Introduction
Protein kinases are central to the regulation of many cellular processes. In recent years they have emerged as one of the most important classes of drug targets for cancer and many other diseases. Here, the kinase Btk (B lymphoid tyrosine kinase), a member of the Src family of protein tyrosine kinases involved in B cell differentiation and proliferation, was assayed using IMAP®, a non-antibody-dependent FP method. IMAP technology enables rapid, non-radioactive assay of a wide array of kinases and is suited to both assay development and high-throughput screening.

As screening laboratories seek to boost their throughput, the demand for automation of assay setup increases. We explored the feasibility of using an automated liquid handling system, the AquaMax® DW4, to assemble IMAP kinase assays and demonstrate that these results compare favorably to those obtained with hand-pipetted assays. Z’ factors were well above 0.5, and IC50 values for Btk were comparable to published values. Staurosporine curves yielded IC50 values similar to those already published. These results demonstrate that automation of assay setup, including dispensing of various reagents and volumes, facilitates higher throughput without sacrificing accurate results.

Materials and Methods
Btk kinase assays were performed using IMAP FP or TR-FRET Progressive Binding System with Tween, and FAM-Btk/Lyntide substrate. Staurosporine, a known inhibitor of Btk, was assayed at concentrations ranging from 0.5 nM to 3 μM. Calibration curves using known mixtures of phosphorylated and non-phosphorylated FAM-Btk/Lyntide were also generated.

Kinase assay setup
5 μL 4x staurosporine in complete reaction buffer
5 μL 4x Btk kinase in complete reaction buffer
10 μL 2x ATP + 2x FAM-Btk/Lyntide substrate
Calibrators
20 μL 0%-100% phosphorylated FAM-Btk/Lyntide
FP Binding Solution
60% Buffer A
40% Buffer B
1:1200 Binding Reagent
TR-FRET Binding Solution
35% Buffer A
65% Buffer B
1:800 Binding Reagent
1:400 Tb Donor

In DW4-dispensed assays, the staurosporine dilution series were manually pipetted into assay plates, then the kinase and ATP/substrate reagents were dispensed automatically using a DW4 with a 384-well dispense head. For calibration curves, calibrators were manually pipetted into assay wells. Following a one-hour kinase assay incubation, 60 μL IMAP Binding Solution was dispensed per assay well using the DW4. In hand-pipetted assays, all reagents were pipetted using multi-channel manual pipettors.

After a 2-hour (FP) or 16-hour (TR-FRET) incubation with Binding Solution, all plates were read on a SpectraMax® M5 multi-detection microplate reader, and data were analyzed using SoftMax Pro® software.

Results: Kinase inhibition assays

Figure 3. Staurosporine inhibition curves. Btk at 80% maximal activity was assayed for staurosporine concentrations ranging from 0.5 nM to 3 μM. IMAP FP (A) or TR-FRET (B) assays dispensed manually (blue circles) or using DW4 (green squares). In FP assays, IC50 values for staurosporine were 21 nM for manually pipetted and 25 nM for DW4-dispensed. In TR-FRET assays, IC50 values were 42 nM (manual) and 30 nM (DW4). Z’ factors ranged from 0.73-0.81.

Results: Calibration curves

Table 1. Data for DW4 and manually dispensed FP calibration curves. Assay windows are nearly identical, and all CV% values are below 7.

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