CAT.&Size: A1080006S (1,000 tests)
A1080006L (20,000 tests)
A1080006B (100,000 tests)
Storage at: 2-8℃

VKEYBIO-03-2025

For Research Use Only
Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET
CDKs Kinase Kit-Rb(S780) substrate
Technical Manual

1. Introduction

KeyTec® TR-FRET CDKs kinase kit is designed for measuring the cyclin-dependent kinases (CDKs) activity. The protein kinase family, which comprises multiple members such as CDK1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, 17 and 18, is known to phosphorylate serine 780 (S780) of the retinoblastoma-associated protein (Rb). The assay kit provides a CDKs-specific substrate labeled with KeyTec® TR-FRET LA*[1] , a phospho-specific antibody labeled with KeyTec® TR-FRET Solar Eu*[2] and detection reagent. These components enable the quantification of kinase activity by detecting the phosphorylated substrate.

This assay is based on a sandwich immunoassay method using KeyTec® TR-FRET technology, offering a simple, rapid, highly specific and sensitive, as well as reproducible detection process. The principle, outlined in Figure 1, involves two main phases:

Kinase Reaction: The specific substrate is phosphorylated by the target kinase in the optimized reaction buffer with ATP.

TR-FRET Detection: Adding an antibody containing EDTA (to terminate the kinase reaction) that specifically binds to the phosphorylation site. This binding brings the TR-FRET donor and acceptor into close proximity, enabling resonance energy transfer (RET) upon excitation and generating a TR-FRET signal. The intensity of TR-FRET signal is directly proportional to the level of phosphorylated substrate.

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*[1] KeyTec® TR-FRET LA: TR-FRET Acceptor

*[2] KeyTec® TR-FRET Solar Eu: TR-FRET Donor

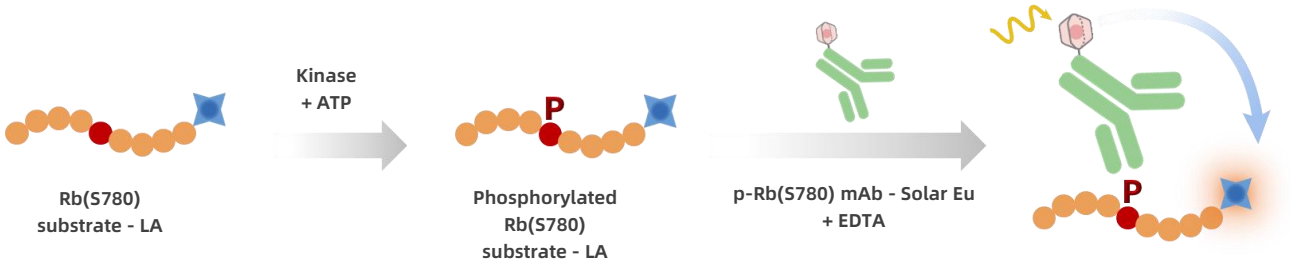

Figure1. The principle of KeyTec® TR-FRET kinase activity detection

2. Components

Components	Storage	A1080006S (1,000 tests[*3])	A1080006L (20,000 tests[*3])	A1080006B (100,000 tests[*3])
Rb(S780) substrate - LA Lyophilized	2-8 ℃	1 vial 1 nmole/vial A1080012S	1 vial 10 nmoles/vial A1080012L	2 vials 20 nmoles/vial A1080012B
p-Rb(S780) mAb - Solar Eu Lyophilized	2-8 ℃	1 vial 1,000 tests/vial	1 vial 20,000 tests/vial	5 vials 20,000 tests/vial
TCEP (1 M)	2-8 ℃	1 vial 120 μL/vial A1010023S	1 vial 600 μL/vial A1010023L	1 vial 3 mL/vial A1010023B
$MgCl_2$ (1 M)	2-8 ℃	1 vial 1.5 mL/vial A1010024S	1 vial 6.0 mL/vial A1010024L	1 bottle 30 mL/bottle A1010024B
Kinase Enzymatic buffer (5X)	2-8 ℃	1 bottle 10 mL/bottle A1010018S	1 bottle 50 mL/bottle A1010018L	1 bottle 250 mL/bottle A1010018B
Kinase Detection buffer (with EDTA)	2-8 ℃	1 bottle 20 mL/bottle A1010019S	1 bottle 250 mL/bottle A1010019L	2 bottles 550 mL/bottle A1010019B

*[3] Tests refer to the number of assay wells that can be performed in 96-well or384-well plates with 20 μL in total reaction volume. The reagents of the kit are suggested to use as recommended.

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3. Storage

- Store all reagents according to the recommended conditions. The products are stable for one year from the date of receipt.
- After thawing, aliquot the stock into single-use volumes (recommended minimum: 10 μL) to avoid repeated freeze-thaw cycles. Store these aliquots at -60 °C and below.

4. Required Components (Not Supplied)

Material	Brand	Catalog
ATP	Bidepharmatech	BD112724
Microplate	VKEY-BIO	M2000102N
Top sealing film	VKEY-BIO	M1000102N
Microplate Reader with TR-FRET module	TECAN	Infinite® 200 PRO

5. Procedure

5.1 Reaction system

Phase	Component	Volume[*4] (20 μL)
Kinase reaction (10 μL)	Test samples or positive control	2 μL[*5]
	Kinase	4 μL[*5]
	Rb(S780) substrate - LA	4 μL (Pre-mix)[*5]
	ATP	
TR-FRET detection (10 μL)	p-Rb(S780) mAb - Solar Eu	10 μL

*[4] Recommended Format: Shallow-well 384-well microplate; For 96-well or 1536-well microplates, proportionally scale the reaction system.

*[5] The proportions of test sample, kinase, substrate and ATP may be adjusted, as long as the concentrations stay the same.

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5.2 Reagent preparation

- Before use, equilibrate all reagents to RT.
- **For lyophilized powder，centrifuge to collect the powder to the bottom（850×g, 1-2minutes）.** It is normal for the fragmentation of lyophilized powder.
- Use the provided/recommended buffers to prepare sample and detection reagents.
- Prepare sample and detection reagent according to the kit technical manual.
- Prepare all reagents immediately before use, unless otherwise specified in the "Working Solution Preparation" section.
- Before use, equilibrate TCEP stock solution to RT and vortex it thoroughly to obtain a homogeneous solution（sonicate if necessary). Notes: Crystallization may occur at low temperature.
- To avoid the degradation of active components，keep 1X Kinase Enzymatic buffer (containing supplemental components) on ice.
- To minimize the aggregation or non-specific binding，It is recommended to add surfactants in Kinase Enzymatic buffer (5X). Notes: Kinase Enzymatic buffer (5X) contained 0.05% BSA.
- Add the KeyTec® TR-FRET detection reagents p-Rb(S780) mAb - Solar Eu and Rb(S780) substrate - LA at the optimized working concentration.
- Avoid vigorous mixing of all reagents.

6. Working Solution Preparation

6.1 Enzyme reaction buffer

- Prepare 1x enzyme reaction buffer: dilute 1 volume of 5X Kinase Enzymatic buffer and kinase supplements required (e.g., $MgCl_2$, TCEP, etc.) with 4 volume of ultrapure water.

6.2 Test sample or positive control preparation

- Dilute the test samples to working concentration (5X). For example, if the final test sample concentration is 10 nM in 10 µL of kinase reaction system，dilute test samples to working concentration (50 nM) using 1X enzyme reaction buffer and add 2 µL/well of test samples. The percentage of DMSO must not exceed 2% for serial dilution.

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6.3 Substrate and ATP pre-mix solution

- **ATP stock solution**: Prepare 100 mM ATP stock solution with ultrapure water, aliquot it into single-use tubes to avoid repeated freeze-thaw cycles. These aliquots can be stored at -80℃ for up to 3 months.

- **Substrate stock solution**: Before reconstitution, centrifuge Rb(S780) substrate - LA to pellet the powder to the bottom (850×g,1-2 minutes). Add the recommended volume of ultrapure water to the powder and mix gently as below.

Size	Substrate Powder	Buffer	Volume	Stock Concentration
1,000 tests	1 nmole/vial	Ultra-pure water	100 µL/vial	10 µM
20,000 tests	10 nmoles/vial	Ultra-pure water	1 mL/vial	10 µM
100,000 tests	20 nmoles/vial	Ultra-pure water	2 mL/vial	10 µM

- **Substrate and ATP pre-mix solution(2.5X)**: Prepare 1 mL substrate and ATP pre-mix Solution(2.5X), dilute 7.5 µL of Rb(S780) substrate - LA stock solution and 2.5 µL of ATP stock solution with 990 µL of 1x enzyme reaction buffer.

Component	Final Conc. µM	Working Conc. µM	Stock Conc. µM	Dilution	Stock Vol. µL	1X Enzyme Reaction Buffer, µL	Total Vol. µL
Rb(S780) substrate - LA	0.03	0.075	10	133.3	7.5	990	1,000
ATP	100	250	100,000	400	2.5		

6.4 Kinase solution

- Dilute the kinase to working concentration (2.5X). For example, if the final kinase concentration is 1 ng/µL in 10 µL of kinase reaction system, dilute 100 ng/µL to working concentration (2.5 ng/µL) using 1X enzyme reaction buffer and add 4 µL/well of kinase solution. **Prepare it immediately prior to addition.**

6.5 Solar Eu - labeled donor

- **p-Rb(S780) mAb - Solar Eu stock solution**: Before reconstitution, centrifuge

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p-Rb(S780) mAb - Solar Eu to pellet the powder to the bottom (850 × g,1-2 minutes). Add the recommended volume of Kinase detection buffer to the powder and mix gently as below.

Size	p-Rb(S780) mAb - Solar Eu Powder	Buffer	Volume	Stock Concentration
1,000 tests	1,000 tests/vial	Kinase Detection buffer	1 mL/vial	10X
20,000 tests	20,000 tests/vial	Kinase Detection buffer	2 mL/vial	100X
100,000 tests	20,000 tests/vial	Kinase Detection buffer	2 mL/vial	100X

- **Working solution(1X)**: Dilute 10X p-Rb(S780) mAb - Solar Eu stock solution to working concentration(1X) for 1,000 tests specification (A1080006S). For example, prepare 1 mL working solution(1X), dilute 100 µL of p-Rb(S780) mAb - Solar Eu stock solution with 900 µL of kinase detection buffer. Working solution(1X) can be stored at 2-8℃ for 24 hours or -60℃ for 3 months .

7. Procedure

- Follow the steps in the table below.

		Kinase Group	Negative Control
Kinase reaction	Step 1	2 µL Test sample (5X) 4 µL Kinase (2.5X) 4 µL Rb(S780) substrate - LA and ATP pre-mix solution (2.5X)	2 µL Test sample (5X) 4 µL Enzymatic buffer 4 µL Rb(S780) substrate - LA and ATP pre-mix solution (2.5X)
	Step 2	Seal the microplate to prevent liquid evaporation, Incubation (RT, 25 ℃) for 60 minutes	
TR-FRET detection	Step 3	Remove the top sealing film, add 10 µL p-Rb(S780) mAb - Solar Eu(1X)	
	Step 4	Seal the microplate to prevent liquid evaporation, Incubation (RT, 25 ℃) for 60 minutes or over-night	
	Step 5	Record the data in microplate reader with top sealing film	

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8. Data Analysis

- Calculate the 665 nm/620 nm Ratio (TR-FRET Ratio) and the percentage coefficient of variation (CV %) for each well.

$$\text{TR-FRET Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10{,}000$$

9. Summary

9.1 Recommended reaction conditions for kinase (Table 1)

Kinase	Cat.# (10 μg)	UniProt	Enzyme (ng/well)	Incubation time (min)	ATP (μM)	Rb(S780) - LA (nM)	Sta. IC_{50} (nM, ON)	S/B (ON)	Buffer additives
CDK1/CycA2	P1HI0331S	P06493/P20248	0.02	60	50	30	11.25	46.04	5 mM $MgCl_2$, 0.5 mM TCEP
CDK1/CycB1	P1HI0332S	P06493/P14635	0.5	60	50	30	24.70	73.33	5 mM $MgCl_2$, 0.5 mM TCEP
CDK1/CycE1	P1HI0333S	P06493/P24864	0.1	60	50	30	54.15	54.73	5 mM $MgCl_2$, 0.5 mM TCEP
CDK1/CycE2	P1HI0347S	P06493/O96020	1.0	60	50	30	24.45	52.06	5 mM $MgCl_2$, 0.5 mM TCEP
CDK3/CycE1	P1HI0336S	Q00526/P24864	1.0	60	50	30	5.02	27.26	5 mM $MgCl_2$, 0.5 mM TCEP
CDK3/CycE2	P1HI0306S	Q00526/O96020	1.0	60	50	30	5.59	30.73	5 mM $MgCl_2$, 0.5 mM TCEP
CDK4/CycD1	P1HI0337S	P11802/P24385	25	60	300	30	331.8	14.88	5 mM $MgCl_2$, 1.0 mM TCEP
CDK4/CycD2	P1HI0352S	P11802/P30279	1	60	100	30	25.40	18.95	5 mM $MgCl_2$, 1.0 mM TCEP
CDK4/CycD3	P1HI0109S	P11802/P30281	1	60	100	30	42.10	68.22	5 mM $MgCl_2$, 1.0 mM TCEP
CDK6/CycD1	P1HI0354S	Q00534/P24385	5	60	50	30	33.93	46.20	5 mM $MgCl_2$, 1.0 mM TCEP
CDK6/CycD2	P1HI0308S	Q00534/P30279	0.5	60	100	30	18.10	51.49	5 mM $MgCl_2$, 1.0 mM TCEP
CDK6/CycD3	P1HI0355S	Q00534/P30281	0.25	60	50	30	32.92	15.33	5 mM $MgCl_2$, 1.0 mM TCEP
CDK7/CycH/MAT1	P1HI0340S	P50613/P51946/P51948	20	60	100	30	31.27	51.28	5 mM $MgCl_2$, 0.5 mM TCEP
CDK9/CycK	P1HI0342S	P50750/O75909	2.0	60	100	30	6.30	17.07	5 mM $MgCl_2$, 1.0 mM TCEP
CDK9/CycT1	P1HI0343S	P50750/O60563	1.5	60	100	30	15.37	21.55	5 mM $MgCl_2$, 1.0 mM TCEP
CDK9/CycT2	P1HI0358S	P50750/O60583	5.0	60	100	30	11.91	12.73	5 mM $MgCl_2$, 1.0 mM TCEP
CDK10/CycM	P1HI0309S	Q15131/08N1B3	10	60	300	30	21.47	36.62	5 mM $MgCl_2$, 1.0 mM TCEP
CDK15/CycY	P1HI0357S	Q96Q40/Q8ND76	10	60	25	30	44.24	36.24	5 mM $MgCl_2$, 0.5 mM TCEP
CDK16/CycY	P1HI0348S	Q00536/Q8ND76	0.5	60	50	30	48.34	54.75	5 mM $MgCl_2$, 0.5 mM TCEP
CDK17/CycY	P1HI0356S	Q00537/Q8ND76	0.5	60	25	30	33.96	48.62	5 mM $MgCl_2$, 0.5 mM TCEP
CDK17/p35	P1HI0250S	Q00537/Q15078	10	60	25	30	22.40	33.14	5 mM $MgCl_2$, 1.0 mM TCEP
CDK18/CycY	P1HI0349S	Q07002/Q8ND76	0.5	60	100	30	161.1	65.41	5 mM $MgCl_2$, 0.5 mM TCEP

Abbreviation: ON stands for overnight.

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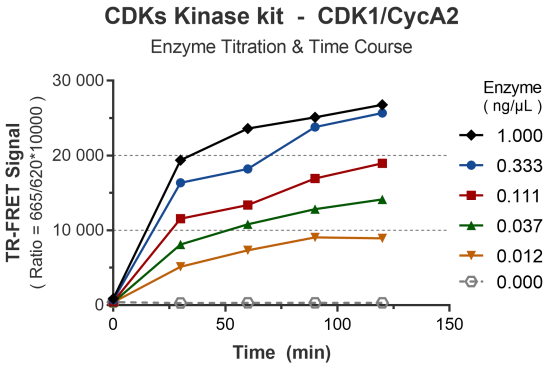
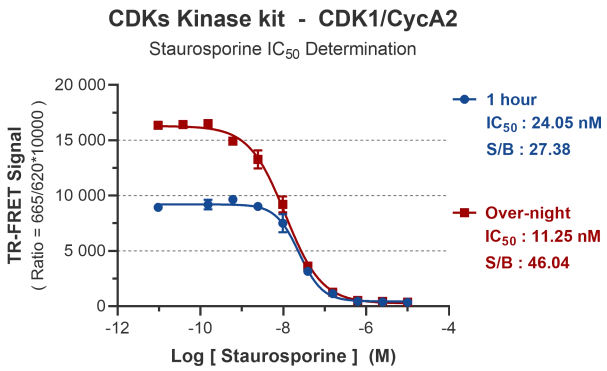
9.2 Results

(1) CDK1/CycA2

P1HI0331S [10 μg] , P1HI0331L [100 μg]

UniProt	P06493/P20248
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.002 ng/μL [final concentration in 10 μL buffer ; 0.02 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 3.46 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP

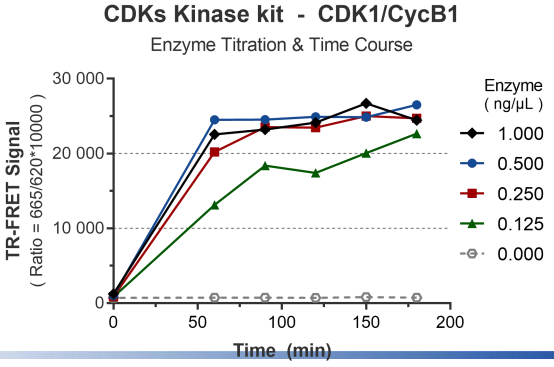


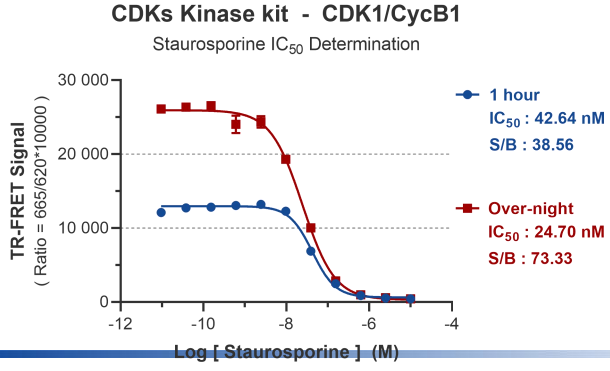


(2) CDK1/CycB1

P1HI0332S [10 μg] , P1HI0332L [100 μg]

UniProt	P06493/P14635
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.05 ng/μL [final concentration in 10 μL buffer ; 0.5 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 17.25 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP





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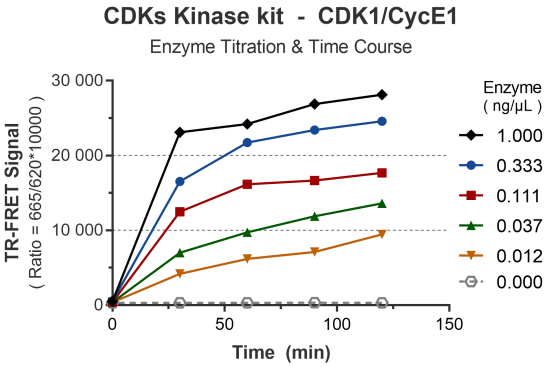
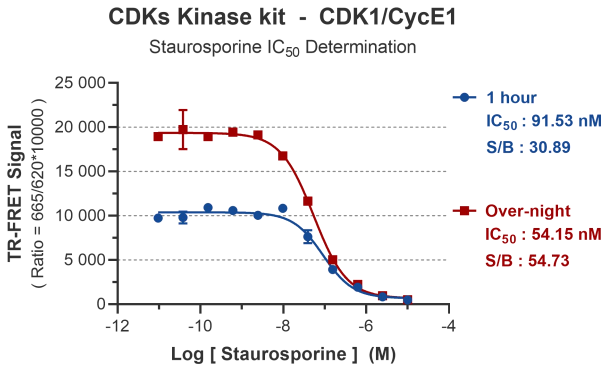
(3) CDK1/CycE1

P1HI0333S [10 μg] , P1HI0333L [100 μg]

UniProt	P06493/P24864
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.01 ng/μL [final concentration in 10 μL buffer ; 0.1 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 20.19 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP

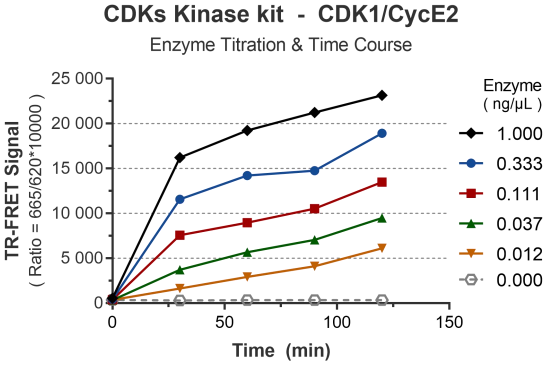


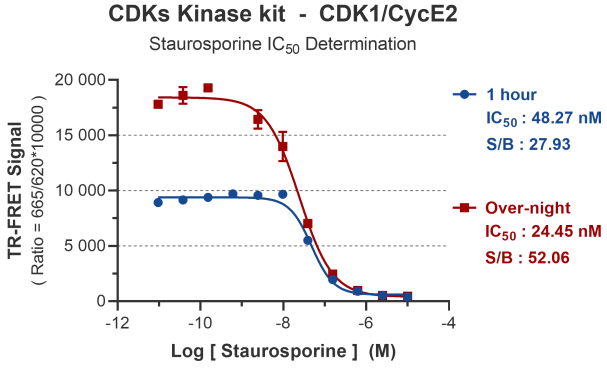


(4) CDK1/CycE2

P1HI0347S [10 μg] , P1HI0347L [100 μg]

UniProt	P06493/O96020
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.1 ng/μL [final concentration in 10 μL buffer ; 1.0 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 3.35 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP





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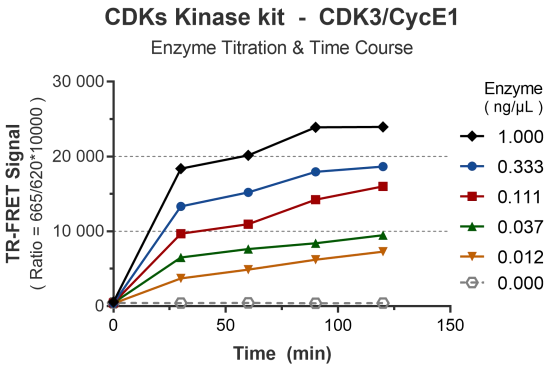
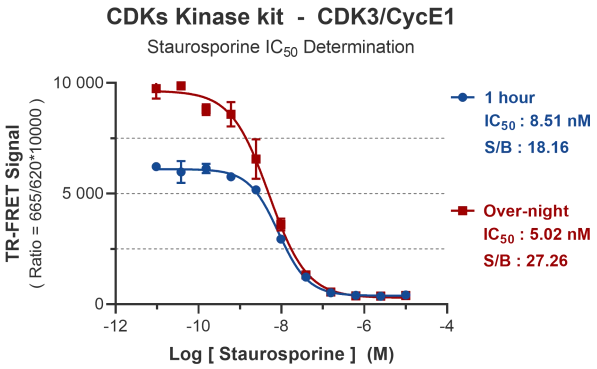
(5) CDK3/CycE1

P1HI0336S [10 μg] , P1HI0336L [100 μg]

UniProt	Q00526/P24864
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.1 ng/μL [final concentration in 10 μL buffer ; 1.0 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 35.91 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP

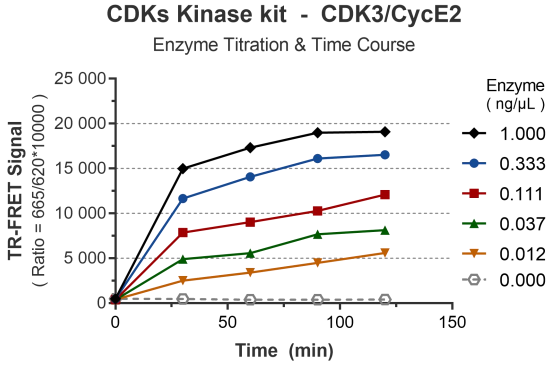


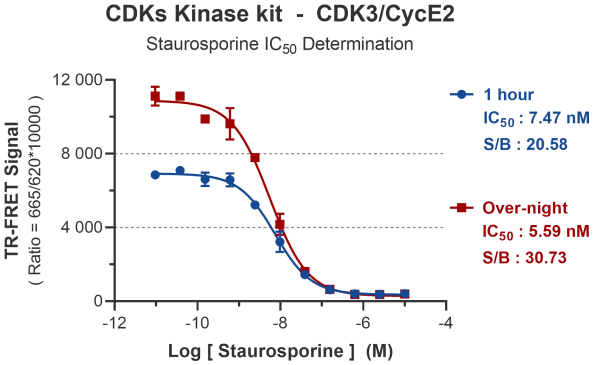


(6) CDK3/CycE2

P1HI0306S [10 μg] , P1HI0306L [100 μg]

UniProt	Q00526/O96020
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.1 ng/μL [final concentration in 10 μL buffer ; 1.0 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 6.85 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP





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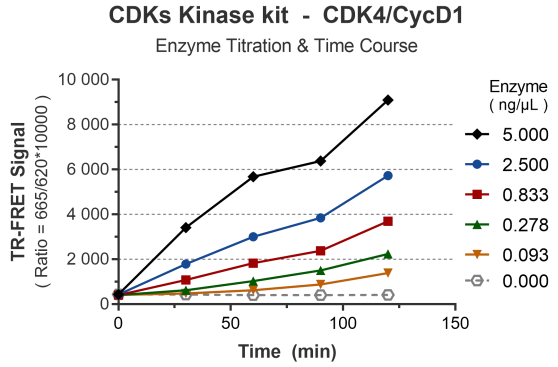
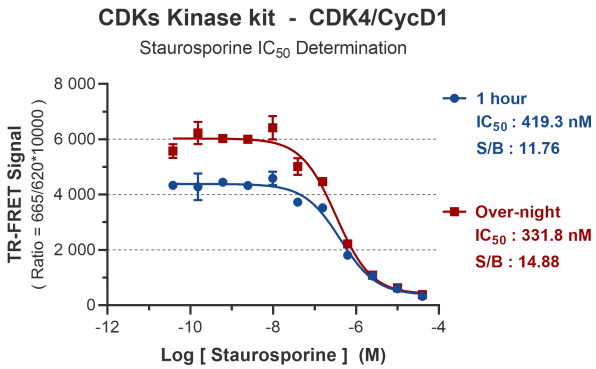
(7) CDK4/CycD1

P1HI0337S [10 μg] , P1HI0337L [100 μg]

UniProt	P11802/P24385
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	2.5 ng/μL [final concentration in 10 μL buffer ; 25 ng/well]
Incubation time at RT	60 min
ATP concentration	300 μM [ATP Km of over-night: 180.7 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP

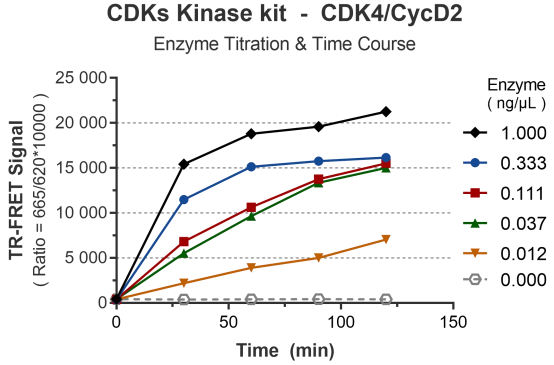


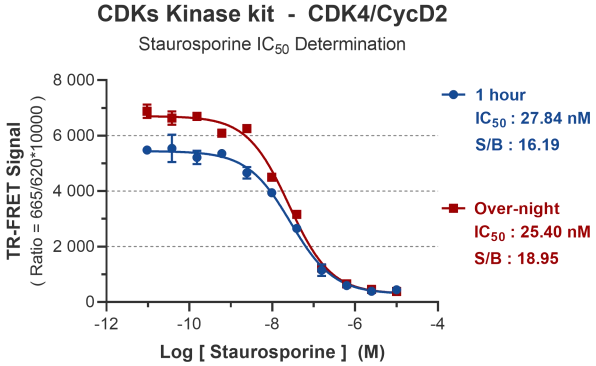


(8) CDK4/CycD2

P1HI0352S [10 μg] , P1HI0352L [100 μg]

UniProt	P11802/P30279
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.1 ng/μL [final concentration in 10 μL buffer ; 1.0 ng/well]
Incubation time at RT	60 min
ATP concentration	100 μM [ATP Km of over-night: 59.77 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP





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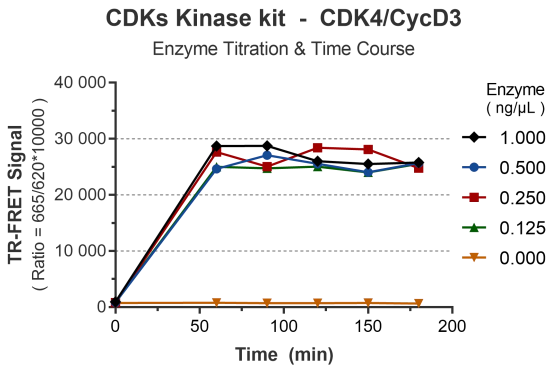
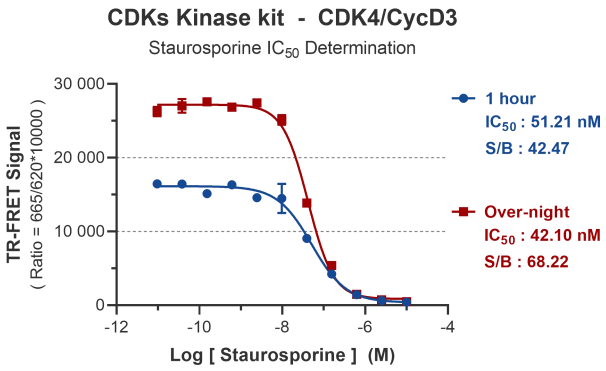
(9) CDK4/CycD3

P1HI0109S [10 μg] , P1HI0109L [100 μg]

UniProt	P11802/P30281
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.1 ng/μL [final concentration in 10 μL buffer ; 1.0 ng/well]
Incubation time at RT	60 min
ATP concentration	100 μM [ATP Km of over-night: 124.9 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP

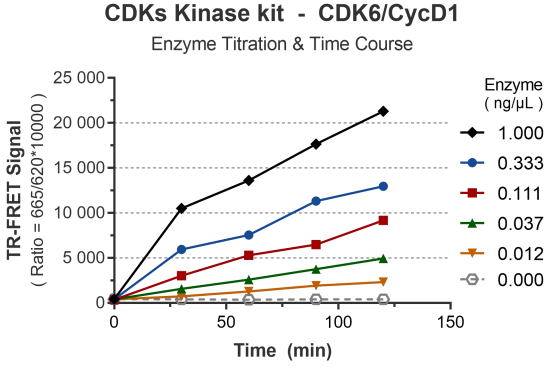


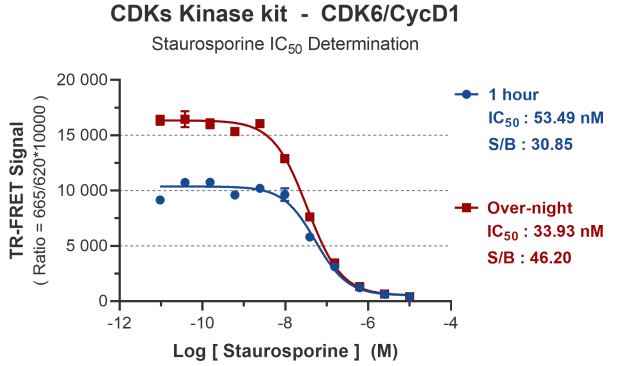


(10) CDK6/CycD1

P1HI0354S [10 μg] , P1HI0354L [100 μg]

UniProt	Q00534/P24385
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.5 ng/μL [final concentration in 10 μL buffer ; 5.0 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 88.65 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP





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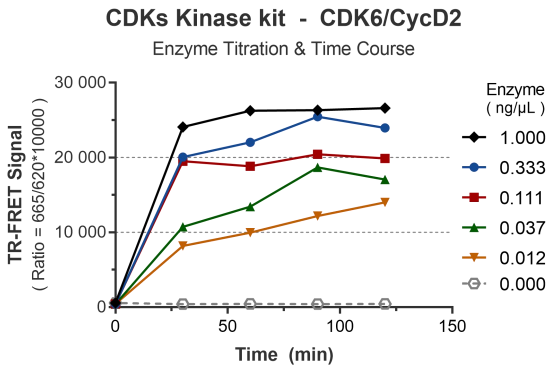
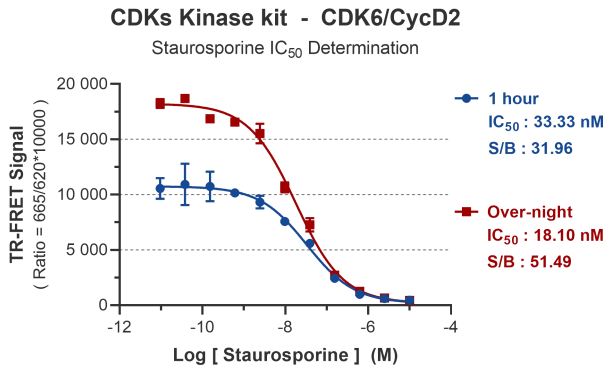
(11) CDK6/CycD2

P1HI0308S [10 μg] , P1HI0308L [100 μg]

UniProt	Q00534/P30279
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.05 ng/μL [final concentration in 10 μL buffer ; 0.5 ng/well]
Incubation time at RT	60 min
ATP concentration	100 μM [ATP Km of over-night: 10.24 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP

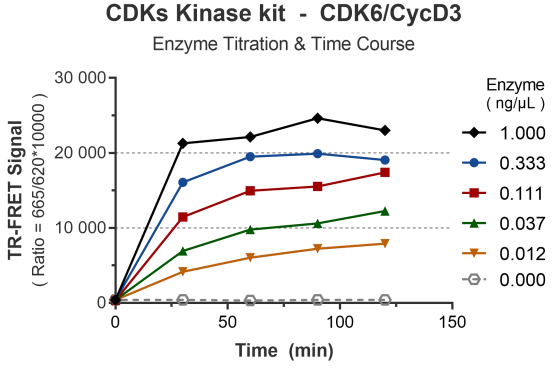


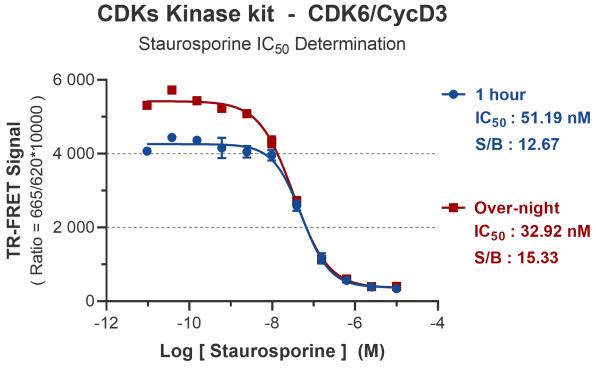


(12) CDK6/CycD3

P1HI0355S [10 μg] , P1HI0355L [100 μg]

UniProt	Q00534/P30281
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.025 ng/μL [final concentration in 10 μL buffer ; 0.25 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 63.45 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP





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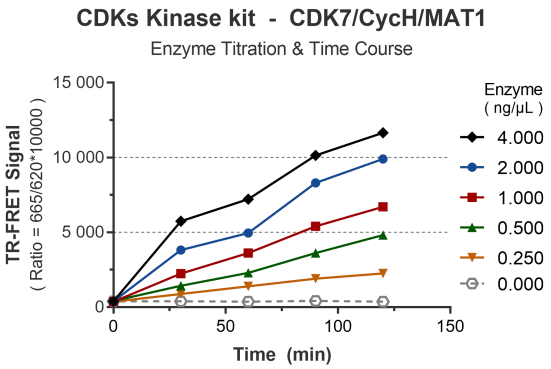
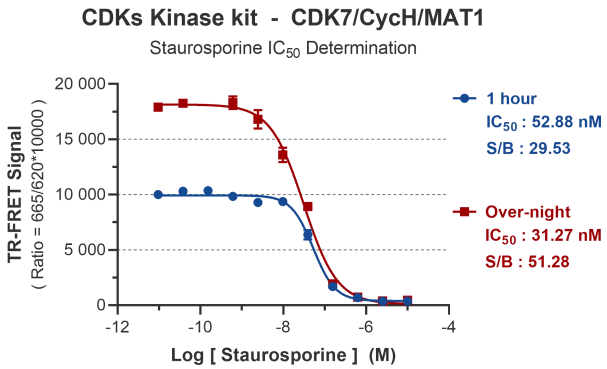
(13) CDK7/CycH/MAT1 P1HI0340S [10 μg] , P1HI0340L [100 μg]

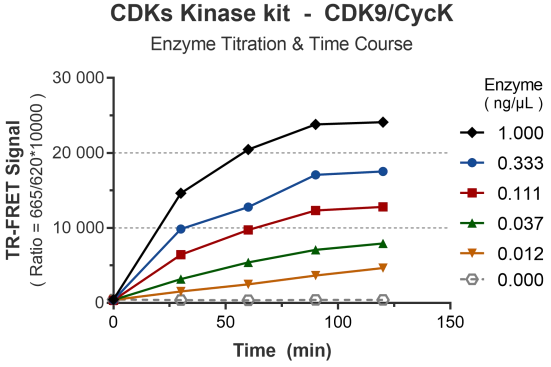
UniProt	P50613/P51946/P51948
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	2.0 ng/μL [final concentration in 10 μL buffer ; 20 ng/well]
Incubation time at RT	60 min
ATP concentration	100 μM [ATP Km of over-night: 88.62 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP

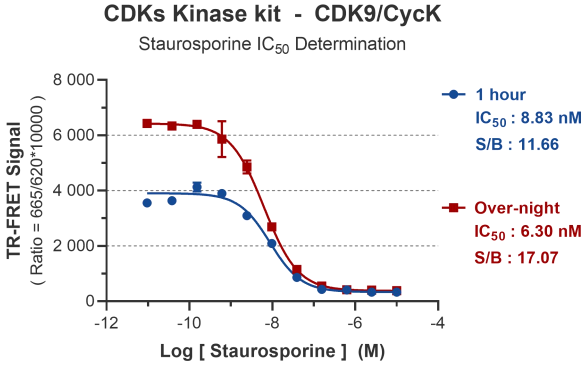




(14) CDK9/CycK P1HI0342S [10 μg] , P1HI0342L [100 μg]

UniProt	P50750/O75909
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.2 ng/μL [final concentration in 10 μL buffer ; 2.0 ng/well]
Incubation time at RT	60 min
ATP concentration	100 μM [ATP Km of over-night: 27.01 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP





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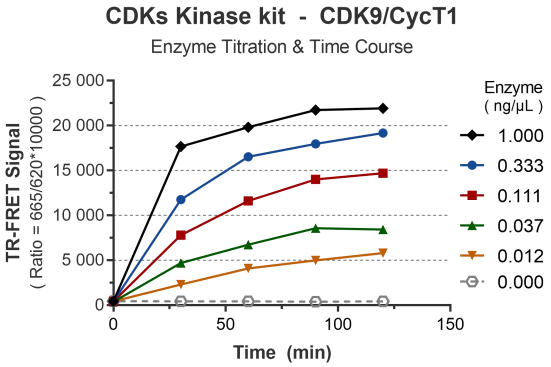
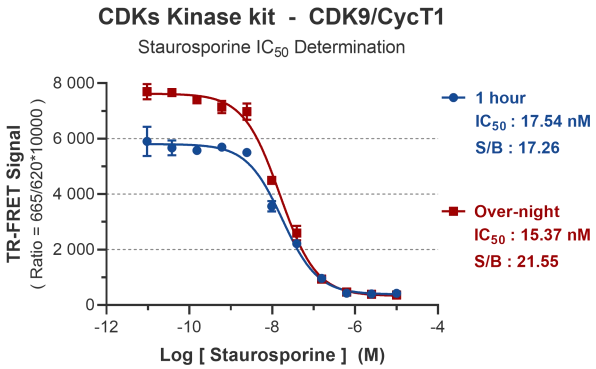
(15) CDK9/CycT1

P1HI0343S [10 μg] , P1HI0343L [100 μg]

UniProt	P50750/O60563
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.15 ng/μL [final concentration in 10 μL buffer ; 1.5 ng/well]
Incubation time at RT	60 min
ATP concentration	100 μM [ATP Km of over-night: 10.64 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP

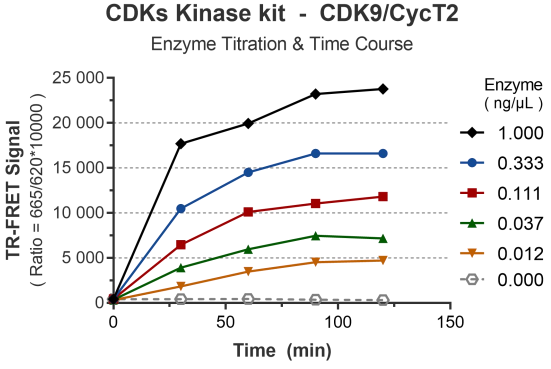


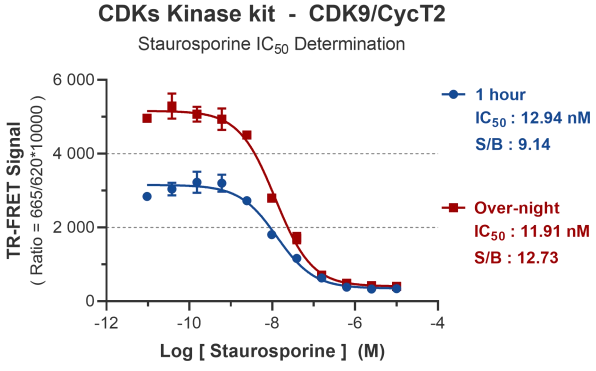


(16) CDK9/CycT2

P1HI0358S [10 μg] , P1HI0358L [100 μg]

UniProt	P50750/O60583
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.5 ng/μL [final concentration in 10 μL buffer ; 5.0 ng/well]
Incubation time at RT	60 min
ATP concentration	100 μM [ATP Km of over-night: 13.38 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP





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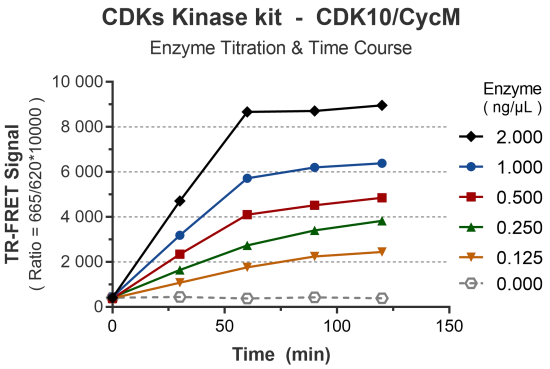
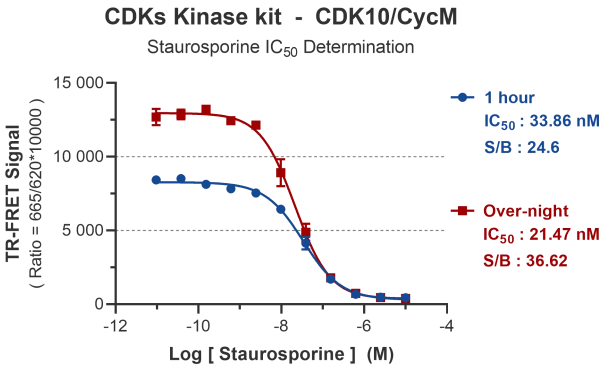
(17) CDK10/CycM

P1HI0309S [10 μg] , P1HI0309L [100 μg]

UniProt	Q15131/08N1B3
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	1.0 ng/μL [final concentration in 10 μL buffer ; 10 ng/well]
Incubation time at RT	60 min
ATP concentration	300 μM [ATP Km of over-night: 32.82 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP

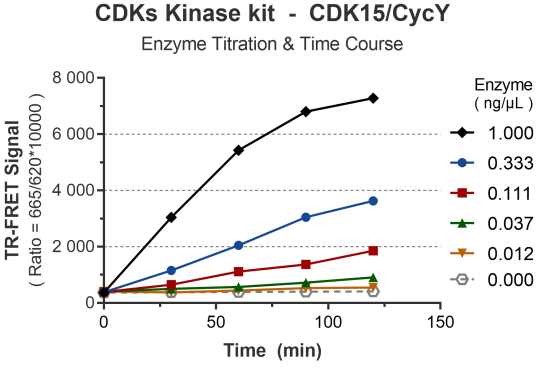


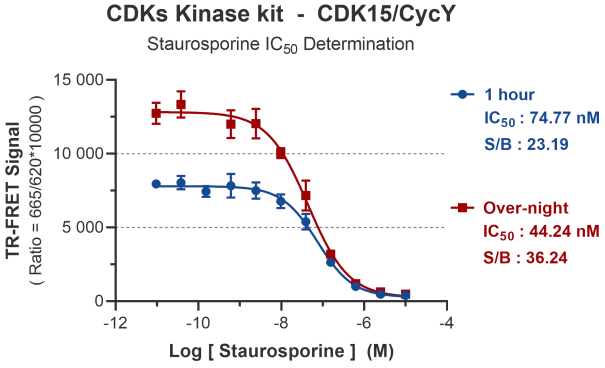


(18) CDK15/CycY

P1HI0357S [10 μg] , P1HI0357L [100 μg]

UniProt	Q96Q40/Q8ND76
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	1.0 ng/μL [final concentration in 10 μL buffer ; 10 ng/well]
Incubation time at RT	60 min
ATP concentration	25 μM [ATP Km of over-night: 23.27 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP





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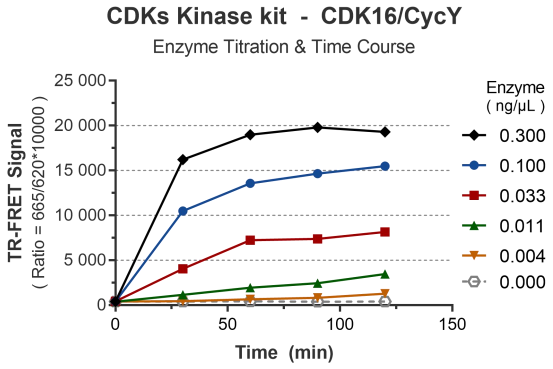
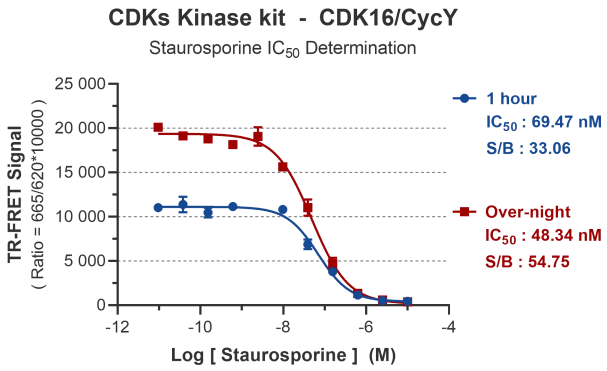
(19) CDK16/CycY

P1HI0348S [10 μg] , P1HI0348L [100 μg]

UniProt	Q00536/Q8ND76
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.05 ng/μL [final concentration in 10 μL buffer ; 0.5 ng/well]
Incubation time at RT	60 min
ATP concentration	50 μM [ATP Km of over-night: 57.74 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP

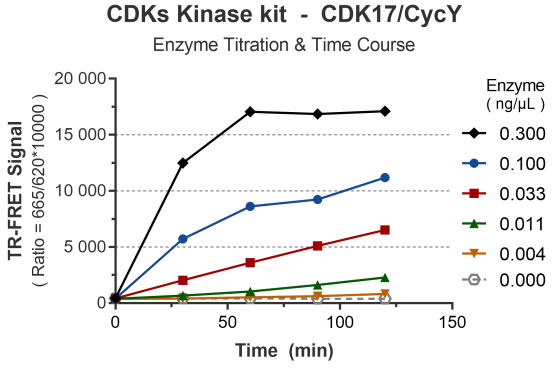


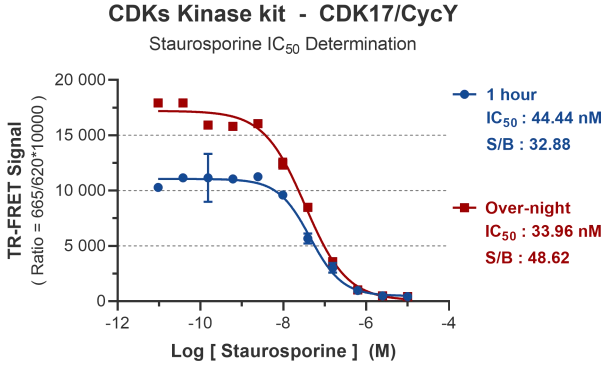


(20) CDK17/CycY

P1HI0356S [10 μg] , P1HI0356L [100 μg]

UniProt	Q00537/Q8ND76
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.05 ng/μL [final concentration in 10 μL buffer ; 0.5 ng/well]
Incubation time at RT	60 min
ATP concentration	25 μM [ATP Km of over-night: 50.91 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP





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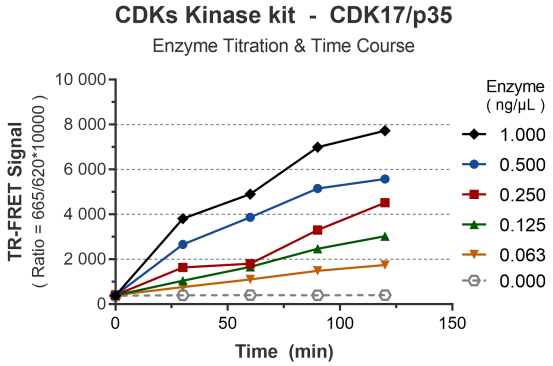
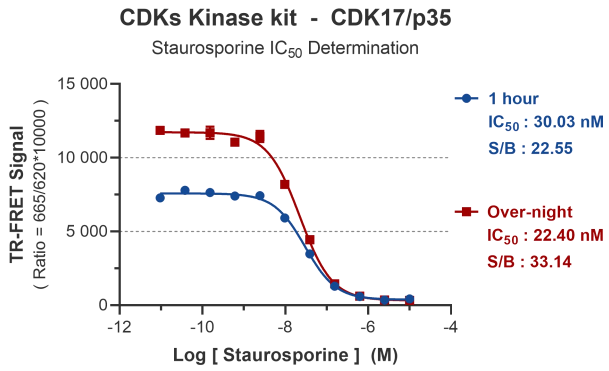
(21) CDK17/p35

P1HI0250S [10 μg] , P1HI0250L [100 μg]

UniProt	Q00537/Q15078
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	1.0 ng/μL [final concentration in 10 μL buffer ; 10 ng/well]
Incubation time at RT	60 min
ATP concentration	25 μM [ATP Km of over-night: 12.34 μM]
Buffer additives	5 mM $MgCl_2$, 1.0 mM TCEP

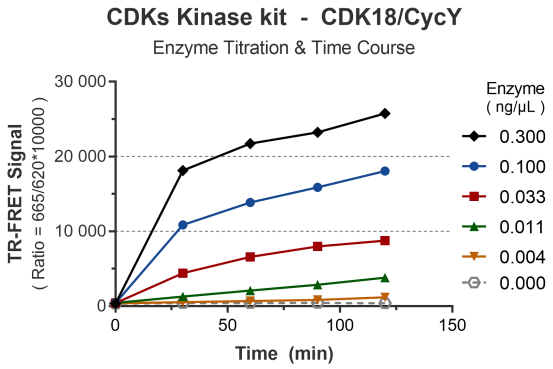


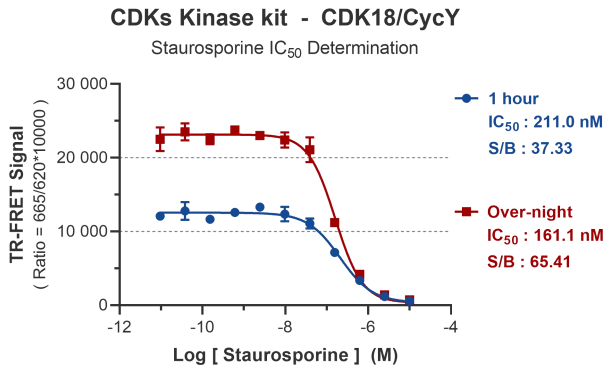


(22) CDK18/CycY

P1HI0349S [10 μg] , P1HI0349L [100 μg]

UniProt	Q07002/Q8ND76
Substrate	Rb(S780) - LA , 30 nM [final concentration in 10 μL buffer]
Detection antibody	p-Rb(S780) mAb - Solar Eu, 1X [Working concentration]
Enzyme concentration	0.05 ng/μL [final concentration in 10 μL buffer ; 0.5 ng/well]
Incubation time at RT	60 min
ATP concentration	100 μM [ATP Km of over-night: 62.33 μM]
Buffer additives	5 mM $MgCl_2$, 0.5 mM TCEP





Note: Exemplary data shown. Results are instrument-dependent.

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10. Instrument Model and Setting

Vendor	TECAN
Instrument model	Infinite® 200 PRO [Ref. 30050303]
Mode	Fluorescence Top Reading
Excitation filter	320 (25) nm [Ref. 30094454]
Emission filter 1	665 (8.5) nm [Ref. 30094518]
Emission filter 2	620 (10) nm [Ref. 30094505]
Mirror	Dichroic 510
Lag time	150 µs
Integration Time	500 µs
Number of reads	5 or user-defined
Gain	150 or optimal
Z -focus (mm)	Can be calculated on the well giving the highest signal

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