

KinEASE™ HTRF®: A new kit for rapid kinase assay development



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Introduction

Upstate and Cisbio have utilized their expertise in the fields of kinases and HTRF® technology to design a universal KinEASE™ HTRF® kit for high-throughput screening of many Serine/Threonine kinases. The kit includes one Europium Cryptate labeled antibody and three substrates (STK Substrate 1, 2, and 3). It currently allows 37 different kinases to be monitored (as depicted in Table 1).

Full assay development with the KinEASE™ HTRF® kit is straightforward and can be rapidly completed. An increase in the HTRF® signal is observed as a result of the kinase activity. In the present study, 6 different Ser/Thr kinase assays were developed using an optimized protocol and were validated by determining IC50 of staurosporine, a general kinase inhibitor.

Assay principle

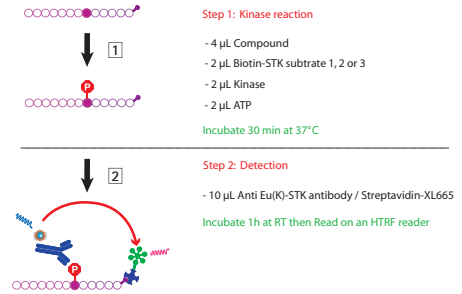
The assay is run in two steps: kinase reaction and detection.

The HTRF signal is proportional to the kinase activity.

The kinase reaction is stopped with the addition of the detection reagents which contain EDTA.

The assay can be run in different plate formats (96 to 1536-well) by simply resizing each reagent volume proportionally.

Protocol in 384-well low volume plate (20 µL):



Upstate Kinase	Recommended substrate
AMPK (rat), CamKII (rat), CamKIV, CHK1, CHK2, RSK1, RSK1(rat), RSK2, RSK3, IKKα, IKKβ, MAPKAPK2, PKCα, β1, βII, γ, δ, ε, eta, iota, μ, θ, zeta, PKD2, PRK2	STK Substrate 1
PKA, PAK2, AuroraA, ROCK-II	STK Substrate 2
MSK1, Pim1, PKBα, β, γ, p70S6K, SGK1	STK Substrate 3

Table 1: List of kinases that can be monitored with KinEASE HTRF®. This list is currently being extended to other kinases.

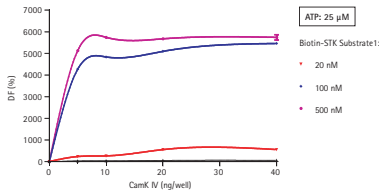
Kinase assay development protocol

The different steps for kinase assay development are depicted for CamK IV using biotin-STK Substrate 1 in a 96 half-well plate format (100 µL final). ROCK II, RSK3, PIM1, PKC beta2 and MAPKAPK2 were optimized following the same protocol. Unknown kinases should be titrated with each STK Substrate (1, 2, and 3) to select the best-suited option.

1- Enzyme titration

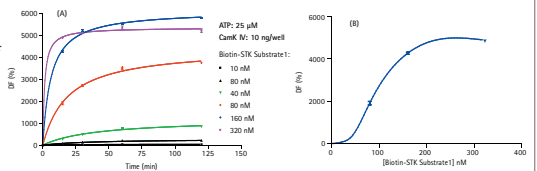
Starting with a fixed and non limiting ATP concentrations (25 µM), the kinase is titrated down from 40 ng/well to 5 ng/well using different substrate concentrations (0.8 nM to 500 nM).

Optimal CamKIV concentration for the assay was determined to be 10 ng/well (7.6 nM kinase reaction step).



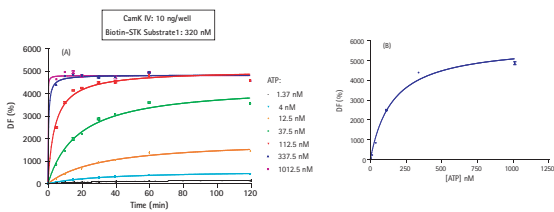
2- Substrate K_M

In order to determine the substrate K_M (app), a time course experiment was run at a fixed concentration of ATP (saturating) and kinase, and substrate concentrations ranging from 10 nM to 320 nM (A). The initial reaction velocity was then plotted versus substrate concentrations (B). Calculated K_M (app) was: 95 nM.



3- ATP K_M

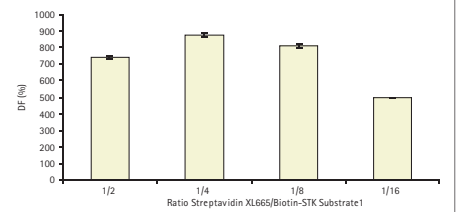
K_M (app) for ATP was measured from a time course experiment run at a fixed concentration of substrate (saturating) and kinase (A), and the replot of ATP concentration against initial reaction velocity (B). Calculated K_M (app) was: 155 nM.



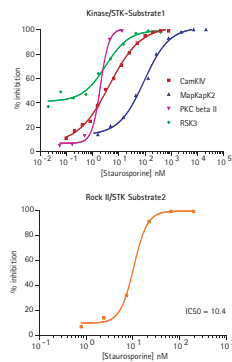
4- Optimal acceptor concentration

Using substrate and ATP at K_M (app) concentration, four different molar ratios of streptavidin-XL665/ Biotin-STK Substrate were tested (1/2, 1/4, 1/8 and 1/16).

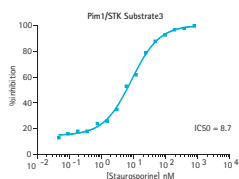
The optimal ratio was: 1/4.



IC50 for staurosporine



To further validate the assays, inhibition studies were conducted with staurosporine using optimal assay parameters for CamK IV, ROCK II, RSK3, PIM1, PKC beta2 and MAPKAPK2 (see section above). As described in Table 2, IC50s obtained with KinEASE HTRF® are similar to the radioactive assay.



	KinEASE™ HTRF® kit		[γ- ³² P] ATP	
	Kinase (nM)	STK Substrate (nM)	IC50 (nM)	IC50 (nM)
CamK IV	7.6	95	4.3	x
MAPKAPK2	5.7	8.3	92	54
PKCβ2	5.1	16.4	6	4.8
RSK3	4.6	120	3.1	0.21
RockII	6.5	156	10.4	0.5
Pim1	6.45	161	8.7	5.5

Table 2: Staurosporine IC50.

Conclusion

- The universal KinEASE™ HTRF® kit is designed to simplify Ser/Thr kinase profiling and HTS.
- 37 different kinase activities can be monitored using this single kit.
- The kit allows straightforward assay development using one antibody and 3 universal substrates.
- The common assay format facilitates kinase profiling.
- The high sensitivity of the HTRF technology allows the assay to be performed with low consumption of kinase and substrate.
- The robustness and the miniaturization capabilities of this technology are particularly well suited to HTS.



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Document reference poster Kinase 2005 v1.0

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