CAT.&Size: A1080001S (1,000 tests)
A1080001L (20,000 tests)
A1080001B (100,000 tests)

Storage at: 2-8℃

VKEYBIO-03-2025

For Research Use Only

Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET
TKs kinase kit
Technical Manual

1. Introduction

KeyTec® TR-FRET TKs kinase kit is designed for measuring the Tyrosine kinase (TKs) activity. Tyrosine kinase family comprises multiple members. This assay kit has been validated for activity measurement of over 60 tyrosine kinases (For more details, please visit our official website or contact our technical support team). The assay provides a biotinylated substrate, Streptavidin-HX*[1] , a phospho Tyr-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 and Streptavidin-HX containing EDTA (to terminate the kinase reaction) that specifically binds to the phosphorylated substrate. 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 HX: TR-FRET Acceptor

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

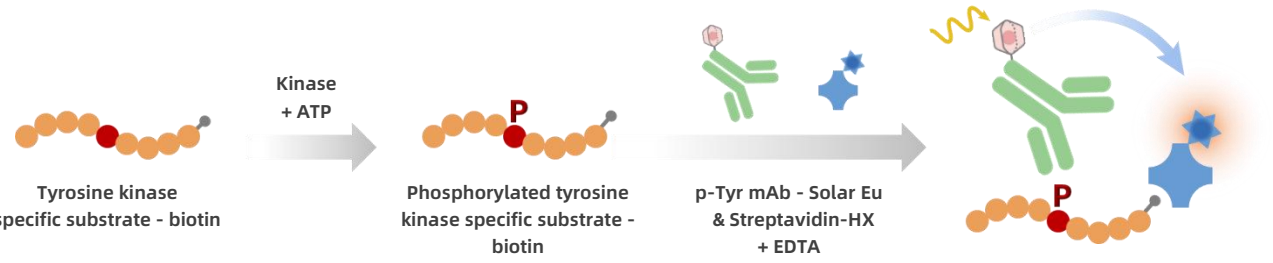

Figure1. The principle of KeyTec® TR-FRET kinase activity detection

2. Components

Components	Storage	A1080001S (1,000 tests[*3])	A1080001L (20,000 tests[*3])	A1080001B (100,000 tests[*3])
p-Tyr mAb - Solar Eu Lyophilized	2-8 ℃	1 vial 1,000 tests/vial	1 vial 20,000 tests/vial	5 vials 20,000 tests/vial
Streptavidin - HX Lyophilized	2-8 ℃	1 vial 1,000 tests/vial A1020024S	2 vials 10,000 tests/vial A1020024L	2 vials 50,000 tests/vial A1020024B
DTT Lyophilized	2-8 ℃	1 vial 120 μmoles/vial A1010022S	1 vial 600 μmoles/vial A1010022L	1 vial 3.0 mmoles/vial A1010022B
$MgCl_2$ (1 M)	2-8 ℃	1 vial 1.5 mL/vial A1010024S	1 bottle 6.0 mL/bottle A1010024L	1 bottle 30 mL/bottle A1010024B
$MnCl_2$ (1 M)	2-8 ℃	1 vial 1.5 mL/vial A1010028S	1 bottle 6.0 mL/bottle A1010028L	1 bottle 30 mL/bottle A1010028B
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	Size/Brand	Catalog
KeyTec® TR-FRET Enzymatic activity booster (EAB)	1.25 nmoles 12.5 nmoles	A1080011S A1080011L
KeyTec® TR-FRET TKs substrate - biotin	25 nmoles 250 nmoles	A1080014S A1080014L
KeyTec® TR-FRET BTKs substrate - biotin	25 nmoles 250 nmoles	A1080015S A1080015L
KeyTec® TR-FRET JAKs substrate - biotin	25 nmoles 250 nmoles	A1080016S A1080016L
KeyTec® TR-FRET FGFRs substrate - biotin	25 nmoles 250 nmoles	A1080017S A1080017L
Please visit our website for the latest version	\	\
ATP	Bidepharmatech	BD112724
Microplate	VKEY-BIO	M2000102N
Top sealing film	VKEY-BIO	M1000102N
Microplate Reader with TR-FRET module	TECAN	Infinite® 200 PRO

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5. Procedure

5.1 Reaction system

Phase	Components	Volume[*4] (20 μL)
Kinase reaction (10 μL)	Test samples or positive control	2 μL[*5]
	Kinase	4 μL[*5]
	Tyrosine kinase specific substrate - biotin	4 μL (Pre-mix)[*5]
	ATP	
TR-FRET detection (10 μL)	p-Tyr mAb - Solar Eu[*6]	5 μL
	Streptavidin - HX[*6]	5 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.

[*6] It is recommended to premix p-Tyr mAb - Solar Eu and Streptavidin - HX, add 10 μL pre-mix solution in TR-FRET detection.

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.
- 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)

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contained 0.05% BSA.

- 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., MgCl2, MnCl2, DTT , EAB etc.) with 4 volume of ultrapure water.

- **DTT stock solution**: Before reconstitution, centrifuge DTT 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. Aliquot DTT stock solution into single-use tubes to avoid repeated freeze-thaw cycles. These aliquots can be stored at -60℃.

DTT Powder	Buffer	Volume	Stock Concentration
120 μmole/vial	Ultra-pure water	120 μL/vial	1 M
600 μmole/vial	Ultra-pure water	600 μL/vial	1 M

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.

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 Tyrosine kinase

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specific substrate - biotin 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.

Substrate Powder	Buffer	Volume	Stock Concentration
25 nmoles/vial	Ultra-pure water	500 µL/vial	50 µM
250 nmoles/vial	Ultra-pure water	500 µL/vial	500 µM

- **Substrate and ATP pre-mix solution(2.5X)**: Prepare 1 mL substrate and ATP pre-mix Solution(2.5X), dilute 20 µL of Tyrosine kinase specific substrate - biotin stock solution and 2.5 µL of ATP stock solution with 977.5 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
Tyrosine kinase specific substrate - biotin	0.4	1.0	50	50	20	977.5	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 Detection solution

- **p-Tyr mAb - Solar Eu stock solution**: Before reconstitution, centrifuge p-Tyr 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.

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Kit Cat.	Size	Buffer	Volume	Stock Concentration
A1080001S	1,000 tests/vial	Kinase Detection buffer	0.5 mL/vial	10X
A1080001L/B	20,000 tests/vial	Kinase Detection buffer	1.0 mL/vial	100X

- **p-Tyr mAb - Solar Eu working solution(1X)**: Dilute 10X p-Tyr mAb - Solar Eu stock solution to working concentration(1X). For example, prepare 1 mL working solution(1X), dilute 100 µL of p-Tyr 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 .

- **Streptavidin - HX stock solution**: Before reconstitution, centrifuge Streptavidin - HX to pellet the powder to the bottom (850 × g,1-2 minutes). Add the recommended volume of Ultra-pure water to the powder and mix gently as below.

Component Cat.	Size	Buffer	Volume	Stock Concentration
A1020024S	1,000 tests/vial	Ultra-pure water	42 µL/vial	20 µM
A1020024L	10,000 tests/vial	Ultra-pure water	420 µL/vial	20 µM
A1020024B	50,000 tests/vial	Ultra-pure water	2.0 mL/vial	20 µM

- **Streptavidin - HX working solution(1X, 100nM)** : The molar ratio of substrate-biotin: Streptavidin-HX has been optimized at 8:1. Dilute Streptavidin-HX stock solution to working concentration (1X). For example, prepare 1 mL working solution(1X), dilute 5 µL of Streptavidin - HX stock solution with 995 µL of kinase detection buffer. Working solution(1X) can be stored at 2-8℃ for 24 hours or -60℃ for 3 months .

- **(Optional) p-Tyr mAb - solar Eu and streptavidin - HX pre-mix solution(0.5X)**: The pre-mix solution should be combined in a 1:1 volume ratio of p-Tyr mAb-Solar Eu working solution and streptavidin-HX working solution.

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Component	Volume	Kinase reaction Conc. (10 μL in total)	Final Conc. (20 μL in total)	Working Conc.
Test samples or positive control	2 μL/well	\	\	\
Kinase	4 μL/well	0.1 ng/μL	\	2.5 ng/μL
Tyrosine kinase specific substrate - biotin	4 μL/well (Premix with ATP)	400 nM	200 nM	1 μM
Streptavidin - HX (1/8*[7])	5 μL/well	\	25 nM*[7]	100 nM*[7]
p-Tyr mAb - Solar Eu	5 μL/well	\	\	1X

*[7] Streptavidin - HX / substrate - biotin = 1/8 (Final Conc.).

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 Tyrosine kinase specific substrate - biotin and ATP pre-mix solution (2.5X)	2 μL Test sample (5X) 4 μL Enzymatic buffer 4 μL Tyrosine kinase specific substrate - biotin 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 detection solution: 5 μL p-Tyr mAb - Solar Eu (1X) 5 μL Streptavidin - HX (1X) or 10 μL p-Tyr mAb - solar Eu and streptavidin - HX pre-mix solution(0.5X)	
	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)	Recommended substrate	Sub. fCo(nM)	Sta. IC_{50} (nM, ON)	S/B (ON)	Buffer additives
EGFR	P1HI0314S	P00533	0.05	60	10	TKs substrate - biotin	400	56.63	38.33	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT
EGFR [d746-750/T790M]	P1HI0315S	P00533	0.05	60	300	TKs substrate - biotin	400	1.33	24.21	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT
EGFR [L858R/T790M]	P1HI0316S	P00533	0.02	60	100	TKs substrate - biotin	400	3.44	44.36	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT
EGFR [T790M/C797S/L858R]	P1HI0317S	P00533	0.02	60	100	TKs substrate - biotin	400	3.80	64.91	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT
FGFR1	P1HI0318S	P11362	0.02	60	50	FGFRs substrate - biotin	400	12.87	44.34	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT
FGFR2	P1HI0319S	P21802	0.2	60	50	FGFRs substrate - biotin	400	7.11	26.56	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT
FGFR3	P1HI0320S	P22607	0.1	60	50	FGFRs substrate - biotin	400	49.87	58.29	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT
FGFR4	P1HI0321S	P22455	0.5	60	100	FGFRs substrate - biotin	400	73.83	39.47	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT
VEGFR1/FLT1	P1HI0322S	P17948	0.2	60	300	TKs substrate - biotin	400	5.89	51.22	20 mM $MgCl_2$, 2 mM DTT
VEGFR2/KDR	P1HI0323S	P35968	0.02	60	300	TKs substrate - biotin	400	3.56	40.37	20 mM $MgCl_2$, 2 mM DTT
FLT3	P1HI0326S	P36888	10	60	100	JAKs substrate - biotin	200	0.55	56.21	10 mM $MgCl_2$, 2 mM DTT
FLT3-ITD-NPOS	P1HI0327S	P36888	5	60	100	JAKs substrate - biotin	200	0.41	58.62	10 mM $MgCl_2$, 2 mM DTT
FLT3-ITD-W51	P1HI0328S	P36888	5	60	100	JAKs substrate - biotin	200	1.27	67.79	10 mM $MgCl_2$, 2 mM DTT
VEGFR3/FLT4	P1HI0324S	P35916	0.1	60	300	TKs substrate - biotin	400	1.20	31.76	20 mM $MgCl_2$, 2 mM DTT
JAK1	P1HI0067S	P23458	10	60	100	JAKs substrate - biotin	200	28.58	39.29	0.5 mM $MnCl_2$, 2 mM DTT
JAK2	P1HI0153S	O60674	2	60	100	JAKs substrate - biotin	200	5.34	45.33	0.5 mM $MnCl_2$, 2 mM DTT
JAK3	P1HI0325S	P52333	0.5	60	100	JAKs substrate - biotin	200	0.48	29.12	0.5 mM $MnCl_2$, 2 mM DTT
BTK	P1HI0329S	Q06187	2	60	50	BTKs substrate - biotin	200	18.61	21.95	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT, 10 nM EAB
BTK [C481R]	P1HI0008S	Q06187	2.5	60	300	BTKs substrate - biotin	200	3,386	43.00	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT, 10 nM EAB
BTK [C481S]	P1HI0330S	Q06187	5	60	50	BTKs substrate - biotin	200	30.33	40.59	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT, 10 nM EAB

Abbreviation: ON stands for overnight.

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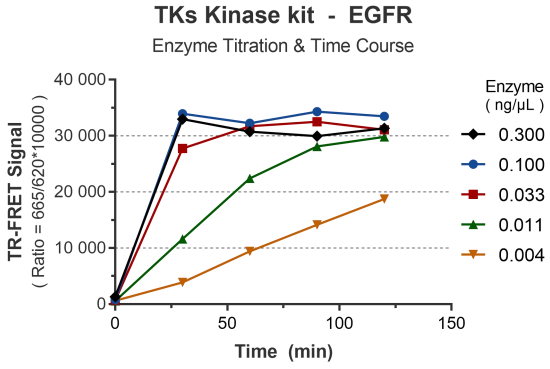
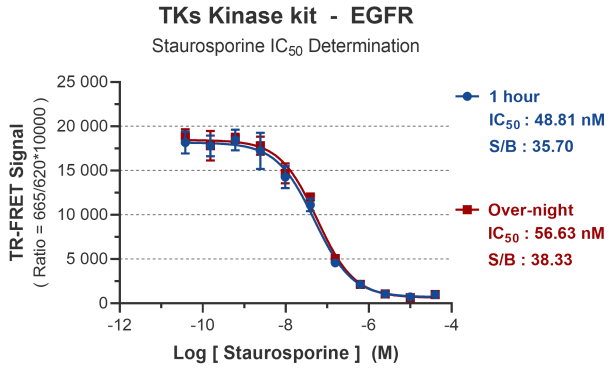
9.2 Results

(1) EGFR

P1HI0314S [10 μg] , P1HI0314L [100 μg]

Enzyme concentration	0.005 ng/μL [Final concentration in 10 μL buffer ; 0.05 ng/well]
Incubation time at RT	60 min
Substrate	TKs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	10 μM [ATP Km of over-night: 2.22 μM]
Buffer additives	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT

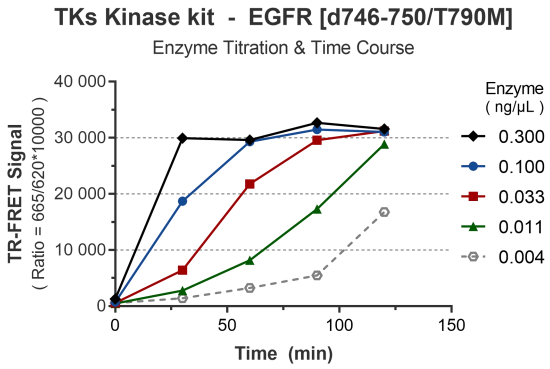


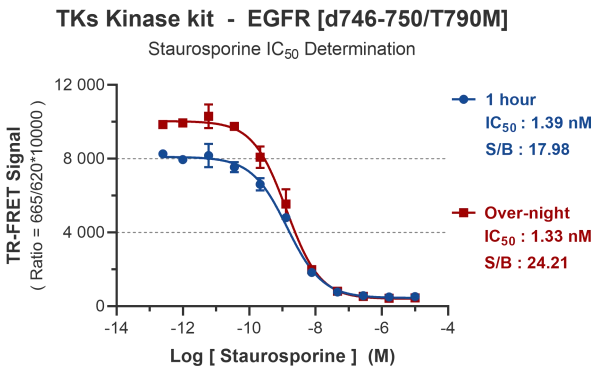


(2) EGFR [d746-750/T790M]

P1HI0315S [10 μg] , P1HI0315L [100 μg]

Enzyme concentration	0.005 ng/μL [Final concentration in 10 μL buffer ; 0.05 ng/well]
Incubation time at RT	60 min
Substrate	TKs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	300 μM [ATP Km of over-night: 10.43 μM]
Buffer additives	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT





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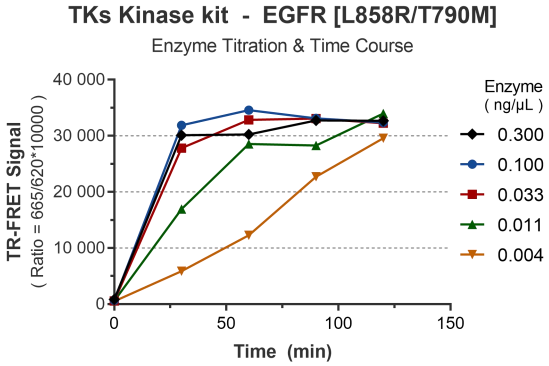
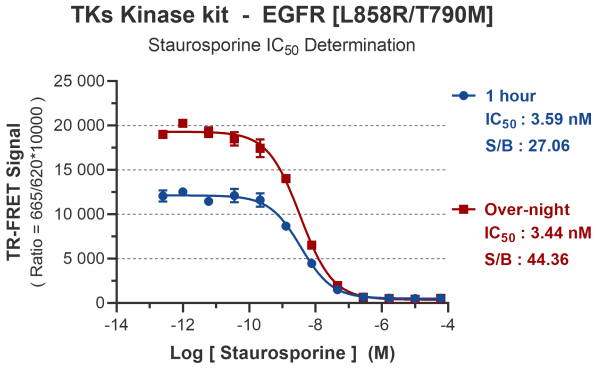
(3) EGFR [L858R/T790M]

P1HI0316S [10 μg] , P1HI0316L [100 μg]

Enzyme concentration	0.002 ng/μL [Final concentration in 10 μL buffer ; 0.02 ng/well]
Incubation time at RT	60 min
Substrate	TKs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 2.07 μM]
Buffer additives	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT

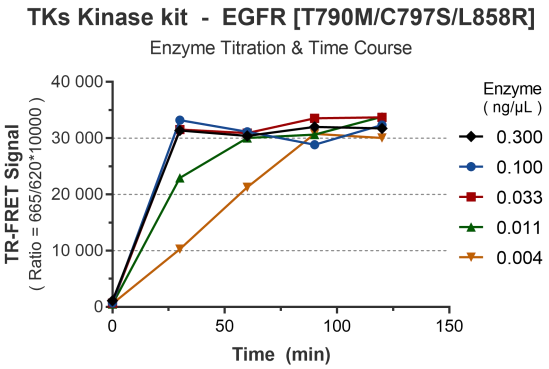


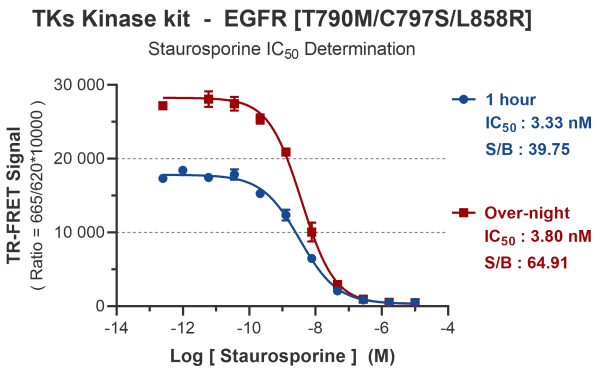


(4) EGFR [T790M/C797S/L858R]

P1HI0317S [10 μg] , P1HI0317L [100 μg]

Enzyme concentration	0.002 ng/μL [Final concentration in 10 μL buffer ; 0.02 ng/well]
Incubation time at RT	60 min
Substrate	TKs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 1.25 μM]
Buffer additives	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT





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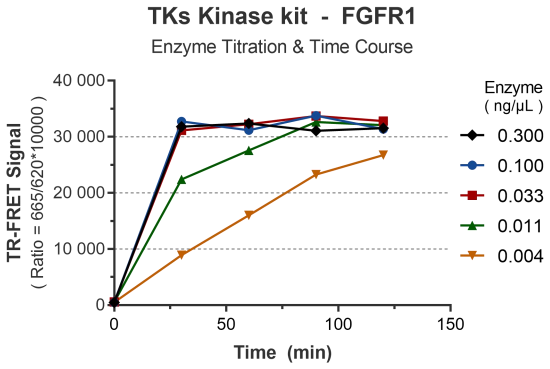
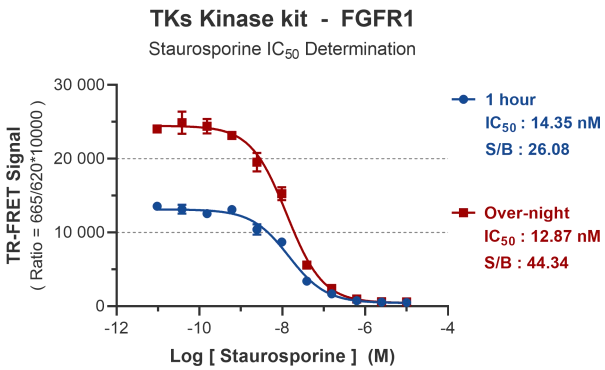
(5) FGFR1

P1HI0318S [10 μg] , P1HI0318L [100 μg]

Enzyme concentration	0.002 ng/μL [Final concentration in 10 μL buffer ; 0.02 ng/well]
Incubation time at RT	60 min
Substrate	FGFRs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	50 μM [ATP Km of over-night: 7.75 μM]
Buffer additives	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT

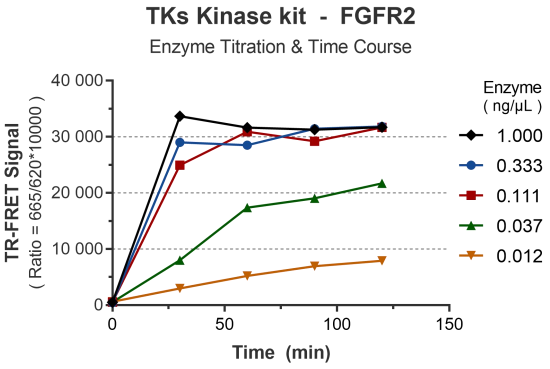


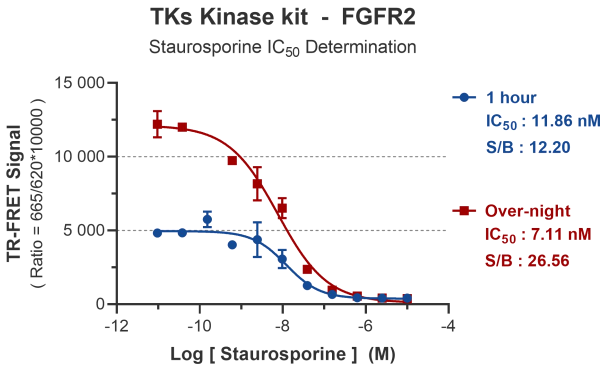


(6) FGFR2

P1HI0319S [10 μg] , P1HI0319L [100 μg]

Enzyme concentration	0.02 ng/μL [Final concentration in 10 μL buffer ; 0.2 ng/well]
Incubation time at RT	60 min
Substrate	FGFRs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	50 μM [ATP Km of over-night: 4.90 μM]
Buffer additives	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT





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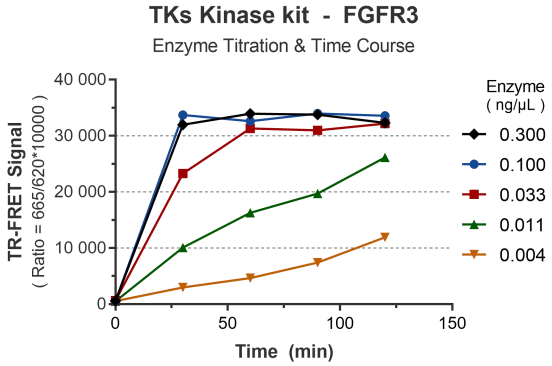
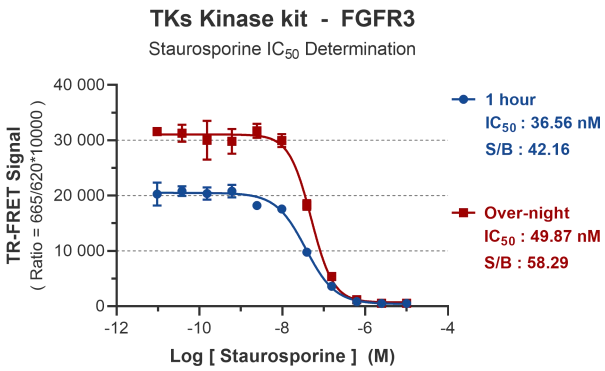
(7) FGFR3

P1HI0320S [10 μg] , P1HI0320L [100 μg]

Enzyme concentration	0.01 ng/μL [Final concentration in 10 μL buffer ; 0.1 ng/well]
Incubation time at RT	60 min
Substrate	FGFRs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	50 μM [ATP Km of over-night: 13.82 μM]
Buffer additives	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT

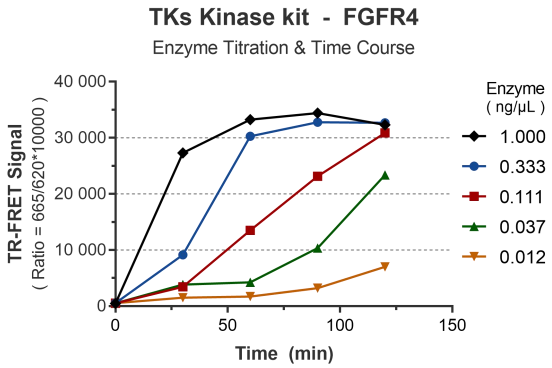


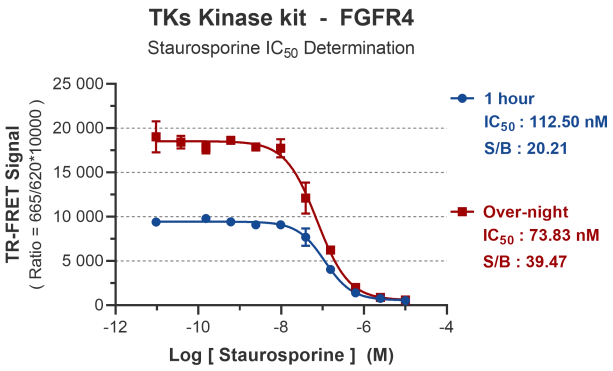


(8) FGFR4

P1HI0321S [10 μg] , P1HI0321L [100 μg]

Enzyme concentration	0.05 ng/μL [Final concentration in 10 μL buffer ; 0.5 ng/well]
Incubation time at RT	60 min
Substrate	FGFRs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 72.01 μM]
Buffer additives	10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT





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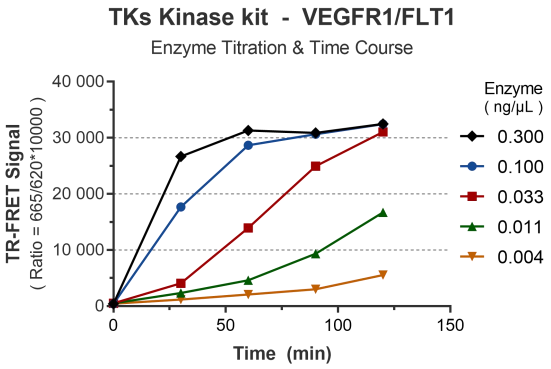
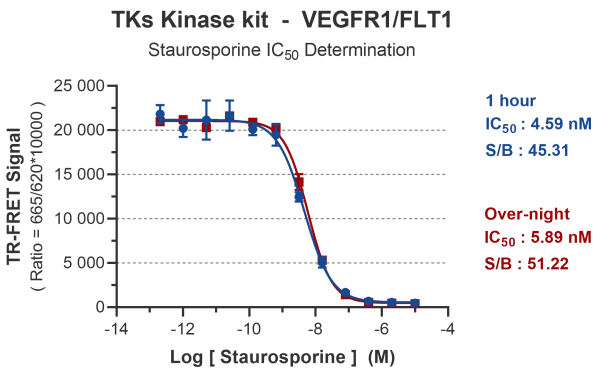
(9) VEGFR1/FLT1

P1HI0322S [10 μg] , P1HI0322L [100 μg]

Enzyme concentration	0.02 ng/μL [Final concentration in 10 μL buffer ; 0.2 ng/well]
Incubation time at RT	60 min
Substrate	TKs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	300 μM [ATP Km of over-night: 93.81 μM]
Buffer additives	20 mM $MgCl_2$, 2 mM DTT

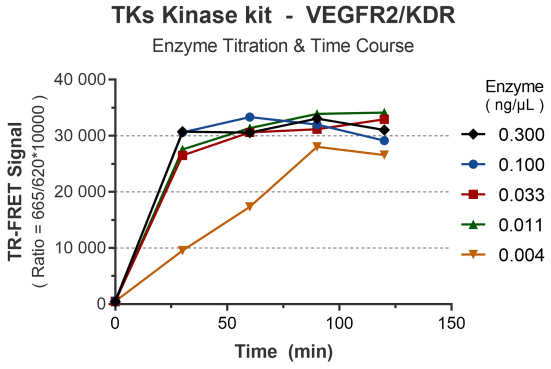


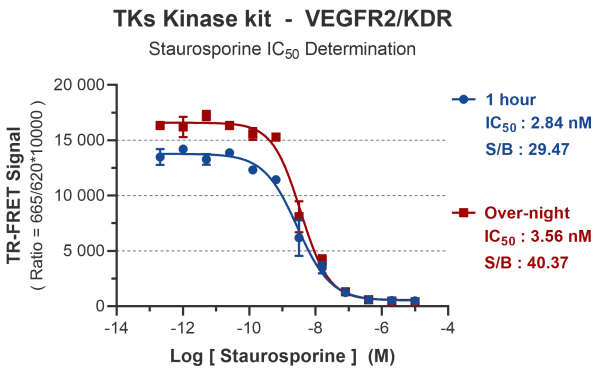


(10) VEGFR2/KDR

P1HI0323S [10 μg] , P1HI0323L [100 μg]

Enzyme concentration	0.002 ng/μL [Final concentration in 10 μL buffer ; 0.02 ng/well]
Incubation time at RT	60 min
Substrate	TKs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	300 μM [ATP Km of over-night: 56.00 μM]
Buffer additives	20 mM $MgCl_2$, 2 mM DTT





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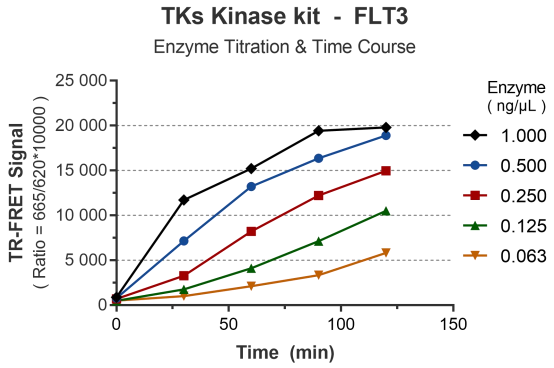
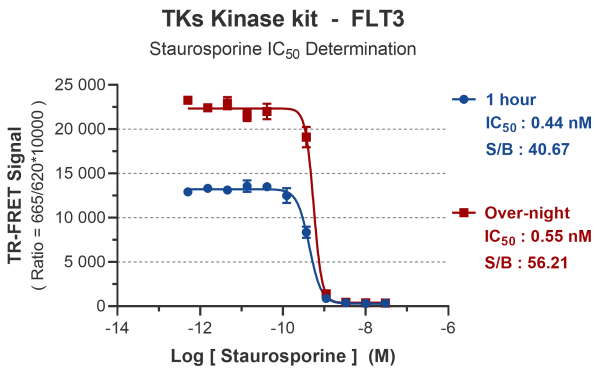
(11) FLT3

P1HI0326S [10 μg] , P1HI0326L [100 μg]

Enzyme concentration	1.0 ng/μL [Final concentration in 10 μL buffer ; 10 ng/well]
Incubation time at RT	60 min
Substrate	JAKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 42.02 μM]
Buffer additives	10 mM $MgCl_2$, 2 mM DTT

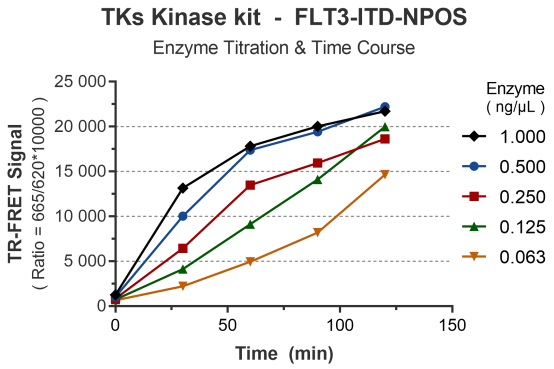


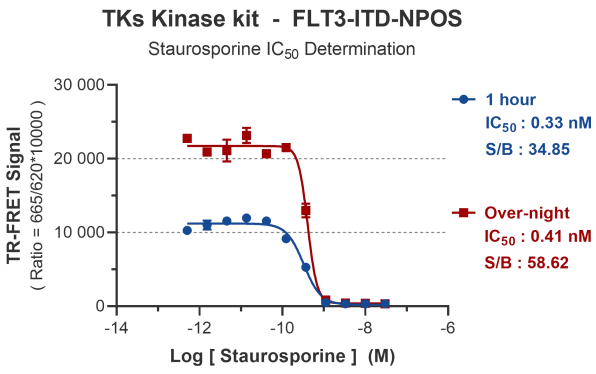


(12) FLT3-ITD-NPOS

P1HI0327S [10 μg] , P1HI0327L [100 μg]

Enzyme concentration	0.5 ng/μL [Final concentration in 10 μL buffer ; 5 ng/well]
Incubation time at RT	60 min
Substrate	JAKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 62.65 μM]
Buffer additives	10 mM $MgCl_2$, 2 mM DTT





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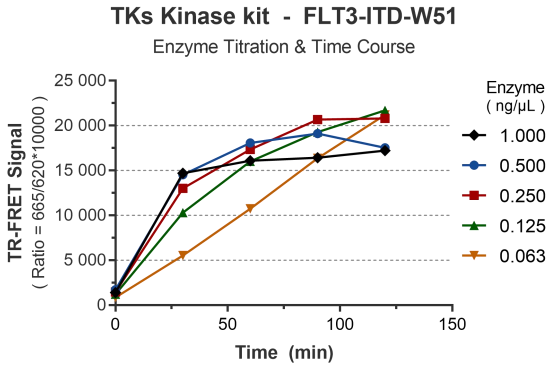
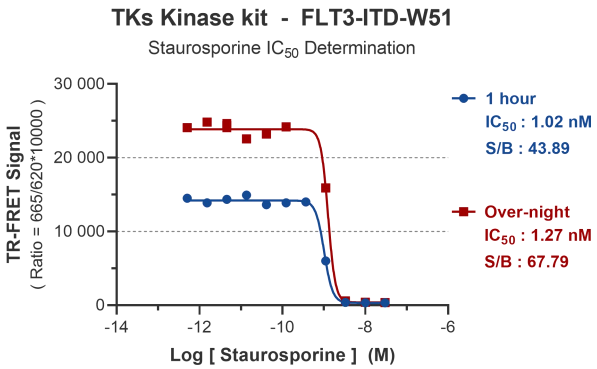
(13) FLT3-ITD-W51

P1HI0328S [10 μg] , P1HI0328L [100 μg]

Enzyme concentration	0.5 ng/μL [Final concentration in 10 μL buffer ; 5 ng/well]
Incubation time at RT	60 min
Substrate	JAKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 14.35 μM]
Buffer additives	10 mM $MgCl_2$, 2 mM DTT

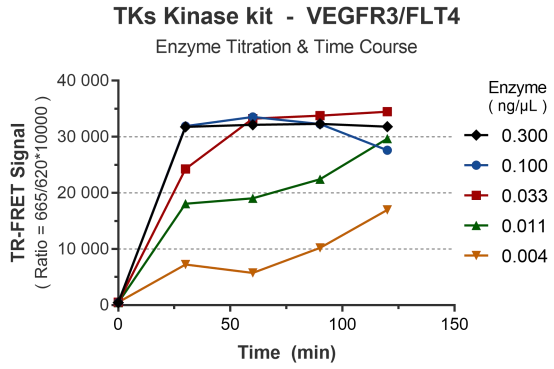


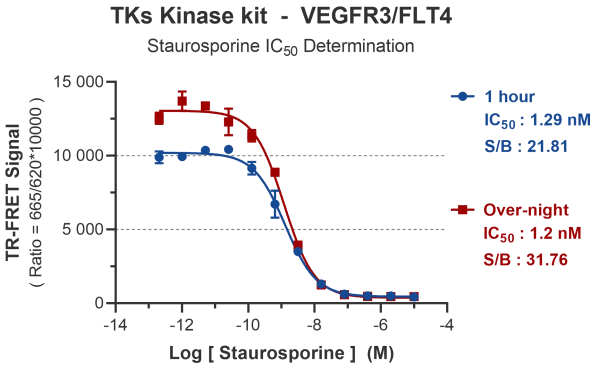


(14) VEGFR3/FLT4

P1HI0324S [10 μg] , P1HI0324L [100 μg]

Enzyme concentration	0.01 ng/μL [Final concentration in 10 μL buffer ; 0.1 ng/well]
Incubation time at RT	60 min
Substrate	TKs substrate - biotin, 400 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	25 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	300 μM [ATP Km of over-night: 125.70 μM]
Buffer additives	20 mM $MgCl_2$, 2 mM DTT





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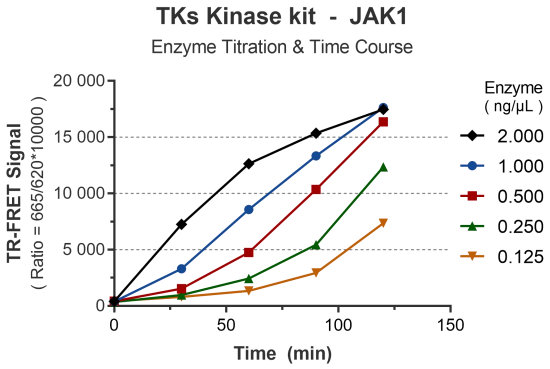
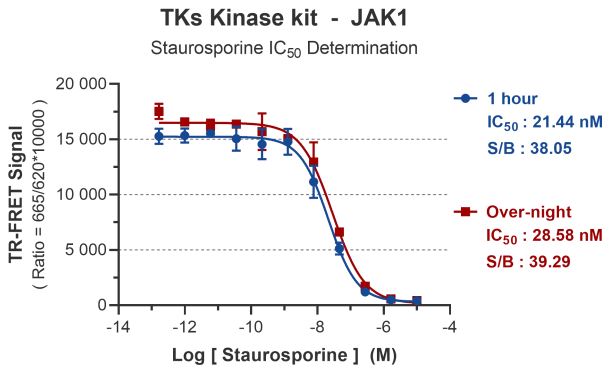
(15) JAK1

P1HI0067S [10 μg] , P1HI0067L [100 μg]

Enzyme concentration	1.0 ng/μL [Final concentration in 10 μL buffer ; 10 ng/well]
Incubation time at RT	60 min
Substrate	JAKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 2.68 μM]
Buffer additives	0.5 mM $MnCl_2$, 2 mM DTT

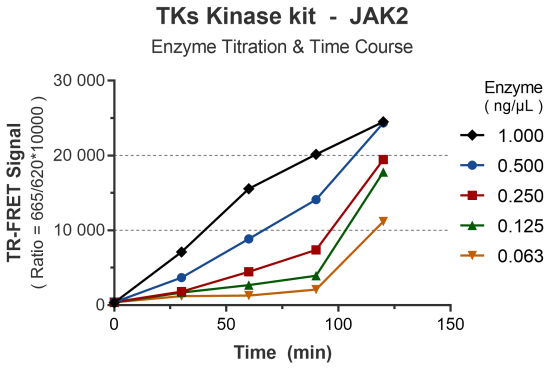


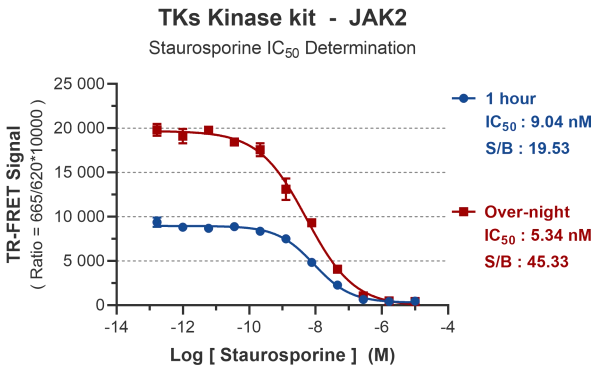


(16) JAK2

P1HI0153S [10 μg] , P1HI0153L [100 μg]

Enzyme concentration	0.2 ng/μL [Final concentration in 10 μL buffer ; 2.0 ng/well]
Incubation time at RT	60 min
Substrate	JAKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 3.926 μM]
Buffer additives	0.5 mM $MnCl_2$, 2 mM DTT





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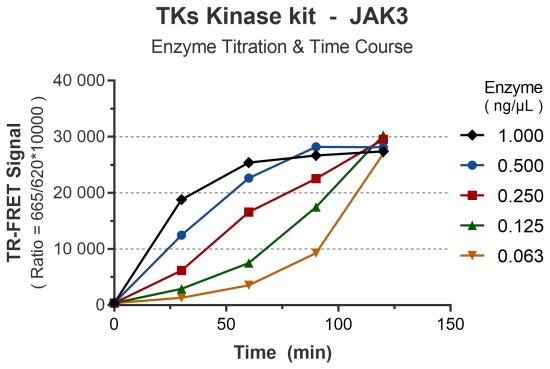
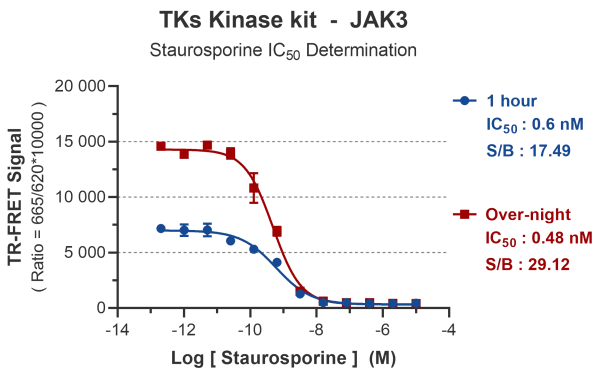
(17) JAK3

P1HI0325S [10 μg] , P1HI0325L [100 μg]

Enzyme concentration	0.05 ng/μL [Final concentration in 10 μL buffer ; 0.5 ng/well]
Incubation time at RT	60 min
Substrate	JAKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	100 μM [ATP Km of over-night: 0.878 μM]
Buffer additives	0.5 mM $MnCl_2$, 2 mM DTT

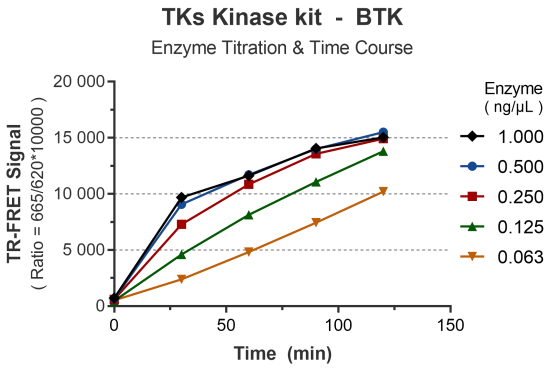


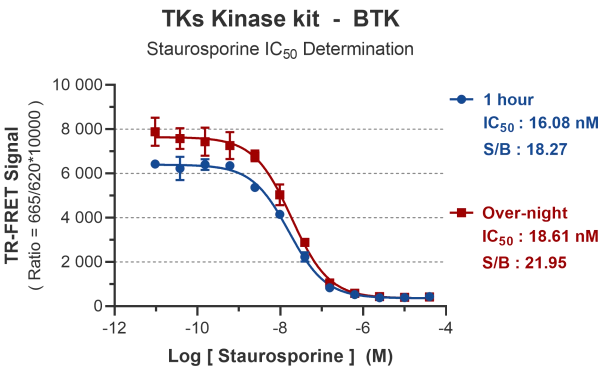


(18) BTK

P1HI0329S [10 μg] , P1HI0329L [100 μg]

Enzyme concentration	0.2 ng/μL [Final concentration in 10 μL buffer ; 2.0 ng/well]
Incubation time at RT	60 min
Substrate	BTKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	50 μM [ATP Km of over-night: 12.64 μM]
Buffer additives	10 mM $MgCl_2$, 2 mM DTT , 10 nM EAB





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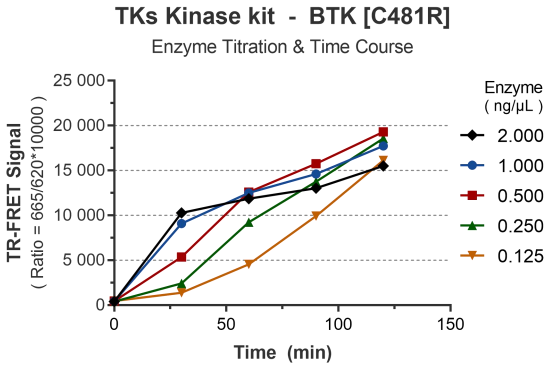
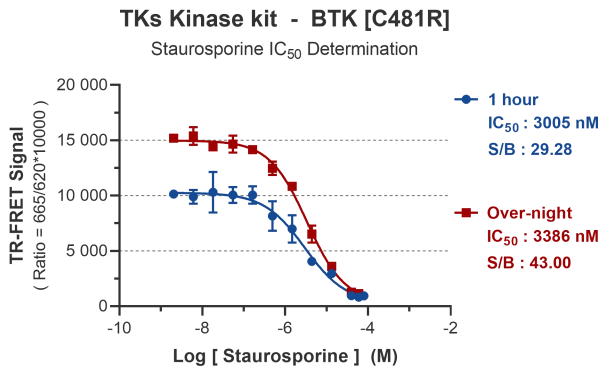
(19) BTK [C481R]

P1HI0008S [10 μg] , P1HI0008L [100 μg]

Enzyme concentration	0.25 ng/μL [Final concentration in 10 μL buffer ; 2.5 ng/well]
Incubation time at RT	60 min
Substrate	BTKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	300 μM [ATP Km of over-night: 204.0 μM]
Buffer additives	10 mM $MgCl_2$, 2 mM DTT , 10 nM EAB

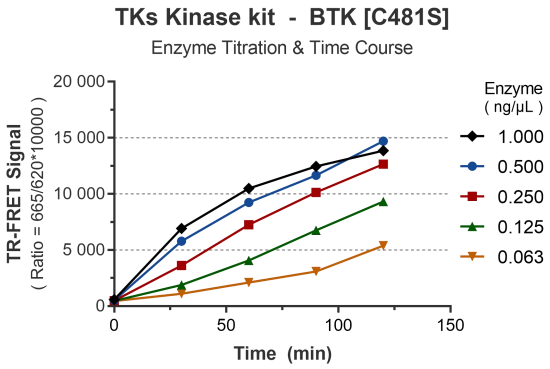


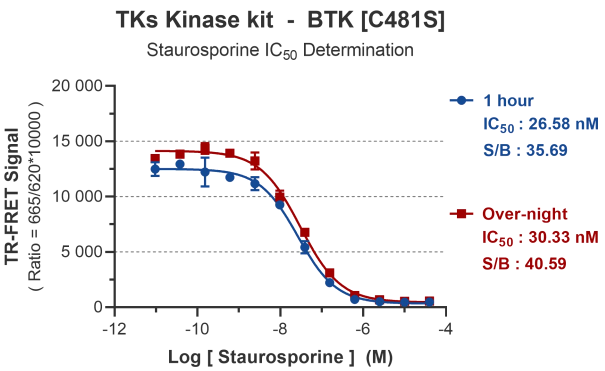


(20) BTK [C481S]

P1HI0330S [10 μg] , P1HI0330L [100 μg]

Enzyme concentration	0.5 ng/μL [Final concentration in 10 μL buffer ; 5.0 ng/well]
Incubation time at RT	60 min
Substrate	BTKs substrate - biotin, 200 nM [Final concentration in 10 μL buffer]
Streptavidin - HX	12.5 nM [SA/Substrate = 1/8 for final concentration in 20 μL buffer]
Detection antibody	p-Tyr mAb - Solar Eu, 1X [Working concentration]
ATP concentration	50 μM [ATP Km of over-night: 9.154 μM]
Buffer additives	10 mM $MgCl_2$, 2 mM DTT , 10 nM EAB





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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