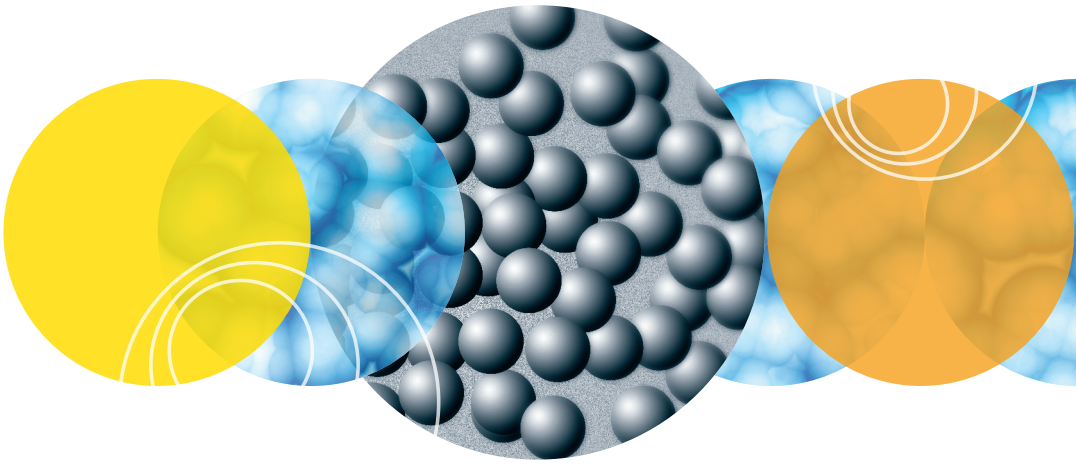


IMAP Technology for kinases, phosphatases and phosphodiesterases

A complete platform for assay development and high-throughput screening

KEY FEATURES

- **No antibodies**
- **Robust fluorescence signal**
- **Complete assay system**
- **Homogeneous assay**
- **Non-radioactive**
- **Sensitive FP and TR-FRET detection**

Until now, assays of kinase activity have been performed using radioactive isotopes or highly specific antibodies. To address this, Molecular Devices introduced its proprietary IMAP® Technology, providing a non-radioactive, homogeneous assay applicable to a wide variety of kinases without regard for the substrate peptide sequences. The assay is a simple "mix-and-read" procedure that allows accurate determination of enzyme activity.

What is IMAP Technology?

IMAP Technology is based on the specific, covalent-coordinate, high-affinity interaction of trivalent metal containing nanoparticles with phosphogroups. These phosphogroups can be free, linked to serines, threonines or tyrosines, or other molecules which make the IMAP Platform a generic to assess kinase, phosphatase and phosphodiesterase activity. This basic principle has been used in the IMAP Binding System using both fluorescence polarization and TR-FRET (as a readout). 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).

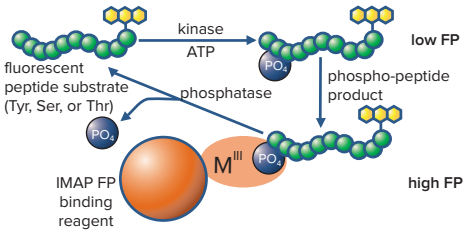


Figure 1. IMAP kinase and phosphatase assays. IMAP Technology principle using FP readout: Binding Solution is added after the kinase reaction using a fluorescently labeled peptide. The small phosphorylated fluorescent substrate binds to the large M(III)-based nanoparticles which reduces its rotational speed of the substrate and thus increases its polarization.

MOLECULAR DEVICES

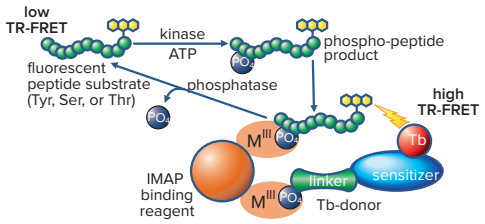
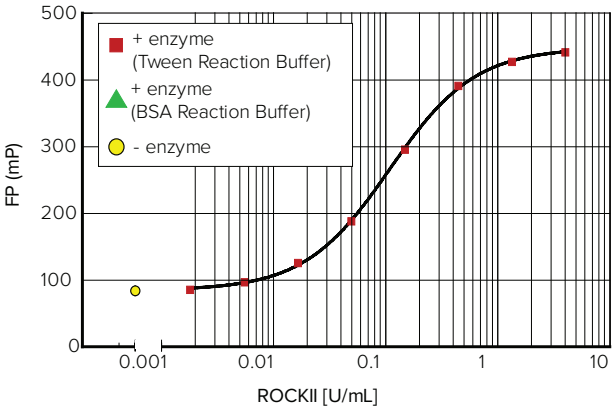
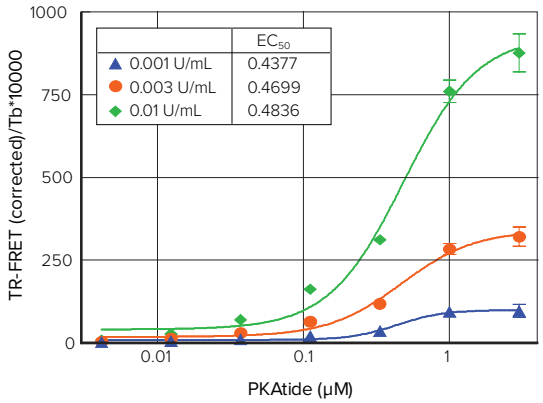


Figure 2. IMAP TR-FRET kinase and phosphatase assays. IMAP principle using TR-FRET readout: Binding Solution is added after the kinase reaction using a fluorescently labeled peptide or protein. In this system, the nanoparticle is spiked with a Tb-Donor molecule. By binding to the spiked M(III)-based nanoparticles, the phosphorylated fluorescent substrate comes into close proximity with the Tb-Donor, which allows measurement of the TR-FRET between the Tb-Donor and the phosphorylated, fluorescent substrate.

Robust data



ROCKII dilution curve. Enzyme dilution curves for ROCKII in BSA and in Tween IMAP Reaction Buffer using the Progressive Binding System. Reaction conditions: ROCKII kinase (Upstate: 14-451) as indicated, 100 nM FAM-S6 derived substrate (5FAM-AKRRRLSSLRA-COOH, R7184), 100 µM ATP. IMAP Binding Solution conditions: 100% Binding Buffer A, Progressive Binding Reagent 1:400.



PKA dilution curve. PKAtide substrate dilution curves with PKA using IMAP TR-FRET. Reaction conditions: PKA kinase at 0.01, 0.003, 0.001 U/mL (Upstate: 14-440), FAM-PKAtide as indicated (5FAM-GRTGRRNSI-NH2, R7250), 100 µM ATP, IMAP Binding Solution: 95% Binding Buffer A, 5% Binding Buffer B, Progressive Binding Reagent 1:400.

Complete solution

Strong signals

Assays using IMAP Technology deliver an intense fluorescent signal that produces high precision and robust results, even in the presence of interfering compounds.

Simple protocol

The IMAP Kinase Assay protocol is easy to use. The kinase reaction mixture is incubated with the binding reagent, then the microplate is read by Molecular Devices SpectraMax i3, SpectraMax Paradigm, or SpectraMax M5 Multi-Mode Microplate Readers.

IMAP Assay Kits

IMAP Assay Kits provide a turnkey solution to evaluate the IMAP Platform and smoothly transition into high-throughput screening. These all-inclusive assay kits are ready to use in assay validation or screening. They also provide the starting point for understanding the IMAP Platform and adapting it to new targets.

Alternate targets

IMAP Technology is designed to be easily adapted to any kinase of interest. With its ease of use, stable signal and inherent flexibility, it is ideal for the rigorous demands of high-throughput screening (HTS).

IMAP Screening Express

IMAP Screening Express consists of the proprietary IMAP beads and buffers for 8,000 data points in a standard 384-well format. This kit is designed for customers who have their own enzyme and substrate. With the IMAP Progressive Binding System, researchers can “fine-tune” the IMAP assay, i.e., adjust the parameters that affect ATP tolerance and background signal, to achieve optimal results according to their individual assay requirements.

IMAP Purchase Plan (IPP)

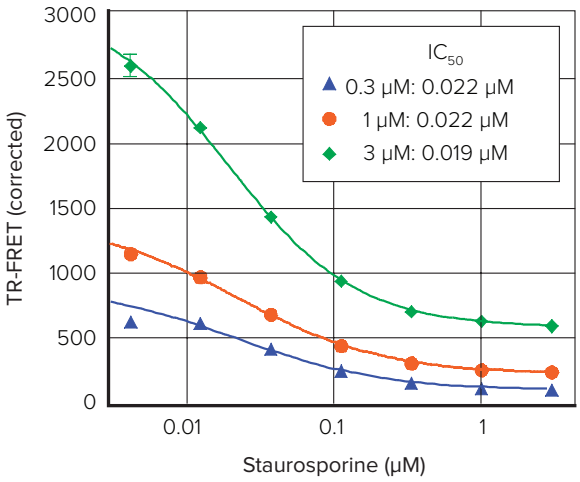
The IMAP Purchase Program (IPP) is a discount program targeted for users in high-throughput environments. IMAP Beads and Buffers can be purchased at preferential prices for a yearly subscription fee. This worldwide program covers all sites within a participating corporation.

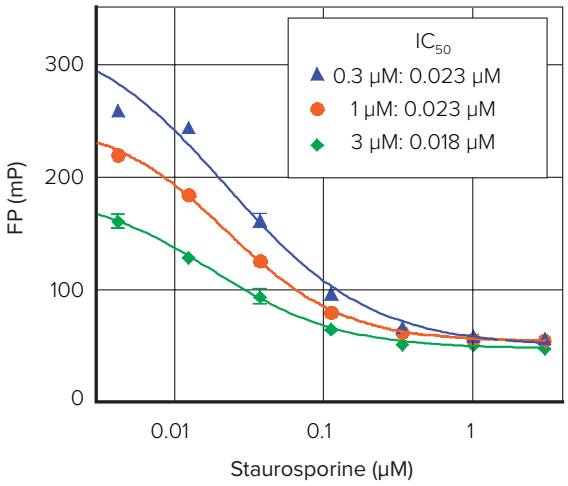
Substrates for IMAP Assays

Substrates are optimized for each enzyme to ensure the finest performance when using the IMAP Platform. Substrates are sold in kits for testing 8,000 and 50,000 data points. Our R&D team is continuously adding to our inventory of pre-validated substrates for use with the IMAP Technology.

IMAP FP Substrate Finder

IMAP FP Substrate Finder plates accelerate the difficult task of finding new substrates for new or proprietary kinases. These kits provide a quick, sensitive, yet inexpensive method to screen dozens of substrates for new kinases of interest. IMAP binding conditions for substrates identified from these FP detection-based plates can be optimized using either FP or TR-FRET detection.





FP vs. TR-FRET detection. Akt inhibition with staurosporine: Comparison of IMAP TR-FRET **(top)** and IMAP FP **(bottom)** detection. Reaction conditions: Akt kinase (0.1U/mL, Upstate: 14-276), 300 nM, 1µM, 3µM FAM-Crosstide (5FAM-GRPRTSSFAEG-COOH, R7110), 10 µM ATP, Staurosporine as indicated. IMAP Binding Solution: 40% Binding Buffer **A,** 60% Binding Buffer **B,** Progressive Binding Reagent 1:400.

IMAP Assay Kits

IMAP Screening Express (8,000 dp/kit)

- Binding System, FP
- Binding System, TR-FRET

IMAP Substrates

- A wide range of validated fluorescently-labeled substrates

IMAP FP Substrate Finder

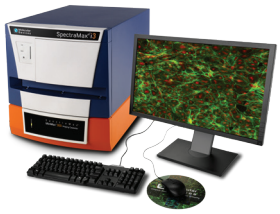
- Ser/Thr Kinase Plate 1 (CAMK/AGC)
- Ser/Thr Kinase Plate 2 (CMGC/CK1/STE/TKL)
- Tyrosine Kinase Plate

Please contact your Molecular Devices sales representative or Customer Service for a current list of IMAP products and details on quantities and bulk discounts.

Ordering information

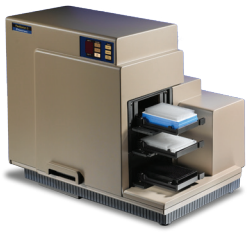
Reagent	Description	Part number
IMAP® FP Evaluation Kit, with Progressive Binding System	For kinase and phosphatase fluorescent polarization assays. Consists of the proprietary IMAP Binding Reagent and Binding Buffers for 800 data points in a standard 384 well format.	R8155
IMAP® TR-FRET Evaluation Kit with Progressive Binding System	For kinase and phosphatase TR-FRET assays. Consists of the proprietary IMAP Binding Reagent and Binding Buffers for 800 data points in a standard 384 well format.	R8161
IMAP® Phosphodiesterase FP Evaluation Kit	For Phosphodiesterase (PDE) fluorescent polarization assays. Consists of the proprietary IMAP Binding Reagent, cAMP and cGMP substrates, and Binding Buffers for 800 data points in a standard 384 well format.	R8175
IMAP® Phosphodiesterase TR-FRET Evaluation Kit	For Phosphodiesterase (PDE) TR-FRET assays. Consists of the proprietary IMAP Binding Reagent, cAMP and cGMP substrates, and Binding Buffers for 800 data points in a standard 384 well format.	R8176
IMAP® FP Screening Express Kit	Fluorescence Polarization Screening Express Kit with Original Binding System. Consists of the proprietary IMAP Binding Reagent and Binding Buffers for 8000 data points in a standard 384 well format.	R8073
IMAP® FP Screening Express Kit with Progressive Binding System	Fluorescence Polarization Screening Express Kit with Progressive Binding System. Consists of the proprietary IMAP Binding Reagent and Binding Buffers for 8000 data points in a standard 384 well format.	R8127
IMAP® TR-FRET Screening Express Kit with Progressive Binding System	TR-FRET Screening Express Kit with Progressive Binding System. Consists of the proprietary IMAP Binding Reagent and Binding Buffers for 8000 data points in a standard 384 well format.	R8160

Compatible with these Molecular Devices systems



SpectraMax® i3/i3x
Multi-Mode Microplate Reader

SpectraMax® Paradigm®
Multi-Mode Microplate Reader



FlexStation® 3
Multi-Mode Microplate Reader

SpectraMax® M Series
Multi-Mode Microplate Readers

Contact Us

Phone: +1-800-635-5577
Web: www.moleculardevices.com
Email: info@moldev.com
Check our website for a current listing
of worldwide distributors.

2/16 0120-1283K
Printed in USA

MOLECULAR DEVICES