The Adapta™ universal kinase assay: A superior alternative to luciferase-based kinase assays

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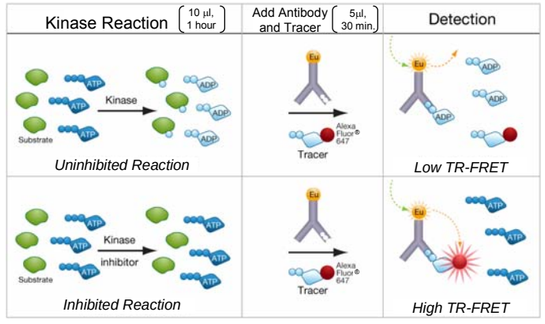
Introduction

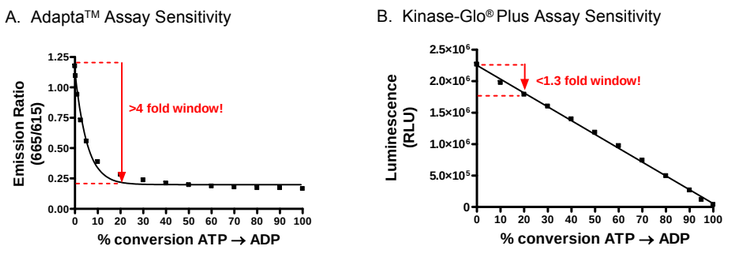
The Adapta™ universal kinase assay* detects ADP formation, the common product of all kinase reactions. The assay is based upon the binding of an Alexa Fluor® 647 labeled ADP analog (tracer) to a europium-labeled anti-ADP antibody. ADP produced by a kinase competes with the ADP-based tracer for binding to the antibody, resulting in a loss of FRET, whereas inhibition of kinase activity results in high FRET. The europium:Alexa Fluor® 647 FRET pair provides a robust signal that is resistant to compound interference, and utilizes common filter sets such as those used for other TR-FRET technologies. In contrast to luciferase-coupled ATP depletion assays, Adapta™ ADP detection assays are sensitive to very low levels of ADP formation, commonly requiring conversion of less than 10% of ATP to ADP to produce a large signal change. Consequently, much less kinase is required relative to ATP-depletion based assays that require consumption of > 50% of the ATP to produce a significant signal change.

Figure 1 – Adapta™ Universal Kinase Assay Schematic



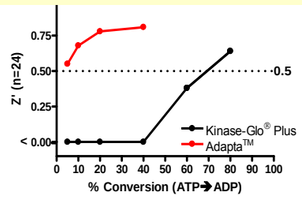
The Adapta™ assay is composed of two steps. First, a standard kinase reaction is performed in the presence or absence of inhibitor. In the second step, formation of ADP is detected by adding a europium-labeled anti-ADP antibody, Alexa Fluor® 647 labeled ADP, and EDTA. ADP formed by uninhibited kinase disrupts antibody-tracer interaction, resulting in a low TR-FRET signal. Inhibited kinase forms less ADP, resulting in an intact antibody-tracer interaction and a high TR-FRET signal.

Figure 2 – Adapta™ Assay Sensitivity Compared to Kinase-Glo® Plus Assay



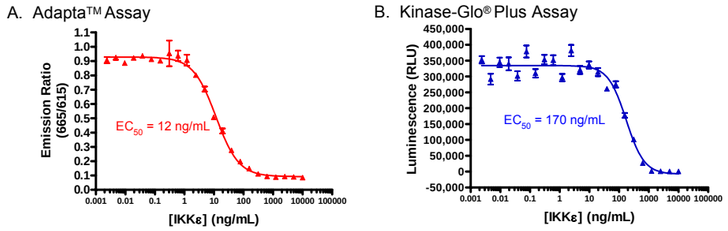
The sensitivity of the Adapta™ ADP detection assay was compared to the Kinase-Glo® Plus luminescent kinase assay from Promega Corporation that measures ATP depletion. In the absence of kinase, varying ratios of ATP and ADP that equaled a total nucleotide concentration of 100 µM were assayed using the standard protocols for each assay technology. Graph A demonstrates that at 20% conversion of 100 µM ATP, the Adapta™ assay has a window of >4-fold, while the Kinase-Glo® Plus assay graph (B) indicates a very small window of less than 1.3 fold at 20% conversion of 100 µM ATP. When running a kinase assay, it is desirable to be at < 20% substrate conversion in order to be within the linear range of the kinase assay.

Figure 3 – Comparison of Z´ Values from Adapta™ and Kinase-Glo® Plus Assays



In an example using IKKε, the Adapta™ universal kinase assay achieves a Z' value of >0.5 at 5% conversion of ATP while the Kinase-Glo® Plus luminescent kinase assay requires nearly 80% ATP conversion to achieve a similar Z' value. The Z' values for each assay technology were determined from 24 replicates at each concentration of an IKKε titration with 10 µM ATP and 200 µM IKKtide. Each assay was developed using the protocol recommended by the manufacturer. The % conversion of ATP to ADP that corresponded to each kinase concentration is plotted versus the calculated Z' value.

Figure 4 – IKKε Titrations Using Adapta™ and Kinase-Glo® Plus Assays

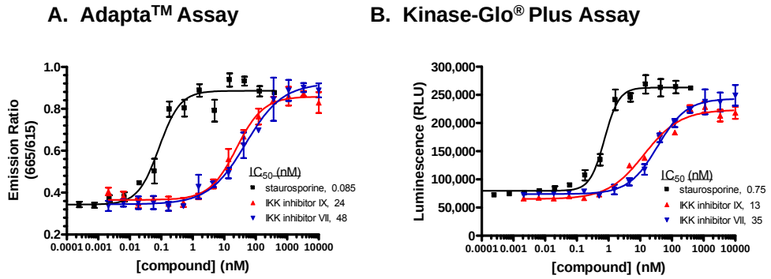


% conversion ATP to ADP	mean Em Ratio	Fold Change	~ ng/mL IKKε
0	0.887	N/A	N/A
5	0.467	1.90	14
10	0.308	2.88	30
20	0.198	4.48	62
40	0.137	6.47	150
60	0.115	7.71	280
80	0.105	8.45	450
100	0.101	8.78	N/A

% conversion ATP to ADP	~ mean RLU	Fold Change	~ ng/mL IKKε
0	334,457	N/A	N/A
5	317,563	1.05	20
10	303,110	1.10	35
20	255,592	1.31	75
40	181,745	1.84	150
60	120,297	2.78	250
80	58,261	5.74	475
100	738	453.19	N/A

IKKε titrations were performed for each assay technology as described in Figure 3. The Adapta™ assay (A) provides a much larger assay window at lower % conversions of ATP to ADP than Kinase-Glo® Plus assay (B). As a result, 10-fold less kinase is required in the Adapta™ assay format relative to that required for the Kinase-Glo® Plus assay format. The corresponding tables that list the fold change for each concentration of kinase and the resulting % conversion of ATP to ADP again highlight the larger assay window for the Adapta™ assay at low % conversion.

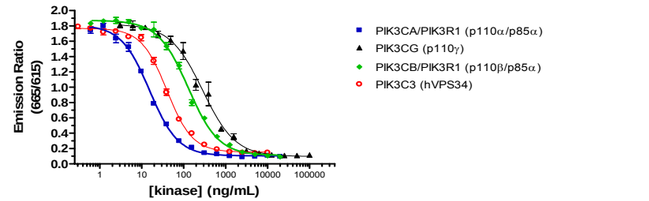
Figure 5 – IC_{50} Values of IKKε Inhibitors Using Adapta™ or Kinase-Glo® Plus Assays



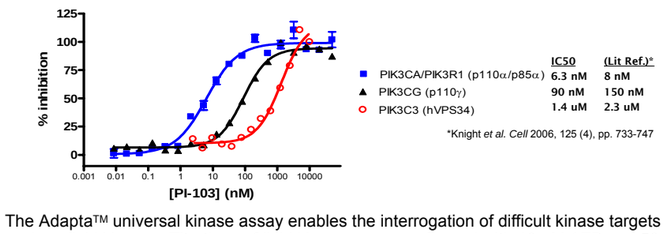
Similar IC_{50} values and rank order potencies were obtained for IKK Inhibitor VII and IX. However, because the Adapta™ assay requires 10 fold less kinase per well, a better indication of the potency of staurosporine is revealed in the Adapta™ assay (85 pM vs 750 pM), which in this case is limited by the amount of kinase present. Kinase assays were performed using a dose response of the inhibitor, the EC_{80} concentration of IKKε, (475 ng/mL for Kinase-Glo® Plus luminescent kinase assay and 40 ng/mL for Adapta™ assay), 10 µM ATP and 200 µM IKKtide.

Figure 6 –Using Adapta™ Assays for Lipid Kinases

A. Lipid Kinase Titrations



B. Lipid Kinase Inhibitors



	IC50	(Lit Ref.)*
PIK3CA/PIK3R1 (p110α/p85α)	6.3 nM	8 nM
PIK3CG (p110γ)	90 nM	150 nM
PIK3C3 (hVPS34)	1.4 uM	2.3 uM

*Knight *et al. Cell* 2006, 125 (4), pp. 733-747

The Adapta™ universal kinase assay enables the interrogation of difficult kinase targets such as lipid kinases. Titrations of lipid kinases were performed with 10 µM ATP and 50 µM PIP2:PS for PIK3CA/PIK3R1, PIK3CG, and PIK3CB/PIK3R1 and 100 µM PI:PS for PIK3C3 . The EC_{80} concentrations for each lipid kinase (40 ng/mL for PIK3CA/PIK3R1, 2000 ng/mL for PIK3Cg, and 150 ng/mL for PIK3C3) were then used to determine the dose response of the inhibitor PI-103 using the same concentration of ATP and lipid substrate. The resulting IC_{50} values were in close agreement with literature values.

Conclusions

- Invitrogen's Adapta™ universal kinase assay format detects the formation of ADP and is much more sensitive than Promega's Kinase-Glo® Plus ATP depletion assay. This increased sensitivity results in Z' values >0.5 at very low substrate conversion; 5% conversion compared to almost 80% for Kinase-Glo® Plus Luminescent Kinase Assay for the IKK ε assay.
- The Adapta™ assay for IKKε requires ten times less kinase than the Kinase-Glo® Plus assay and allows inhibitor dose response data to be obtained at less than 20% substrate conversion while Kinase-Glo® Plus assay requires closer to 80% conversion.
- Because the Adapta™ assay uses 10 times less kinase than Kinase-Glo® Plus Luminescent Kinase assay, tight binding inhibitors can be better resolved from weaker inhibitors.
- The Adapta™ assay format is a suitable assay choice for any enzyme reaction that produces ADP. This makes it an excellent choice for the interrogation of difficult kinase targets such as lipid kinases. We demonstrated this application with examples of kinase titrations and inhibitor IC_{50} determinations for Class I and Class III PI3 kinases.
- Invitrogen offers a wide selection of lipid kinase products, including purified lipid kinases, optimized lipid substrates and the Adapta™ universal kinase assay kit. For more information, visit www.invitrogen.com/adapta.

*Adapta™ assays utilize Transcreener™ HTS Assay Platform technology (patent pending) under license from Bellbrook Labs, LLC.