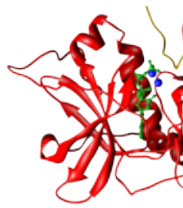
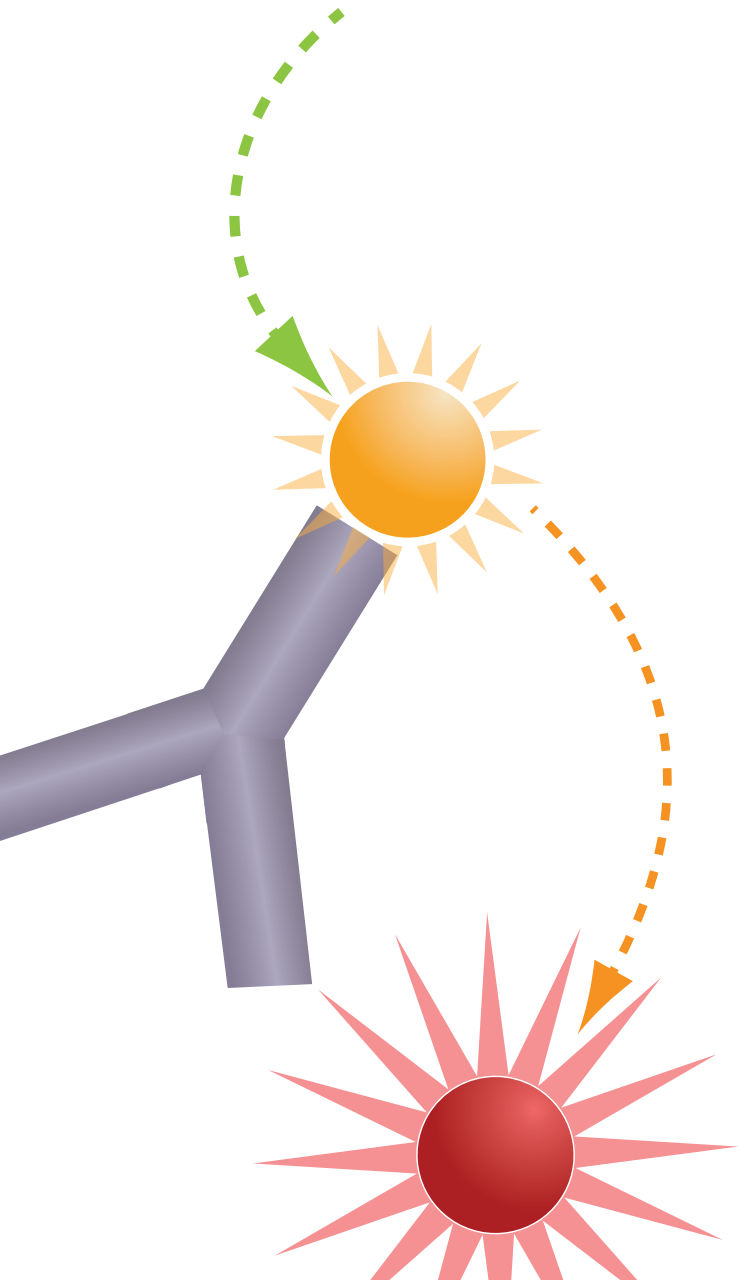
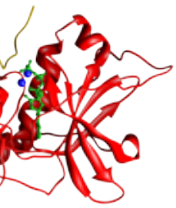


Be sensitive.
Be adaptable.
Be ahead.

Adapta™ Universal Kinase Assay



invitrogen™



Adapta™ Universal Kinase Assay

- → High Z´-factors at low percent conversion of ATP to ADP using less kinase than ATP depletion assays
- → One assay suitable for all kinases, including difficult-to-assay targets such as lipid kinases
- → Best-in-class Alexa Fluor® dyes enable a superior red-shifted TR-FRET assay
- → Performance validation using our portfolio of specialized substrates and optimized assay buffers

A growing collection of Invitrogen kinases have been validated for use with the Adapta™ Universal Kinase Assay Kit and corresponding substrates. To see if your kinase has been validated with the Adapta™ assay, visit www.invitrogen.com/adapta.

Increase assay performance with TR-FRET detection

The Adapta™ Universal Kinase Assay uses time-resolved fluorescence resonance energy transfer (TR-FRET) to monitor ADP formation as the kinase assay progresses. TR-FRET detection technology utilizes a lanthanide chelate rather than a more traditional organic fluorophore to measure interactions between binding partners. As with other TR-FRET systems, the Eu (europium) donor is excited using a 340 nm excitation filter with a 30 nm bandpass. Eu chelates can effectively conduct energy transfer to a range of suitable far-red acceptors, such as Alexa Fluor® 647, that are readily detected in the silent regions between the Eu emission peaks. Following energy transfer, the emission from the Alexa Fluor® 647 can be measured using a filter centered at 665 nm with a 10 nm bandpass. This signal is then referenced (or "ratioed") to the emission from the Eu, which is detected using a 615 nm filter with a 10 nm bandpass (Figure 1).

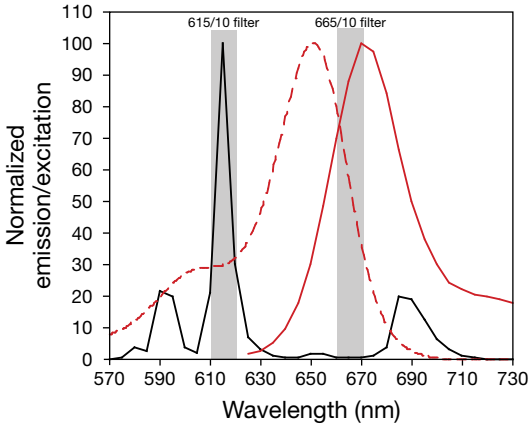


Figure 1—Excitation and emission spectra of the Adapta™ Universal Kinase Assay. The emission spectrum of the Eu donor (in black) overlaps with the excitation spectrum for the Alexa Fluor® 647 dye (dashed red line). Following energy transfer, the emission from the Alexa Fluor® 647 dye (solid red line) is detected with a filter centered at 665 nm. This signal is then referenced (or "ratioed") to the Eu emission signal measured using a filter centered at 615 nm.

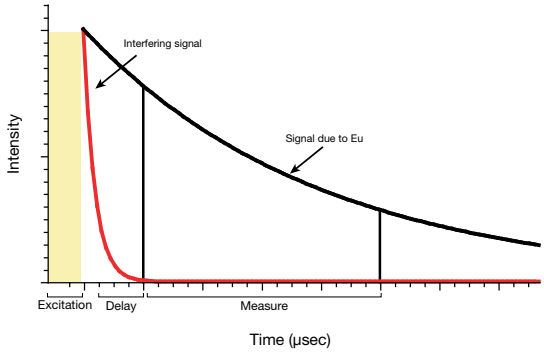


Figure 2—Schematic of measurement setup for detection of TR-FRET in HTS assays. The Adapta™ assay format is based on the use of a long-lifetime Eu chelate as the donor species and Alexa Fluor® 647 as the acceptor species. When Eu- and Alexa Fluor®–labeled molecules are brought into proximity, energy transfer takes place, causing an increase in acceptor fluorescence and a decrease in donor fluorescence. These fluorescent signals can be read in a time-resolved manner to reduce assay interference and increase data quality.

The "emission ratio" is calculated as the 665 nm signal divided by the 615 nm signal.

Since the Eu donor used in the Adapta™ assay has a fluorescence lifetime many orders of magnitude longer than background fluorescence due to scattered light or autofluorescent compounds, energy transfer can be measured after the interfering signal has completely decayed (Figure 2).

The Adapta™ assay can be divided into two phases: a kinase reaction phase and an ADP detection phase (Figure 3). In the kinase reaction phase, all kinase reaction components are added to the well and the reaction is allowed to incubate for a set period of time, typically 60 minutes. After the reaction, a detection solution of Eu-labeled anti-ADP antibody, Alexa Fluor® 647–labeled ADP tracer, and EDTA (to stop the kinase reaction) are added to the assay well. ADP formed by the kinase reaction will displace the Alexa Fluor® 647–labeled ADP tracer from the antibody, resulting in a decrease in 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 maintains a high TR-FRET signal.

Most instruments, settings, and filters that work with other Eu-based TR-FRET assay systems can be used with the Adapta™ Universal Kinase Assay. For assistance in determining if your instrument is suitable to read this assay, or for assistance with instrument setup, please call us in North America at +1 760 603 7200 (select option 3, then enter ext. 40266).

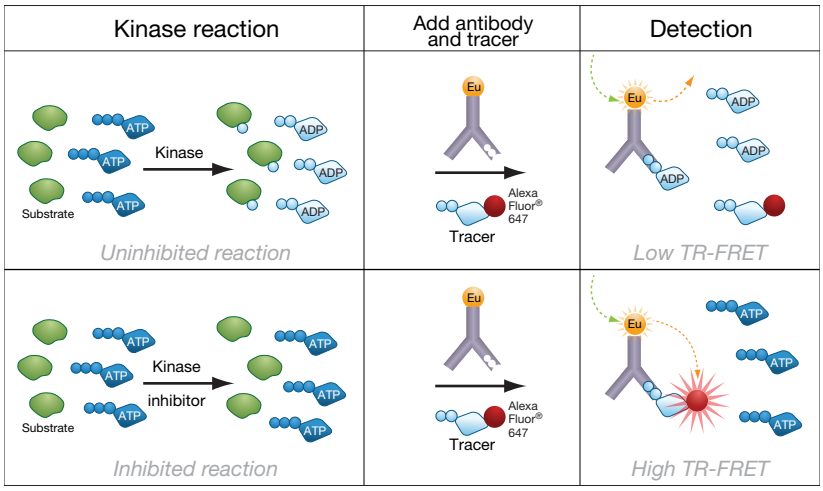


Figure 3—Schematic of the Adapta™ Universal Kinase Assay.

Achieve optimal results with less kinase

The Adapta™ Universal Kinase Assay measures kinase activity by detecting ADP formation. Most of the signal change in the assay occurs in the first 10–20% of conversion of ATP to ADP (Figure 4). This is in sharp contrast to kinase assays that measure ATP depletion, in which 20% conversion of ATP to ADP results in only a 20% signal change. As a result, the Adapta™ Universal Kinase Assay produces high Z′-factors at low percent ATP conversion (Figure 5). Because reduced amounts of kinase are required to achieve an optimal assay window, the assay is ideal for kinases with low activity.

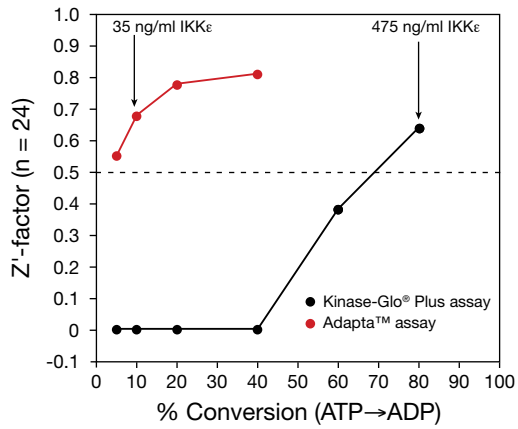


Figure 5—Comparison of Z′-factors for the Adapta™ Universal Kinase Assay and Kinase-Glo® Plus Luminescent Kinase Assay. Due to the sensitivity of the Adapta™ Universal Kinase Assay to small changes in ADP formation, Z′-factors of ≥0.5 are achieved with as little as 5% conversion of ATP to ADP. For the Kinase-Glo® Plus Luminescent Kinase Assay (Promega Corp.), a much higher conversion (~80%) is necessary to achieve similar Z′-factors. This makes the Adapta™ assay ideally suited for use with kinases with low activity, since less kinase has to be used to achieve an optimal assay window.

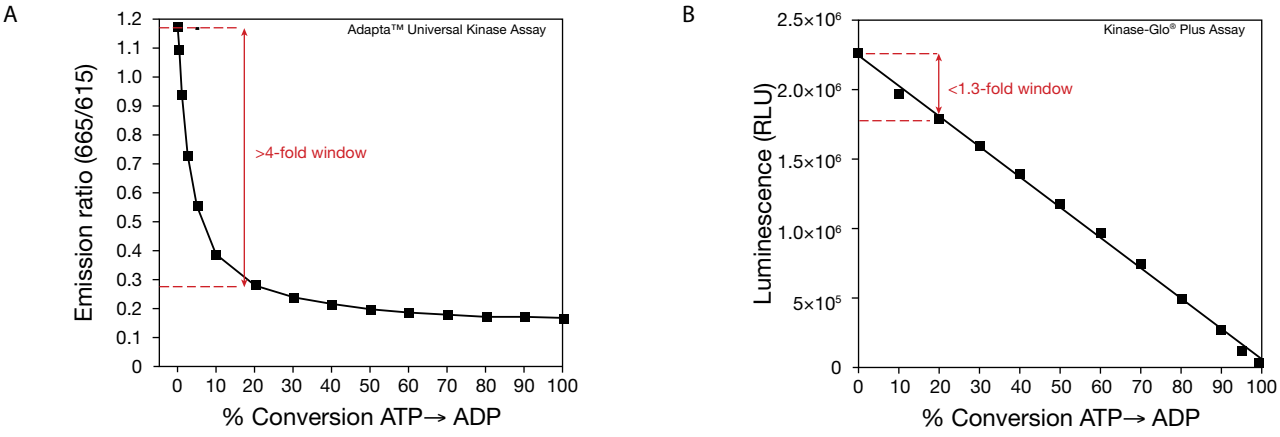
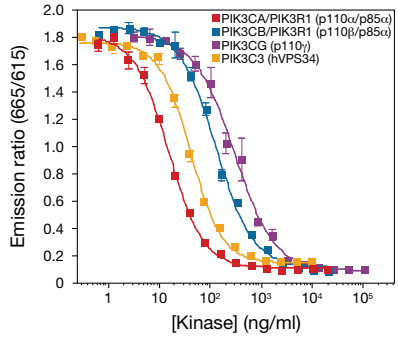


Figure 4—Assay signal as a function of percent conversion of ATP to ADP. **A.** A representative ATP→ADP titration curve highlights the large change in assay signal in the Adapta™ assay at only 20% conversion of ATP to ADP. **B.** In a typical ATP depletion assay (Kinase-Glo® Plus Luminescent Kinase Assay, Promega Corp.), the relative change in the assay window at 20% conversion is considerably smaller.

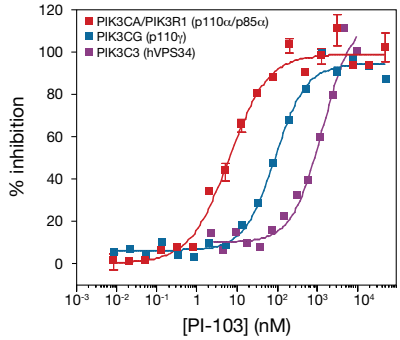
Obtain robust and relevant data

The Adapta™ Universal Kinase Assay has been successfully tested with both lipid kinases (Figure 6) and protein kinases (Figure 7). The experimental results obtained demonstrate a clear correlation with the established pharmacology of the kinases and inhibitors tested.

A



B



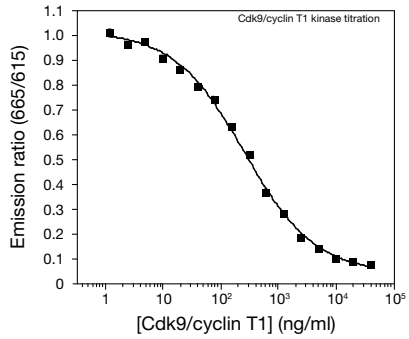
C

Kinase	Inhibitor	IC_{50} (nM) Literature*	Observed
PIK3CA/PIK3R1 (p110α/p85α)	PI-103	8	6.3
PIK3CG (p110γ)	PI-103	150	89.2
PIK3C3 (hVPS34)	PI-103	2,300	1,324

* Knight, Z.A. et al. (2006) *Cell* 125 (4):733-747.

Figure 6—Assaying class I and class III lipid kinases. The Adapta™ Universal Kinase Assay can be used to assay both class I and class III PI3 kinases. The performance of the Adapta™ assay with some common PI3K lipid kinases **(A)** was examined. Inhibitor titrations performed with PI-103 **(B)** display the anticipated pharmacology **(C)**.

A



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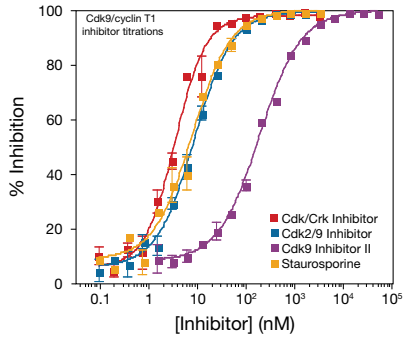

C

Kinase	Inhibitor	IC_{50} (nM) Literature*	Observed
Cdk9/cyclin T1	Cdk/Crk Inhibitor	6–10	3.1
	Cdk2/9 Inhibitor	4	7.7
	Cdk9 Inhibitor II	350	182.1
	Stauro-sporine	NR	5.7

NR = No value reported. * Caligiuri, M. et al. (2005) *Chem Biol* 12:1103-1115; Chao, S. et al. (2000) *J Biol Chem* 275:28345-28348; Wang, S. et al. (2004) *J Med Chem* 47:1662-1675; Krystof, V. et al. (2006) *J Med Chem* 49:6500-6509.

Figure 7—Assaying protein kinases. In addition to lipid kinases, the Adapta™ Universal Kinase Assay can be used to identify inhibitors for difficult or low-activity protein kinases. For example, following a titration of Cdk9 with the Adapta™ assay **(A),** known Cdk inhibitors were evaluted **(B).** The resulting IC_{50} values have a strong correlation to literature values **(C)**.

Substrate and buffer availability for a complete assay solution

In addition to the Adapta™ Universal Kinase Assay Kit, we offer a selection of lipid- and peptide-based substrates for use with the Adapta™ assay. Both the lipid and peptide substrates were designed and optmized with assay performance in mind.

Our lipid substrates were designed for optimal performance in the Adapta™ Universal Kinase Assay with our PI3 kinases.

- → No rehydration, sonication, or extrusion needed—the lipid substrates are ready for immediate use in your kinase assay
- → Formulated for optimal performance with PI3 kinases
- → Get reliable, reproducible performance each and every time, even after multiple freeze-thaw cycles

Since lipid kinases are often highly sensitive to reaction conditions, such as the type and concentration of detergents and salts, we have optimized buffers for each PI3 kinase in our collection.

To view a list of substrates and buffers currently available from Invitrogen for use in the Adapta™ Universal Kinase Assay, visit www.invitrogen.com/adapta.

Ordering information

Visit www.invitrogen.com/adapta to order or learn more about our Adapta™ Universal Kinase Assay and our collection of lipid and peptide substrates.

The Adapta™ assay utilizes Transcreener™ HTS Assay Platform technology (7332278) under license from BellBrook Labs, LLC.

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