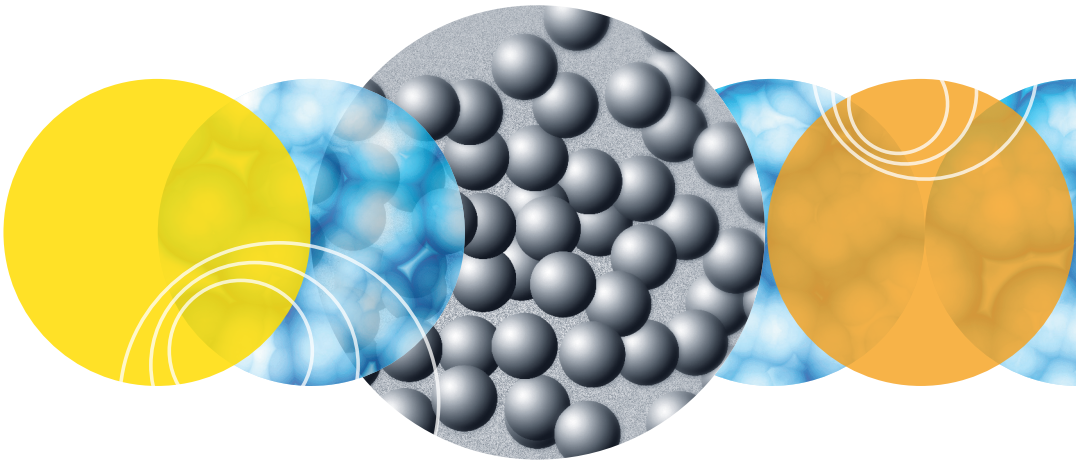


IMAP FP Substrate Finder

Accelerating kinase assay development

KEY FEATURES

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

Introduction

The IMAP® Platform provides advanced tools for successful assay development and high-throughput screening (HTS) of kinases, one of the most important target classes in drug discovery. The IMAP technology has a simple "mix-and-read" protocol: after the kinase reaction has been completed in a microtiter plate, the IMAP Binding Solution is added to stop the reaction and enable detection by fluorescence polarization.

IMAP FP Substrate Finder

The IMAP FP Substrate Finder allows researchers to quickly test one or more kinases against dozens of peptides which are included in a 384-well microtiter plate to identify potential substrates for subsequent IMAP screens. This new assay development tool significantly limits the time spent searching for a suitable substrate. Once the substrates are identified from the FP detection-based plates, IMAP binding conditions can be optimized using either FP or TR-FRET detection. Additionally, substrate specificity may be evaluated between different kinase variants or among different mutants of the same kinase. The IMAP Substrate Finder allows researchers to screen through dozens of predefined potential substrates for less than the cost of a few unknown potential substrates.

There are three IMAP Substrate Finder kits: two focus on the Serine/Threonine kinases of the human kinome, and the third Substrate Finder concentrates on Tyrosine kinases. All peptide substrates contained in these plates have been published and shown to be valid kinase substrates (literature links are provided with the IMAP Substrate Mapper). The first Serine/Threonine plate covers the CAMK, AGC portions of the kinome and contains 56 kinase substrates and three phosphorylated substrates as positive controls. The second Serine/Threonine plate covers the CMGC, CK1, STE, TKL and contains 61 kinase substrates and six phosphorylated substrates as positive controls. The Tyrosine plate consists of 57 kinase substrates and five phosphorylated substrates. All peptides are represented in quadruplicate to allow for numerous controls or to enable testing of additional kinases, according to the user's preference.

MOLECULAR DEVICES

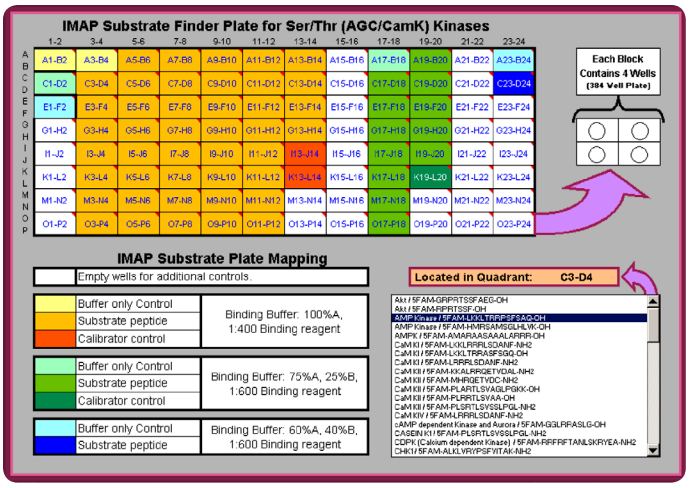


Figure 1. IMAP Substrate Mapper plate layout for Ser/Thr CAMK and AGC kinases. An illustration of the plate layout from the IMAP FP Substrate Mapper provided with IMAP FP Substrate Finder Kits..

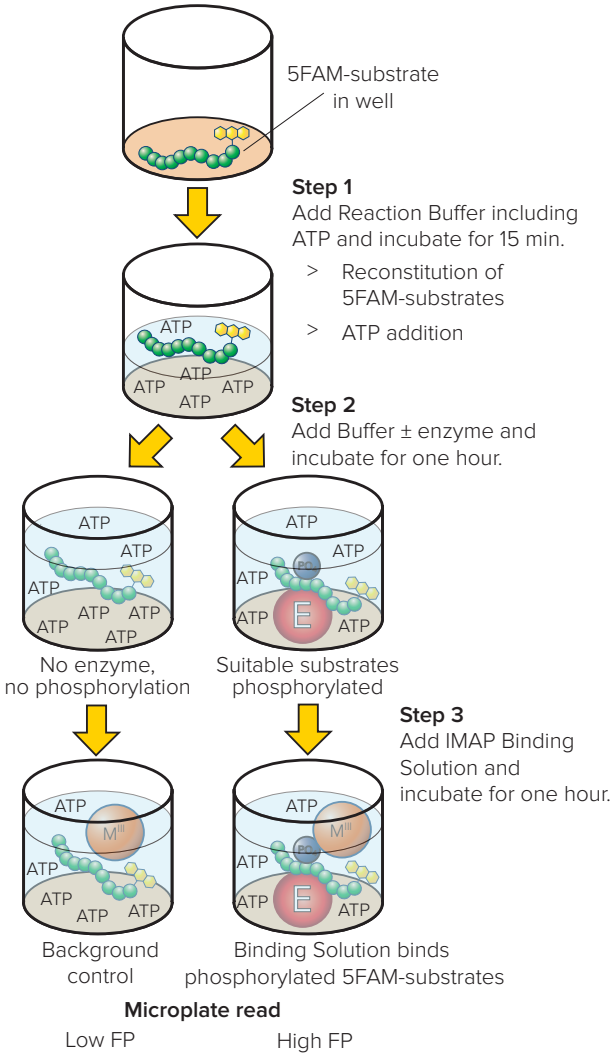


Figure 2. IMAP FP Substrate Finder assay principle.

Simple protocol to characterize your assays

IMAP Substrate Mapper

The IMAP Substrate Mapper (Figure 1) is an intuitive electronic map to assist in navigating the substantial amount of information that comes with the IMAP FP Substrate Finder: sequences of the peptide substrates, initial target kinase and location on the plate, literature references, recommended Progressive Binding Buffer ratios and substrate part numbers to assist with ordering the desired substrate.

Simple protocol

The fluorescein- (5FAM-) labeled substrates are lyophilized in a 384-well microtiter plate (Figure 2). Upon reconstitution with 10 µL buffer, including ATP or other activators needed for the reaction (Figure 2, Step 1), the peptides are at an appropriate concentration (100 nM) for an IMAP Fluorescence Polarization (FP) assay. Next, 10 µL of Buffer with or without enzyme is added into the plate (Figure 2, Step 2). Two of the replicate wells can be incubated with the kinase of interest, while the other two wells contain background controls to assess the (FP) background specific for this particular peptide. The IMAP Binding System is added and the change in fluorescence polarization is measured (Figure 2, Step 3). With four replicates of each substrate on a single plate, several initial parameters can be assessed against dozens of peptides in a single assay.

If more than one potential substrate is found with the IMAP FP Substrate Finder, we recommend evaluating the assay with the two or three substrates that have the highest ΔmP, i.e., the biggest change between no-enzyme control and enzyme. During the assay development process, one of the other substrates may demonstrate an advantage over the initial one that had the highest FP on the IMAP FP Substrate Finder plate.

Characterize your kinase

The IMAP FP Substrate Finder can also help characterize the kinase. In one set of experiments, the IMAP FP Substrate Finder revealed differences in substrate specificity between a wild-type and mutant form of a target kinase. This application could be easily configured to measure similar results in response to various lead compounds of interest. A novel kinase could be characterized according to its phosphorylation site preference in a very short amount of time. Alternatively, various enzyme preparations could be assayed for relative purity based on their phosphorylation patterns. These are just a few examples of how the IMAP FP Substrate Finder can enable new discoveries.

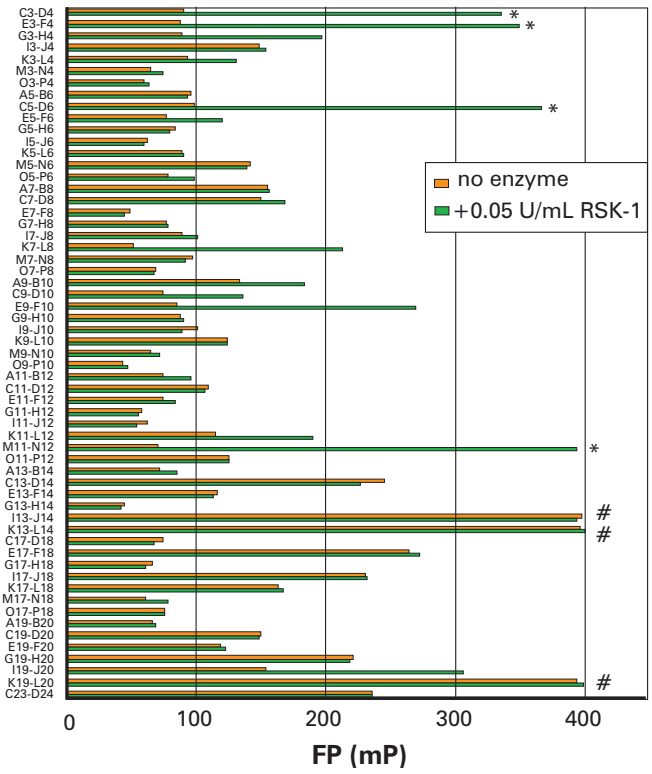


Figure 3. IMAP FP Substrate Finder sample. Screening using the IMAP FP Substrate Finder and 0.05 U/mL RSK-1 at 100 µM ATP. *Good substrate ΔmP > 200. #positive controls (phosphorylated sequence).

Results

Figure 3 shows the results from a standard screen for a kinase substrate. The Ser/Thr Kinase RSK-1 was assayed at 0.05 U/mL and 100 µM ATP. Eight substrates were identified that demonstrated a polarization change ΔmP (plus enzyme - background) of > 100 mP, one of which had a ΔmP of > 300 mP.

Note: While each of the peptides in the Substrate Finder was chosen with great care, Molecular Devices cannot guarantee that a substrate will be found for each kinase tested.

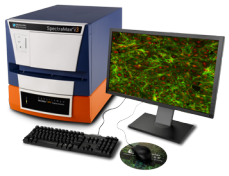
Ordering information

Reagent	Description	Part number
Substrate Finder Kit for Ser/Thr Kinases 1 (CAMK/AGC)	Serine/Threonine Kinase Plate 1 contains 59 substrates: 56 unphosphorylated substrates and 3 phosphorylated substrates (as controls)	R8131
Substrate Finder Kit for Ser/Thr Kinases 2 (CMGC/CK1/ STE/TKL)	Serine/Threonine Kinase Plate 2 contains 67 substrates: 61 unphosphorylated substrates and 6 phosphorylated substrates (as controls)	R8140
Substrate Finder Kit for Tyrosine kinases	Tyrosine Kinase Plate consists of 62 substrates: 57 unphosphorylated substrates and 5 phosphorylated substrates (as controls)	R8134

All IMAP FP Substrate Finder Kits include:

- (2) 384-well plates
- Each substrate is represented in quadruplicate on each plate
- Sufficient IMAP FP beads and buffers to run both plates including added controls
- IMAP Substrate Mapper (electronic file)
- IMAP FP Substrate Finder Product Insert; contains directions and a sample protocol

Compatible with these Molecular Devices systems



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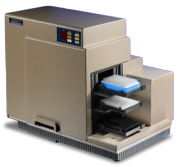
SpectraMax® Paradigm® Multi-Mode Microplate Reader



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