b. To make the Complete Reaction Buffer, you may need to add other cofactors or DTT to a final concentration of 1 mM in the 1X solution of Reaction Buffer, depending on the requirements of the enzyme. Prepare Complete Reaction Buffer fresh for each day of assay. Approximately 15 mL is required per 384 wells. Scale up or down, as needed.

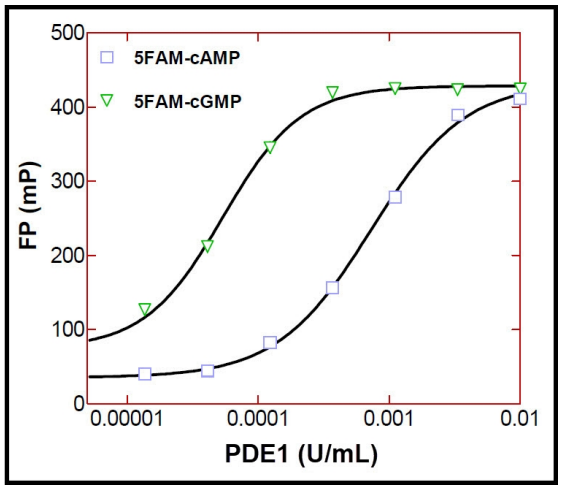
2. Prepare the cGMP and cAMP Substrate Working Solution:
   Add 3 µL of the 100 µM cGMP or 100 µM cAMP substrate solution per 1500 µL of Complete Reaction Buffer each to make a substrate working solution (200 nM, 2x of final) for each.
3. Design the assay plate:
   a. Use the SoftMax® Pro Data Acquisition and Analysis Software to analyze the results. You can set up a template before or after you read the plate. If you set up the plate before, you can print out a template to help you pipette the plate.
   b. The following is an example of an enzyme dilution curve for PDE1 with 3 replicates:

Column→ Row ↓	1	2	3	4	5	6	7	8	9
A	0.01 units/mL	0.0033 units/mL	0.0011 units/mL	0.00037 units/mL	0.00012 units/mL	0.00004 units/mL	0.000014 units/mL	0 enzyme	Buffer Only control
B									
C									

4. Prepare the enzyme working stock solution:
   If you use PDE1 (P9529), reconstitute the lyophilized material in 50% Glycerol to 1 U/mL as recommended by the supplier. Aliquot and freeze at -20°C.
   To prepare the enzyme dilution buffer add $CaCl_2$ to 200 µM and Calmodulin (Sigma) to 5,000 U/mL to the IMAP Reaction Buffer you prepared in step 1.
   To prepare an enzyme dilution curve as shown in step 3b, make a 180 µL stock of approximately 0.02 units/mL* in enzyme dilution buffer and serially transfer 60 µL of this stock to 120 µL of enzyme dilution buffer. The resulting dilutions are 2x the final reaction concentrations of 0.01, 0.0033, 0.0011, 0.00037, 0.00012, 0.00004, 0.000014, and 0 units/mL.
   *One unit is defined as the amount of PDE1 (SIGMA: P9529) that hydrolyzes one µmole of cAMP per minute at 30°C and pH 7.5.
5. Add components to the 384-well assay plate:
   a. Add 10 µL of each enzyme dilution prepared in step 4 to the appropriate wells.
   b. Add 10 µL of the substrate solution prepared in step 2 to the appropriate wells.
   c. For the Buffer Only background control, add 20 µL of Complete Reaction Buffer to the appropriate wells. Each well of the assay should now have 20 µL volume.
6. Cover the plate and protect from light. Incubate at room temperature for 60 minutes. You may need to optimize reaction time for your needs.

7. Prepare sufficient IMAP Binding Solution:
    a. Make a 1X solution of Progressive Binding Buffer A and Buffer B by adding 120 mL of 5X Progressive Binding Buffer A or B to 480 mL of purified water each. When stored at 4°C, the 1X solutions of Progressive Binding Buffer A or B are stable for 6 months.
    b. The optimal Binding Buffer composition for FAM-cAMP is: 85% Binding Buffer A, 15% Binding Buffer B and Binding Reagent 1:600. To make, for example, 20 mL of this Binding Solution combine: 17 mL 1x Binding Buffer A, 3 mL 1x Binding Buffer B mix thoroughly and add 33µL Progressive Binding Reagent and mix again.
    c. The optimal Binding Buffer composition for FAM-cGMP is: 75% Binding Buffer A, 25% Binding Buffer B and Binding Reagent 1:600. To make for example 20 mL of this Binding Solution combine: 15 mL 1x Binding Buffer A, 5 mL 1x Binding Buffer B mix thoroughly and add 33 µL Progressive Binding Reagent and mix again.
    d. Add 60 µL of Binding Solution to each assay well, including the Buffer Only wells.
8. Cover the plate and protect from light. Incubate at room temperature for 1 - 2 hours. Longer incubation times can provide a slight increase in response.
9. The SoftMax Pro Software settings to read the plate depend upon the microplate reader you use. See the *Configuring SpectraMax and FlexStation Multi-Mode Microplate Readers for IMAP Assays Setup Guide* document for details.
10. Analyze the results:
    a. Calculate the average background (= Buffer Only wells) for both S and P fluorescent intensity data.
    b. Subtract the background value from both S and P raw data.
    c. Calculate FP and plot FP against enzyme concentration.

Chapter 4: Sample Data



Titration of PDE1 with the IMAP Phosphodiesterase Assay Kit in the presence of 2.5 mM $CaCl_2$ and 2500 U/mL calmodulin

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

Visit our website for a current listing of worldwide distributors.