Assay Theory

The Adapta universal kinase assay is a homogenous, fluorescent based immunoassay for the detection of ADP. In contrast to ATP depletion assays, the Adapta assay is extremely sensitive to ADP formation such that a majority of the signal change occurs in the first 10-20% conversion of ATP to ADP. This makes the Adapta universal kinase assay ideally suited for use with low activity kinases.

The principle of the Adapta universal kinase assay is outlined below. The assay itself can be divided into two phases: a kinase reaction phase, and an ADP detection phase. In the kinase reaction phase, all components required for the kinase reaction are added to the well, and the reaction is allowed to incubate for 60 minutes. After the reaction, a detection solution consisting of a europium labeled anti-ADP antibody, an Alexa Fluor™ 647 labeled ADP tracer, and EDTA (to stop the kinase reaction) is added to the assay well. ADP formed by the kinase reaction (in the absence of an inhibitor) will displace the Alexa Fluor 647 labeled ADP tracer from the antibody, resulting in a decrease in the TR-FRET signal. In the presence of an inhibitor, the amount of ADP formed by the kinase reaction is reduced, and the resulting intact antibody-tracer interaction results in a high TR-FRET signal.

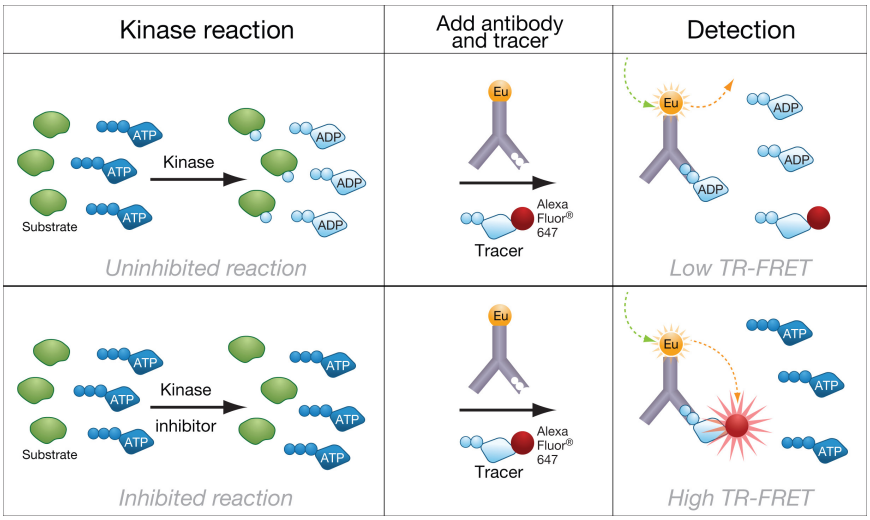

Figure 1. Schematic of the Adapta Universal Kinase assay

ADP formation is determined by calculating the emission ratio from the assay well. The emission ratio is calculated by dividing the intensity of the tracer (acceptor) emission by the intensity of the Eu (donor) emission at 615 nm as shown in the equation below.

$$\textbf{Emission Ratio} = \frac{\text{AlexaFluor647 Emission (665 nm)}}{\text{Europium Emission (615 nm)}}$$

Since the Adapta technology measures ADP formation (i.e. conversion of ATP to ADP) it can be used to measure any type of ATP hydrolysis, including intrinsic ATPase activity of kinases. In this case, the substrate is water, not a lipid or peptide. The SelectScreen service screens CHUK in this way, so a substrate is not included in the kinase reaction. A reference for using intrinsic ATPase activity to screen for kinase inhibitors is provided below.

Kashem, MA *et al.* (2007) *J. Biomol. Screen.* **12(1)**:70-83

Adapta Assay Conditions

Test Compounds
The Test Compounds are screened in 1% DMSO (final) in the well. For 10 point titrations, 3-fold serial dilutions are conducted from the starting concentration of the customer's choosing.

Substrate/Kinase Mixtures
All Substrate/Kinase Mixtures are diluted to a 2X working concentration in the appropriate Kinase Buffer (see section *Kinase Specific Assay Conditions* for a complete description).

ATP Solution
All ATP Solutions are diluted to a 4X working concentration in water.

ATP Km apparent is previously determined using a radiometric assay except when no substrate is available in which case an Adapta assay is conducted.

Detection Mix
The Detection Mix is prepared in TR-FRET Dilution Buffer. The Detection mix consists of EDTA (30 mM), Eu-anti-ADP antibody (6 nM) and ADP tracer. The detection mix contains the EC_{60} concentration of tracer for 5-150 μM ATP.

Assay Protocol
Bar-coded Corning, low volume, white 384-well plate (Corning Cat. #4512)
1. 100 nL – 100X Test Compound in 100% DMSO
2. 2.4 µL – 30 mM HEPES
3. 2.5 µL – 4X ATP Solution
4. 5 µL – 2X Substrate/Kinase Mixture
5. 30-second plate shake
6. 1-minute centrifuge at 1000 x g
7. 60-minute Kinase Reaction incubation at room temperature
8. 5 µL – Detection Mix
9. 30-second plate shake
10. 1-minute centrifuge at 1000 x g
11. 60-minute Detection Mix equilibration at room temperature
12. Read on fluorescence plate reader and analyze the data

Adapta Assay Controls

The following controls are made for each individual kinase and are located on the same plate as the kinase:

0% Conversion Control (100% Inhibition Control)
The maximum Emission Ratio is established by the 0% Conversion Control (100% Inhibition Control), which contains no ATP in the kinase reaction and therefore exhibits no kinase activity. After addition of the Detection Mix containing EDTA, ATP is added to these wells. ATP addition is required for the 0% conversion controls wells because the ADP antibody binds ATP with low affinity. The ATP in wells with maximum kinase inhibition will displace the ADP tracer slightly, though much less efficiently than ADP.

100% Conversion Control
The 100% Conversion Control wells contain ADP instead of ATP and are designed to allow for the calculation of percent ATP conversion.

The 0% Conversion and 100% Conversion Controls allow one to estimate the percent ATP Conversion achieved in a specific reaction well. Control wells do not include any kinase inhibitors.

0% Inhibition Control
The minimum Emission Ratio in a screen is established by the 0% Inhibition Control, which contains active kinase. This control is designed to produce < 40%* ATP conversion in the Kinase Reaction.

*The range of ATP conversion allowed is different for each kinase and set in the linear region.

Known Inhibitor
A known inhibitor control standard curve, 10 point titration, is run for each individual kinase on the same plate as the kinase to ensure the kinase is inhibited within an expected IC_{50} range previously determined.

Adapta Data Analysis

The following equations are used for each set of data points:

We run Adapta assays in the linear range determined for each kinase. Full ATP/ADP standard curves are run during validation to define this range. In addition, ATP/ADP standard curves are used to calculate the percent ATP conversion of each sample.

	Equation
Emission Ratio	$\frac{\text{AF647 Emission (665 nm)}}{\text{Europium Emission (615 nm)}}$
% Conversion	$\left\{ \frac{\text{EC}_{50\ SC}}{\left(\frac{\text{Top}_{SC} - \text{Bottom}_{SC}}{\text{Emission Ratio}_{Sample} - \text{Bottom}_{SC}}\right) - 1 \wedge \left(\frac{1}{\text{Hillslope}_{SC}}\right)} \right\} * 100$
% Inhibition	$\left\{ 1 - \frac{\%\ \text{Conversion}_{Sample}}{\%\ \text{Conversion}_{0\%\ \text{Inhibition Ctrl}}} \right\} * 100$
Difference Between Data Points (single point only)	$\lvert \%\ \text{Inhibition}_{Point\ 1} - \%\ \text{Inhibition}_{Point\ 2} \rvert$
Test Compound Interference	For each emission wavelength, fluorescence interference is flagged for a compound well that is more than 20% outside the range of the controls.
Z´ (using Emission Ratio values)	$1 - \frac{3 * \text{Stdev}_{0\%\ \text{Conv Ctrl}} + 3 * \text{Stdev}_{0\%\ \text{Inhibition}}}{\lvert \text{Mean}_{0\%\text{Conv Ctrl}} - \text{Mean}_{0\%\ \text{Inhibition}} \rvert}$

* SC = Standard Curve

Graphing Software

SelectScreen Kinase Profiling Service uses XL*fit* from IDBS. The ATP/ADP standard curve is fit to model number 205 (sigmoidal dose-response model). The dose response curve is also curve fit to model number 205. If the bottom of the curve does not fit between -20% & 20% inhibition, it is set to 0% inhibition. If the top of the curve does not fit between 70% and 130% inhibition, it is set to 100% inhibition.

Kinase-Specific Assay Conditions

Note about Lipid Substrates

Lipid substrates are prepared by creating lipid vesicles. In some cases, these vesicles include a carrier lipid, such as phosphatidylserine (PS). In the assay conditions section below, "PIP2:PS" refers to large unilamellar vesicles (LUVs) containing five mole percent L-α-Phosphatidylinositol-4,5-bisphosphate and ninety-five percent phosphatidylserine. The concentration listed refers only to the PIP2 substrate, not the PS carrier lipid.

CAMK1 (CaMK1)
The 2X CAMK1 (CaMK1) / ZIPtide mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 4 mM CaCl2, 800 U/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.25 - 1.2 ng CAMK1 (CaMK1) and 200 µM ZIPtide in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 400 U/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

CDK4/cyclin D1
The 2X CDK4/cyclin D1 / Rb Substrate mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 7.5 - 30 ng CDK4/cyclin D1 and 1 µM Rb Substrate in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MnCl2, 0.5 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

CDK4/cyclin D3
The 2X CDK4/cyclin D3 / Rb Substrate mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 25 - 100 ng CDK4/cyclin D3 and 1 µM Rb Substrate in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MnCl2, 0.5 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

CDK6/cyclin D1
The 2X CDK6/cyclin D1 / Rb Substrate mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 3 - 12 ng CDK6/cyclin D1 and 1 µM Rb Substrate in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MnCl2, 0.5 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

CDK7/cyclin H/MNAT1
The 2X CDK7/cyclin H/MNAT1 / CDK7/9tide mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 7.5 - 38.75 ng CDK7/cyclin H/MNAT1 and 200 µM CDK7/9tide in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

CDK9/cyclin T1
The 2X CDK9/cyclin T1 / CDK7/9tide mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5 - 40 ng CDK9/cyclin T1 and 200 µM CDK7/9tide in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

CHUK (IKK alpha)
The 2X CHUK (IKK alpha) is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 50 - 250 ng CHUK (IKK alpha) in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA. No substrate is required, as this assay measures the ability of a compound to inhibit the kinase's intrinsic ATPase activity. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

DAPK1
The 2X DAPK1 / ZIPtide mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 4 mM CaCl2, 800 U/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.45 - 5.4 ng DAPK1 and 200 µM ZIPtide in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 400 U/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

GSG2 (Haspin)
The 2X GSG2 (Haspin) / Histone H3 (1-20) peptide mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.25 - 1 ng GSG2 (Haspin) and 100 µM Histone H3 (1-20) peptide in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

IRAK1
The 2X IRAK1 / Histone H3 (1-20) peptide mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.5 - 30.5 ng IRAK1 and 100 µM Histone H3 (1-20) peptide in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

LRRK2
The 2X LRRK2 / ERM (LRRKtide) mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 7.5 - 70 ng LRRK2 and 200 µM ERM (LRRKtide) in 25 mM Tris / 7.5 mM HEPES pH 8.2, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

LRRK2 FL
The 2X LRRK2 FL / ERM (LRRKtide) mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 7.5 - 40 ng LRRK2 FL and 200 µM ERM (LRRKtide) in 25 mM Tris / 7.5 mM HEPES pH 8.2, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

LRRK2 G2019S
The 2X LRRK2 G2019S / ERM (LRRKtide) mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 6 - 24 ng LRRK2 G2019S and 200 µM ERM (LRRKtide) in 25 mM Tris / 7.5 mM HEPES pH 8.2, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

LRRK2 G2019S FL
The 2X LRRK2 G2019S FL / ERM (LRRKtide) mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 5 - 25 ng LRRK2 G2019S FL and 200 µM ERM (LRRKtide) in 25 mM Tris / 7.5 mM HEPES pH 8.2, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

LRRK2 I2020T
The 2X LRRK2 I2020T / ERM (LRRKtide) mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 4 - 110 ng LRRK2 I2020T and 200 µM ERM (LRRKtide) in 25 mM Tris / 7.5 mM HEPES pH 8.2, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

LRRK2 R1441C
The 2X LRRK2 R1441C / ERM (LRRKtide) mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 12.5 - 60 ng LRRK2 R1441C and 200 µM ERM (LRRKtide) in 25 mM Tris / 7.5 mM HEPES pH 8.2, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

NUAK1 (ARK5)
The 2X NUAK1 (ARK5) / CHKtide mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3 - 30 ng NUAK1 (ARK5) and 200 µM CHKtide in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PI4K2A (PI4K2 alpha)
The 2X PI4K2A (PI4K2 alpha) / PI Lipid Substrate mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 7.5 - 40 ng PI4K2A (PI4K2 alpha) and 100 µM PI Lipid Substrate in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MnCl2, 0.5 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PI4K2B (PI4K2 beta)
The 2X PI4K2B (PI4K2 beta) / PI Lipid Substrate mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 3.75 - 20 ng PI4K2B (PI4K2 beta) and 100 µM PI Lipid Substrate in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MnCl2, 0.5 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PI4KA (PI4K alpha)
The 2X PI4KA (PI4K alpha) / PI Lipid Substrate mixture is prepared in 20 mM Tris, pH 7.5, 0.4% Triton X-100, 5 mM MgCl2, 0.5 mM EGTA. The final 10 µL Kinase Reaction consists of 75 - 300 ng PI4KA (PI4K alpha) and 100 µM PI Lipid Substrate in 7.5 mM HEPES, 10 mM Tris, pH 7.5, 0.2% Triton X-100, 2.5 mM MgCl2, 0.25 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PI4KB (PI4K beta)
The 2X PI4KB (PI4K beta) / PI Lipid Substrate mixture is prepared in 50 mM HEPES pH 7.5, 0.1% CHAPS, 1 mM EGTA, 4 mM MgCl2. The final 10 µL Kinase Reaction consists of 7.5 - 60 ng PI4KB (PI4K beta) and 100 µM PI Lipid Substrate in 32.5 mM HEPES pH 7.5, 0.05% CHAPS, 0.5 mM EGTA, 2 mM MgCl2. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3C2A (PI3K-C2 alpha)
The 2X PIK3C2A (PI3K-C2 alpha) / PI Lipid Substrate mixture is prepared in 100 mM HEPES pH 7.5, 200 mM NaCl, 0.06% CHAPS, 2 mM EGTA, 6 mM MgCl2, 4 mM DTT. The final 10 µL Kinase Reaction consists of 10 - 120 ng PIK3C2A (PI3K-C2 alpha) and 100 µM PI Lipid Substrate in 57.5 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 1 mM EGTA, 3 mM MgCl2, 2 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3C2B (PI3K-C2 beta)
The 2X PIK3C2B (PI3K-C2 beta) / PI Lipid Substrate mixture is prepared in 100 mM HEPES pH 7.5, 200 mM NaCl, 0.06% CHAPS, 2 mM EGTA, 6 mM MgCl2, 4 mM DTT. The final 10 µL Kinase Reaction consists of 50 - 200 ng PIK3C2B (PI3K-C2 beta) and 100 µM PI Lipid Substrate in 57.5 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 1 mM EGTA, 3 mM MgCl2, 2 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3C2G (PI3K-C2 gamma)
The 2X PIK3C2G (PI3K-C2 gamma) / PI Lipid Substrate mixture is prepared in 100 mM HEPES pH 7.5, 200 mM NaCl, 0.06% CHAPS, 2 mM EGTA, 6 mM MgCl2, 4 mM DTT. The final 10 µL Kinase Reaction consists of 1 - 28 ng PIK3C2G (PI3K-C2 gamma) and 100 µM PI Lipid Substrate in 57.5 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 1 mM EGTA, 3 mM MgCl2, 2 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3C3 (hVPS34)
The 2X PIK3C3 (hVPS34) / PI Lipid Substrate mixture is prepared in 100 mM HEPES pH 7.5, 0.2% CHAPS, 10 mM MnCl2, 2 mM EGTA. The final 10 µL Kinase Reaction consists of 6 - 24 ng PIK3C3 (hVPS34) and 100 µM PI Lipid Substrate in 57.5 mM HEPES pH 7.5, 0.1% CHAPS, 5 mM MnCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3CA E542K/PIK3R1 (p110 alpha E542K/p85 alpha)
The 2X PIK3CA E542K/PIK3R1 (p110 alpha E542K/p85 alpha) / PIP2:PS mixture is prepared in 100 mM HEPES pH 7.5, 200 mM NaCl, 0.06% CHAPS, 2 mM EGTA, 6 mM MgCl2, 4 mM DTT. The final 10 µL Kinase Reaction consists of 7.5 - 30 ng PIK3CA E542K/PIK3R1 (p110 alpha E542K/p85 alpha) and 50 µM PIP2:PS in 57.5 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 1 mM EGTA, 3 mM MgCl2, 2 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3CA E545K/PIK3R1 (p110 alpha E545K/p85 alpha)
The 2X PIK3CA E545K/PIK3R1 (p110 alpha E545K/p85 alpha) / PIP2:PS mixture is prepared in 50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 3 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2 - 8 ng PIK3CA E545K/PIK3R1 (p110 alpha E545K/p85 alpha) and 50 µM PIP2:PS in 32.5 mM HEPES pH 7.5, 50 mM NaCl, 0.015% CHAPS, 1.5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3CA/PIK3R1 (p110 alpha/p85 alpha)
The 2X PIK3CA/PIK3R1 (p110 alpha/p85 alpha) / PIP2:PS mixture is prepared in 50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 3 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.25 - 2 ng PIK3CA/PIK3R1 (p110 alpha/p85 alpha) and 50 µM PIP2:PS in 32.5 mM HEPES pH 7.5, 50 mM NaCl, 0.015% CHAPS, 1.5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3CA/PIK3R3 (p110 alpha/p55 gamma)
The 2X PIK3CA/PIK3R3 (p110 alpha/p55 gamma) / PIP2:PS mixture is prepared in 50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 3 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2 - 12 ng PIK3CA/PIK3R3 (p110 alpha/p55 gamma) and 50 µM PIP2:PS in 32.5 mM HEPES pH 7.5, 50 mM NaCl, 0.015% CHAPS, 1.5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3CB/PIK3R1 (p110 beta/p85 alpha)
The 2X PIK3CB/PIK3R1 (p110 beta/p85 alpha) / PIP2:PS mixture is prepared in 50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 3 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 6.25 - 80 ng PIK3CB/PIK3R1 (p110 beta/p85 alpha) and 50 µM PIP2:PS in 32.5 mM HEPES pH 7.5, 50 mM NaCl, 0.015% CHAPS, 1.5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3CB/PIK3R2 (p110 beta/p85 beta)
The 2X PIK3CB/PIK3R2 (p110 beta/p85 beta) / PIP2:PS mixture is prepared in 50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 3 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10 - 60 ng PIK3CB/PIK3R2 (p110 beta/p85 beta) and 50 µM PIP2:PS in 32.5 mM HEPES pH 7.5, 50 mM NaCl, 0.015% CHAPS, 1.5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3CD/PIK3R1 (p110 delta/p85 alpha)
The 2X PIK3CD/PIK3R1 (p110 delta/p85 alpha) / PIP2:PS mixture is prepared in 50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 3 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.3 - 3 ng PIK3CD/PIK3R1 (p110 delta/p85 alpha) and 50 µM PIP2:PS in 32.5 mM HEPES pH 7.5, 50 mM NaCl, 0.015% CHAPS, 1.5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIK3CG (p110 gamma)
The 2X PIK3CG (p110 gamma) / PIP2:PS mixture is prepared in 50 mM HEPES pH 7.5, 1 mM EGTA, 3 mM MgCl2. The final 10 µL Kinase Reaction consists of 7.16 - 56 ng PIK3CG (p110 gamma) and 50 µM PIP2:PS in 32.5 mM HEPES pH 7.5, 0.5 mM EGTA, 1.5 mM MgCl2. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIP4K2A
The 2X PIP4K2A / PI(5)P mixture is prepared in 50 mM HEPES pH 7.5, 0.1% CHAPS, 1 mM EGTA, 4 mM MgCl2. The final 10 µL Kinase Reaction consists of 1.5 - 6 ng PIP4K2A and 50 µM PI(5)P in 32.5 mM HEPES pH 7.5, 0.05% CHAPS, 0.5 mM EGTA, 2 mM MgCl2. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIP5K1A
The 2X PIP5K1A / PI(3;4)P2 mixture is prepared in 100 mM HEPES pH 7.5, 200 mM NaCl, 0.06% CHAPS, 2 mM EGTA, 6 mM MgCl2, 4 mM DTT. The final 10 µL Kinase Reaction consists of 2 - 8 ng PIP5K1A and 50 µM PI(3;4)P2 in 57.5 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 1 mM EGTA, 3 mM MgCl2, 2 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIP5K1B
The 2X PIP5K1B / PI(3;4)P2 mixture is prepared in 100 mM HEPES pH 7.5, 200 mM NaCl, 0.06% CHAPS, 2 mM EGTA, 6 mM MgCl2, 4 mM DTT. The final 10 µL Kinase Reaction consists of 3.75 - 15 ng PIP5K1B and 50 µM PI(3;4)P2 in 57.5 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 1 mM EGTA, 3 mM MgCl2, 2 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

PIP5K1C
The 2X PIP5K1C / PI(3;4)P2 mixture is prepared in 100 mM HEPES pH 7.5, 200 mM NaCl, 0.06% CHAPS, 2 mM EGTA, 6 mM MgCl2, 4 mM DTT. The final 10 µL Kinase Reaction consists of 4 - 16 ng PIP5K1C and 50 µM PI(3;4)P2 in 57.5 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 1 mM EGTA, 3 mM MgCl2, 2 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

SPHK1
The 2X SPHK1 / Sphingosine Lipid Substrate mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.05 - 0.2 ng SPHK1 and 50 µM Sphingosine Lipid Substrate in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

SPHK2
The 2X SPHK2 / Sphingosine Lipid Substrate mixture is prepared in 50 mM HEPES pH 7.5, 1 mM EGTA, 3 mM MgCl2. The final 10 µL Kinase Reaction consists of 50 - 200 ng SPHK2 and 50 µM Sphingosine Lipid Substrate in 32.5 mM HEPES pH 7.5, 0.5 mM EGTA, 1.5 mM MgCl2. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

Table of Kinase ATP Km Bins and Inhibitor Validation

The table below provides specifications and data around each kinase. The representative IC50 value with a known inhibitor for each kinase was determined at the ATP bin nearest to the ATP Km app, unless indicated with an asterisk (*) in which case the IC50 value was determined at 10 µM ATP.

Kinase	Substrate	ATP Km app (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)	
CAMK1 (CaMK1)	ZIPtide	845	N/A	Staurosporine	2.36	*
CDK4/cyclin D1	Rb Substrate	1	N/A	Staurosporine	138	*
CDK4/cyclin D3	Rb Substrate	1	N/A	Staurosporine	133	*
CDK6/cyclin D1	Rb Substrate	1	N/A	Staurosporine	71.8	*
CDK7/cyclin H/MNAT1	CDK7/9tide	153	150	Staurosporine	44.6	
CDK9/cyclin T1	CDK7/9tide	32	25	Staurosporine	3.92	
CHUK (IKK alpha)	None	8.8	10	Staurosporine	48.2	
DAPK1	ZIPtide	5.8	5	Staurosporine	2.84	
GSG2 (Haspin)	Histone H3 (1-20) peptide	32	25	Staurosporine	7.69	
IRAK1	Histone H3 (1-20) peptide	36.5	25	Staurosporine	27.1	
LRRK2	ERM (LRRKtide)	70	75	Staurosporine	1.64	
LRRK2 FL	ERM (LRRKtide)	44	50	Staurosporine	1.79	
LRRK2 G2019S	ERM (LRRKtide)	100	100	Staurosporine	0.721	
LRRK2 G2019S FL	ERM (LRRKtide)	134	150	Staurosporine	0.809	
LRRK2 I2020T	ERM (LRRKtide)	7.5	10	Staurosporine	0.966	
LRRK2 R1441C	ERM (LRRKtide)	60	50	Staurosporine	2.40	
NUAK1 (ARK5)	CHKtide	35	25	Staurosporine	3.02	
PI4K2A (PI4K2 alpha)	PI Lipid Substrate	2	5	Staurosporine	35200	
PI4K2B (PI4K2 beta)	PI Lipid Substrate	4	5	None	---	
PI4KA (PI4K alpha)	PI Lipid Substrate	132	N/A	None	---	*
PI4KB (PI4K beta)	PI Lipid Substrate	6.7	5	PIK-93	8.01	
PIK3C2A (PI3K-C2 alpha)	PI Lipid Substrate	19	25	PI-103	172	
PIK3C2B (PI3K-C2 beta)	PI Lipid Substrate	150	N/A	PI-103	4.71	*
PIK3C2G (PI3K-C2 gamma)	PI Lipid Substrate	128	150	PI-103	19.6	
PIK3C3 (hVPS34)	PI Lipid Substrate	9.3	10	PIK-93	1640	
PIK3CA E542K/PIK3R1 (p110 alpha E542K/p85 alpha)	PIP2:PS	1	N/A	PI-103	3.57	*
PIK3CA E545K/PIK3R1 (p110 alpha E545K/p85 alpha)	PIP2:PS	98	100	PI-103	7.63	
PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	PIP2:PS	25	25	PI-103	5.08	
PIK3CA/PIK3R3 (p110 alpha/p55 gamma)	PIP2:PS	77	75	PI-103	6.61	

PIK3CB/PIK3R1 (p110 beta/p85 alpha)	PIP2:PS	166	150	PI-103	25.9	
PIK3CB/PIK3R2 (p110 beta/p85 beta)	PIP2:PS	171	150	PI-103	28.9	
PIK3CD/PIK3R1 (p110 delta/p85 alpha)	PIP2:PS	63	75	PI-103	7.25	
PIK3CG (p110 gamma)	PIP2:PS	31	25	PI-103	36.6	
PIP4K2A	PI(5)P	1	N/A	Staurosporine	29000	*
PIP5K1A	PI(3;4)P2	1	N/A	PP242	220	*
PIP5K1B	PI(3;4)P2	1	N/A	PP242	235	*
PIP5K1C	PI(3;4)P2	1	N/A	PP242	57.0	*
SPHK1	Sphingosine Lipid Substrate	10.3	10	PF-543	510	
SPHK2	Sphingosine Lipid Substrate	214	N/A	PF-543	27500	*