LanthaScreen™ Eu Kinase Binding Assay Screening Protocol and Assay Conditions

Assay Theory

The principle of the LanthaScreen Eu Kinase Binding Assay is shown in Figure 1. Binding of an Alexa Fluor™ conjugate or "tracer" to a kinase is detected by addition of a Eu-labeled anti-tag antibody. Binding of the tracer and antibody to a kinase results in a high degree of FRET, whereas displacement of the tracer with a kinase inhibitor results in a loss of FRET. Unlike many kinase activity assays, this assay is a simple mix-and-read assay, with no development steps.

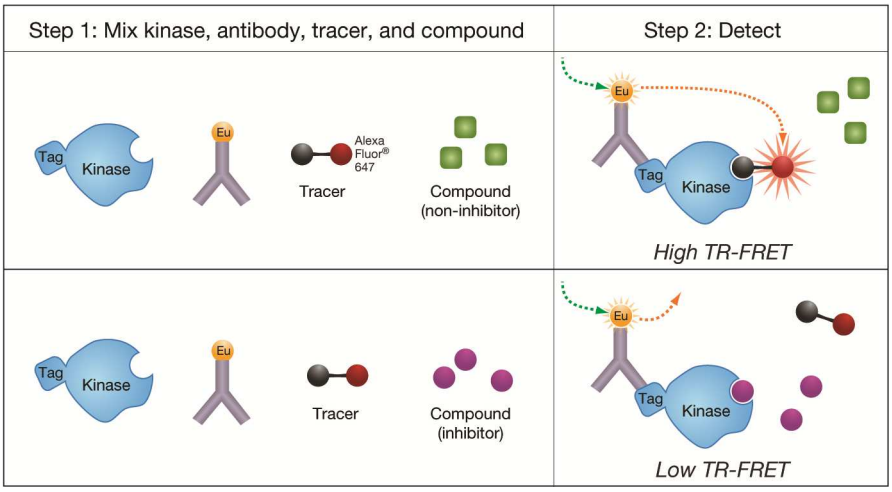

Figure 1. Schematic of the LanthaScreen Eu Kinase Binding assay

Life Technologies' Kinase Tracers are based on ATP-competitive kinase inhibitors, making them suitable for detection of any compounds that bind to the ATP site. Inhibitors that bind the ATP site include both Type I kinase inhibitors, which bind solely to the ATP site, and Type II inhibitors (e.g., Gleevec®/Imatinib, Sorafenib, BIRB-796), which bind to both the ATP site and a second site often referred to as the allosteric site.

LanthaScreen Eu Kinase Binding Assay Conditions

Test Compounds
The Test Compounds are screened in 1% DMSO (final) in the well. For 10 point titrations, 3-fold serial dilutions are conducted from the starting concentration of the customer's choosing.

Kinase/Antibody Mixtures
All Kinase/Antibody Mixtures are diluted to a 2X working concentration in the appropriate Kinase Buffer (see section *Kinase Specific Assay Conditions* for a complete description).

Tracer
The 4X AlexaFluor labeled Tracer is prepared in Kinase Buffer.

Assay Protocol
Bar-coded, low volume, white 384-well plate (Greiner Cat. #784207)
1. 160 nL – 100X Test Compound in 100% DMSO
2. 3.84 µL – Kinase Buffer
3. 8.0 µL – 2X Kinase/Antibody Mixture
4. 4.0 µL – 4X Tracer
5. 30-second plate shake
6. 60-minute incubation at room temperature
7. Read on fluorescence plate reader and analyze the data

LanthaScreen Eu Kinase Binding Assay Controls

The following controls are made for each individual kinase and are located on the same plate as the kinase:

0% Displacement Control
The maximum Emission Ratio is established by the 0% Displacement Control wells, which do not contain known inhibitor in the reaction and therefore exhibits no displacement of the tracer.

100% Displacement Control
The minimum Emission Ratio is established by the 100% Displacement Control wells, which contain the highest concentration of the known inhibitor used in that assay.

Known Inhibitor
A known inhibitor control standard curve, 10 point titration, is run for each individual kinase on the same plate as the kinase to ensure the inhibitor is displaced within an expected IC_{50} range previously determined.

LanthaScreen Eu Kinase Binding Assay Data Analysis

The following equations are used for each set of data points:

	Equation
Emission Ratio (ER)	$\frac{\text{AF647 Emission (665 nm)}}{\text{Europium Emission (615 nm)}}$
% Displacement	$\left\{ \frac{ER_{0\%\ Disp\ Ctrl} - ER_{Sample}}{ER_{0\%\ Disp\ Ctrl} - ER_{100\%\ Disp\ Ctrl}} \right\} * 100$
Difference Between Data Points (single point only)	$\lvert \%\ Displacement_{Point\ 1} - \%\ Displacement_{Point\ 2} \rvert$
Test Compound Interference	For each emission wavelength, fluorescence interference is flagged for a compound well that is more than 20% outside the range of the controls.
Z´ (using Emission Ratio values)	$1 - \frac{3 * Stdev_{0\%\ Disp\ Ctrl} + 3 * Stdev_{100\%\ Disp\ Ctrl}}{\lvert Mean_{0\%Disp\ Ctrl} - Mean_{100\%\ Disp\ Ctrl} \rvert}$

Graphing Software

SelectScreen Kinase Profiling Service uses XL*fit* from IDBS. The dose response curve is curve fit to model number 205. If the bottom of the curve does not fit between -20% & 20% inhibition, it is set to 0% inhibition. If the top of the curve does not fit between 70% and 130% inhibition, it is set to 100% inhibition.

Kinase-Specific Assay Conditions

Kinase	Kinase Conc (nM)	Antibody	Antibody Conc (nM)	Tracer	Tracer Conc (nM)	Tracer Kd (nM)	Buffer	Known Inhibitor	IC50 (nM)
AAK1	5	Eu-anti-GST	2	Tracer 222	100	72	A	Sunitinib	46.7
ABL1 H396P	10	Eu-anti-GST	2	Tracer 1710	100	277	A	PP121	6.89
ABL1 M351T	10	Eu-anti-GST	2	Tracer 1710	100	250	A	PP121	7.01
ABL1 Q252H	10	Eu-anti-GST	2	Tracer 1710	100	229	A	PP121	6.67
ACVR1 (ALK2)	5	Eu-anti-GST	2	Tracer 236	100	76	A	Staurosporine	45.7
ACVR1 (ALK2) R206H	5	Eu-anti-GST	2	Tracer 236	30	44	A	Staurosporine	23.4
ACVR2A	5	Eu-anti-GST	2	Tracer 178	50	51	A	Dasatinib	74.1
ACVR2B	10	Eu-anti-GST	2	Tracer 178	50	157	A	Dasatinib	217
ACVRL1 (ALK1)	20	Eu-anti-GST	2	Tracer 178	20	19	A	Dasatinib	80.1
ADCK3	5	Eu-anti-GST	2	Tracer 178	100	100	A	Dasatinib	321
ALK C1156Y	5	Eu-anti-GST	2	Tracer 236	10	9.6	A	Staurosporine	2.09
ALK F1174L	5	Eu-anti-GST	2	Tracer 236	10	12	A	Staurosporine	2.14
ALK L1196M	5	Eu-anti-GST	2	Tracer 236	10	17	A	Staurosporine	3.81
ALK R1275Q	5	Eu-anti-GST	2	Tracer 236	10	9.9	A	Staurosporine	2.97
ALK T1151_L1152insT	5	Eu-anti-GST	2	Tracer 236	10	12	J	Staurosporine	3.90
AMPK (A1/B1/G2)	2.5	Eu-anti-His	2	Tracer 236	5	7.4	A	Staurosporine	2.34
AMPK (A1/B1/G3)	2.5	Eu-anti-His	2	Tracer 236	5	6.4	A	Staurosporine	0.927
AMPK (A1/B2/G1)	2.5	Eu-anti-His	2	Tracer 236	5	7.5	A	Staurosporine	1.13
AMPK (A2/B2/G1)	2.5	Eu-anti-His	2	Tracer 236	5	6.4	A	Staurosporine	1.09
AMPK (A2/B2/G2)	2.5	Eu-anti-His	2	Tracer 236	5	5.9	A	Staurosporine	0.992
AXL R499C	5	Eu-anti-GST	2	Tracer 236	10	8.5	A	Staurosporine	8.25
BMPR1A (ALK3)	10	Eu-anti-GST	2	Tracer 178	50	56	A	Dasatinib	852
BMPR1B (ALK6)	5	Eu-anti-GST	2	Tracer 178	20	36	A	Dasatinib	161
BMPR2	5	Eu-anti-His	2	Tracer 236	100	70	A	Staurosporine	765
BRAF	5	Eu-anti-GST	2	Tracer 178	20	20	A	Dasatinib	67.1
BRAF V599E	5	Eu-anti-GST	2	Tracer 178	20	33	A	Dasatinib	59.6
BRSK2	5	Eu-anti-GST	2	Tracer 236	100	100	A	Staurosporine	11.5
CAMK2G (CaMKII gamma)	1	Eu-anti-GST	2	Tracer 236	1	2	A	Staurosporine	0.324
CAMKK1 (CAMKKA)	5	Eu-anti-His	2	Tracer 236	100	266	A	Staurosporine	2.03
CAMKK2 (CaMKK beta)	5	Eu-anti-GST	2	Tracer 236	10	9.8	A	Staurosporine	1.19
CASK	5	Eu-anti-GST	2	Tracer 236	30	56	A	Staurosporine	11.2
CDC7/DBF4	0.5	Eu-anti-GST	2	Tracer 236	1	2.7	A	Staurosporine	1.90
CDK1/cyclin A2	10	Eu-anti-GST	2	Tracer 236	100	132	A	Staurosporine	3.38
CDK11 (Inactive)	5	Eu-anti-GST	2	Tracer 236	10	15	A	Staurosporine	25.4

Kinase	Kinase Conc (nM)	Antibody	Antibody Conc (nM)	Tracer	Tracer Conc (nM)	Tracer Kd (nM)	Buffer	Known Inhibitor	IC50 (nM)
CDK11/cyclin C	1	Eu-anti-GST	2	Tracer 236	5	8.2	A	Staurosporine	3.83
CDK13/cyclin K	5	Eu-anti-GST	2	Tracer 236	100	165.4	A	Staurosporine	57.3
CDK14 (PFTK1)/cyclin Y	2.5	Eu-anti-GST	2	Tracer 236	100	157	A	Staurosporine	14.8
CDK16 (PCTK1)/cyclin Y	5	Eu-anti-GST	2	Tracer 236	250	251	A	Staurosporine	11.6
CDK2/cyclin A1	2.5	Eu-anti-GST	2	Tracer 236	30	25	A	Staurosporine	2.11
CDK2/cyclin E1	2.5	Eu-anti-GST	2	Tracer 236	100	102	A	Staurosporine	2.60
CDK2/cyclin O	2.5	Eu-anti-GST	2	Tracer 236	30	46	A	Staurosporine	1.69
CDK3/cyclin E1	10	Eu-anti-GST	2	Tracer 236	100	147	A	Staurosporine	3.71
CDK5 (Inactive)	5	Eu-anti-GST	2	Tracer 236	100	152	A	Staurosporine	13.2
CDK8/cyclin C	5	Eu-anti-His	2	Tracer 236	10	12	A	Staurosporine	9.81
CDK9 (Inactive)	5	Eu-anti-GST	2	Tracer 236	30	26	A	Staurosporine	6.81
CDK9/cyclin K	5	Eu-anti-His	2	Tracer 236	100	49	A	Staurosporine	12.0
CLK4	5	Eu-anti-GST	2	Tracer 236	30	28	A	Staurosporine	5.39
DAPK2	5	Eu-anti-His	2	Tracer 222	100	66	A	Sunitinib	214
DDR1	5	Eu-anti-GST	2	Tracer 178	10	19	A	Dasatinib	7.04
DDR2	5	Eu-anti-GST	2	Tracer 178	10	14	A	Dasatinib	16.1
DDR2 N456S	1	Eu-anti-GST	2	Tracer 236	1	1.8	A	Staurosporine	0.103
DDR2 T654M	20	Eu-anti-GST	2	Tracer 178	100	166	A	Dasatinib	132
DMPK	2.5	Eu-anti-GST	2	Tracer 236	30	43	A	Staurosporine	0.870
DYRK2	5	Eu-anti-GST	2	Tracer 236	100	314	A	Staurosporine	94.1
EGFR (ErbB1) d746-750	10	Eu-anti-GST	2	Tracer 199	10	10	A	SB202190	69.6
EGFR (ErbB1) d747-749 A750P	5	Eu-anti-GST	2	Tracer 236	10	19.5	A	Staurosporine	8.06
EIF2AK2 (PKR)	20	Eu-anti-GST	2	Tracer 236	100	486	K	Staurosporine	30.9
EPHA3	10	Eu-anti-His	2	Tracer 236	100	328	A	Staurosporine	160
EPHA6	5	Eu-anti-GST	2	Tracer 236	30	23	A	Staurosporine	21.9
EPHA7	5	Eu-anti-GST	2	Tracer 236	100	79	A	Staurosporine	40.8
ERN1	5	Eu-anti-GST	2	Tracer 236	100	160	A	Staurosporine	5.58
ERN2	5	Eu-anti-GST	2	Tracer 236	100	108	A	Staurosporine	29.6
FGFR1 V561M	1	Eu-anti-GST	2	Tracer 236	1	0.89	A	Staurosporine	0.279
FGFR3 G697C	5	Eu-anti-GST	2	Tracer 236	100	111	A	Staurosporine	19.6
FGFR3 K650M	5	Eu-anti-GST	2	Tracer 236	10	20	A	Staurosporine	0.953
FLT3 ITD	2.5	Eu-anti-GST	2	Tracer 236	1	1.6	A	Staurosporine	0.186
FYN A	5	Eu-anti-GST	2	Tracer 236	100	74	A	Staurosporine	14.9
GAK	5	Eu-anti-GST	2	Tracer 236	100	120	A	Staurosporine	12.5

Kinase	Kinase Conc (nM)	Antibody	Antibody Conc (nM)	Tracer	Tracer Conc (nM)	Tracer Kd (nM)	Buffer	Known Inhibitor	IC50 (nM)
GRK1	1	Eu-anti-GST	2	Tracer 236	1	1.3	A	Staurosporine	1.07
HUNK	5	Eu-anti-GST	2	Tracer 236	10	15	A	Staurosporine	20.1
ICK	5	Eu-anti-GST	2	Tracer 236	30	38	A	Staurosporine	7.47
IRAK3	1	Eu-anti-GST	2	Tracer 236	1	2.7	A	Staurosporine	0.263
KIT A829P	10	Eu-anti-GST	2	Tracer 222	10	9.3	A	Sunitinib	158
KIT D816H	5	Eu-anti-GST	2	Tracer 222	30	29	A	Sunitinib	69.1
KIT D816V	5	Eu-anti-GST	2	Tracer 222	100	67	A	Sunitinib	134
KIT D820E	5	Eu-anti-GST	2	Tracer 222	5	5.8	A	Sunitinib	15.6
KIT N822K	5	Eu-anti-GST	2	Tracer 222	10	14	A	Sunitinib	23.3
KIT T670E	5	Eu-anti-GST	2	Tracer 236	30	45	A	Staurosporine	118
KIT V559D T670I	1	Eu-anti-GST	2	Tracer 222	1	1.7	A	Sunitinib	1.31
KIT V654A	5	Eu-anti-His	2	Tracer 178	10	10	A	Dasatinib	18.0
KIT Y823D	20	Eu-anti-GST	2	Tracer 222	30	25	A	Sunitinib	40.5
LATS1	5	Eu-anti-GST	2	Tracer 236	100	47	A	Staurosporine	13.0
LATS2	5	Eu-anti-GST	2	Tracer 236	10	8	A	Staurosporine	3.88
LIMK1	5	Eu-anti-His	2	Tracer 236	100	111	A	Staurosporine	24.7
LIMK2	5	Eu-anti-GST	2	Tracer 178	50	37	A	Dasatinib	244
MAP2K1 (MEK1)	5	Eu-anti-His	2	Tracer 236	100	63	A	Staurosporine	8.18
MAP2K1 (MEK1) S218D S222D	5	Eu-anti-His	2	Tracer 236	30	40	A	Staurosporine	4.84
MAP2K2 (MEK2)	5	Eu-anti-His	2	Tracer 236	100	53	A	Staurosporine	11.0
MAP2K4 (MEK4)	5	Eu-anti-GST	2	Tracer 236	30	34	A	Staurosporine	13.5
MAP2K5 (MEK5)	5	Eu-anti-GST	2	Tracer 178	20	23	A	Dasatinib	31.3
MAP2K6 (MKK6)	5	Eu-anti-His	2	Tracer 236	100	76	A	Staurosporine	5.01
MAP2K6 (MKK6) S207E T211E	20	Eu-anti-His	2	Tracer 236	100	60	A	Staurosporine	5.69
MAP3K10 (MLK2)	5	Eu-anti-GST	2	Tracer 236	10	11	A	Staurosporine	4.80
MAP3K11 (MLK3)	2.5	Eu-anti-GST	2	Tracer 236	10	5.1	A	Staurosporine	1.37
MAP3K14 (NIK)	10	Eu-anti-GST	2	Tracer 236	100	61	A	Staurosporine	29.1
MAP3K2 (MEKK2)	15	Eu-anti-GST	2	Tracer 236	100	121	A	Staurosporine	8.09
MAP3K3 (MEKK3)	15	Eu-anti-GST	2	Tracer 236	100	184	A	Staurosporine	7.16
MAP3K5 (ASK1)	20	Eu-anti-GST	2	Tracer 236	100	308	A	Staurosporine	11.0
MAP3K7/MAP3K7IP1 (TAK1-TAB1)	10	Eu-anti-His	2	Tracer 222	30	87	A	Sunitinib	16.4
MAP4K1 (HPK1)	5	Eu-anti-GST	2	Tracer 222	10	16	A	Sunitinib	7.13
MAP4K3 (GLK)	5	Eu-anti-GST	2	Tracer 236	10	6.1	A	Staurosporine	0.559
MAPK10 (JNK3)	5	Eu-anti-GST	2	Tracer 236	30	11	A	Staurosporine	158

Kinase	Kinase Conc (nM)	Antibody	Antibody Conc (nM)	Tracer	Tracer Conc (nM)	Tracer Kd (nM)	Buffer	Known Inhibitor	IC50 (nM)
MAPK15 (ERK7)	5	Eu-anti-His	2	Tracer 236	30	26	A	Staurosporine	4.79
MAPK8 (JNK1)	5	Eu-anti-His	2	Tracer 236	100	29	A	Staurosporine	108
MAPK9 (JNK2)	5	Eu-anti-His	2	Tracer 199	5	5.2	A	SB202190	161
MASTL	5	Eu-anti-GST	2	Tracer 236	100	117	A	Staurosporine	28.4
MERTK (cMER) A708S	5	Eu-anti-GST	2	Tracer 236	30	29	A	Staurosporine	17.9
MET D1228H	5	Eu-anti-GST	2	Tracer 236	10	15	A	Staurosporine	29.0
MKNK2 (MNK2)	5	Eu-anti-GST	2	Tracer 236	100	235	A	Staurosporine	8.96
MLCK (MLCK2)	5	Eu-anti-GST	2	Tracer 236	30	46	A	Staurosporine	13.3
MLK4	5	Eu-anti-GST	2	Tracer 236	30	28	K	Staurosporine	2.58
MYLK (MLCK)	5	Eu-anti-GST	2	Tracer 236	30	25	A	Staurosporine	11.2
MYLK4	5	Eu-anti-GST	2	Tracer 222	100	173	A	Sunitinib	19.9
MYO3A (MYO3 alpha)	5	Eu-anti-GST	2	Tracer 236	30	41.3	A	Staurosporine	27.0
MYO3B (MYO3 beta)	5	Eu-anti-GST	2	Tracer 236	10	13	A	Staurosporine	5.82
NEK8	5	Eu-anti-GST	2	Tracer 236	30	53	A	Staurosporine	13.3
NLK	5	Eu-anti-GST	2	Tracer 178	50	67	A	Dasatinib	500
NUAK2	20	Eu-anti-GST	2	Tracer 222	100	57	A	Sunitinib	225
PKMYT1	2.5	Eu-anti-GST	2	Tracer 178	1	1.1	A	Dasatinib	60.3
PKN2 (PRK2)	1	Eu-anti-GST	2	Tracer 236	1	1.1	A	Staurosporine	0.514
PLK4	1	Eu-anti-GST	2	Tracer 236	1	1.7	A	Staurosporine	1.11
PRKACB (PRKAC beta)	1	Eu-anti-GST	2	Tracer 236	1	1.6	A	Staurosporine	0.757
PRKACG (PRKAC gamma)	5	Eu-anti-GST	2	Tracer 236	10	9.4	A	Staurosporine	1.61
RAF1 (cRAF) Y340D Y341D	5	Eu-anti-GST	2	Tracer 178	10	10	A	Dasatinib	110
RET G691S	5	Eu-anti-GST	2	Tracer 236	10	12	A	Staurosporine	2.96
RET M918T	5	Eu-anti-GST	2	Tracer 236	10	11	A	Staurosporine	2.38
RET V804M	2.5	Eu-anti-GST	2	Tracer 236	1	2.2	A	Staurosporine	0.535
RIPK2	5	Eu-anti-His	2	Tracer 178	10	8	A	Dasatinib	3.39
RIPK3	5	Eu-anti-GST	2	Tracer 178	20	23	A	Dasatinib	21.3
SIK1	5	Eu-anti-GST	2	Tracer 236	10	17	A	Staurosporine	0.788
SIK3	5	Eu-anti-GST	2	Tracer 236	1	4.3	A	Staurosporine	0.403
SLK	5	Eu-anti-GST	2	Tracer 236	10	11	A	Staurosporine	2.21
STK16 (PKL12)	10	Eu-anti-His	2	Tracer 236	100	141	A	Staurosporine	74.3
STK17A (DRAK1)	10	Eu-anti-GST	2	Tracer 236	30	65	A	Staurosporine	5.23
STK17B (DRAK2)	5	Eu-anti-GST	2	Tracer 222	100	107	W	Sunitinib	95.9
STK32B (YANK2)	5	Eu-anti-GST	2	Tracer 236	10	11	A	Staurosporine	10.5

Kinase	Kinase Conc (nM)	Antibody	Antibody Conc (nM)	Tracer	Tracer Conc (nM)	Tracer Kd (nM)	Buffer	Known Inhibitor	IC50 (nM)
STK32C (YANK3)	5	Eu-anti-GST	2	Tracer 236	100	85	A	Staurosporine	29.6
STK33	5	Eu-anti-His	2	Tracer 236	30	25	A	Staurosporine	0.783
STK38 (NDR)	5	Eu-anti-GST	2	Tracer 236	100	92	A	Staurosporine	39.8
STK38L (NDR2)	5	Eu-anti-GST	2	Tracer 236	100	81	A	Staurosporine	31.0
STK39 (STLK3)	20	Eu-anti-His	2	Tracer 236	250	480	R	Staurosporine	94.4
TAOK1	2.5	Eu-anti-GST	2	Tracer 236	1	2.9	A	Staurosporine	0.280
TAOK3 (JIK)	5	Eu-anti-GST	2	Tracer 236	100	67	A	Staurosporine	7.72
TEC	1	Eu-anti-His	2	Tracer 178	1	1	A	Dasatinib	70.9
TEK (TIE2) R849W	5	Eu-anti-GST	2	Tracer 236	30	48	A	Staurosporine	9.16
TEK (TIE2) Y1108F	10	Eu-anti-GST	2	Tracer 236	100	94	A	Staurosporine	15.4
TESK1	1	Eu-anti-GST	2	Tracer 178	1	2.3	A	Dasatinib	23.4
TGFBR1 (ALK5)	5	Eu-anti-GST	2	Tracer 178	10	30	A	Dasatinib	39.8
TGFBR2	5	Eu-anti-GST	2	Tracer 199	100	166	A	SB202190	390
TLK1	5	Eu-anti-GST	2	Tracer 236	10	16	A	Staurosporine	4.07
TLK2	5	Eu-anti-GST	2	Tracer 236	10	16	A	Staurosporine	0.957
TNIK	2.5	Eu-anti-GST	2	Tracer 236	5	6.6	A	Staurosporine	2.15
TNK2 (ACK)	5	Eu-anti-GST	2	Tracer 236	30	23	A	Staurosporine	4.52
TTK	5	Eu-anti-GST	2	Tracer 236	30	59	A	Staurosporine	34.2
ULK1	5	Eu-anti-GST	2	Tracer 236	10	18	A	Staurosporine	6.12
ULK2	2.5	Eu-anti-GST	2	Tracer 236	10	9.6	A	Staurosporine	1.80
ULK3	5	Eu-anti-His	2	Tracer 236	30	54	K	Staurosporine	9.74
VRK2	5	Eu-anti-GST	2	Tracer 178	100	106	J	Dasatinib	2,060
WEE1	5	Eu-anti-GST	2	Tracer 178	50	24	A	Dasatinib	257
WNK1	5	Eu-anti-His	2	Tracer 1710	250	254.6	A	PP121	39.6
WNK2	5	Eu-anti-GST	2	Tracer 178	50	24	A	Dasatinib	735
WNK3	20	Eu-anti-His	2	Tracer 1710	100	156	K	PP121	46.0
ZAK	5	Eu-anti-GST	2	Tracer 178	50	6.7	A	Dasatinib	33.0

Buffer A: 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA
Buffer J: 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 1 mM DTT
Buffer K: 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.01% NaN3
Buffer R: 32.5 mM HEPES pH 7.5, 50 mM NaCl, 0.015% CHAPS, 1.5 mM MgCl2, 0.5 mM EGTA
Buffer W: 57.5 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 1 mM EGTA, 3 mM MgCl2, 2 mM DTT