



ASSAY THEORY _____ **2**

LANTHASCREEN® EU KINASE BINDING ASSAY CONDITIONS _____ **3**

LANTHASCREEN® EU KINASE BINDING ASSAY CONTROLS _____ **3**

LANTHASCREEN® EU KINASE BINDING ASSAY DATA ANALYSIS _____ **4**

KINASE-SPECIFIC ASSAY CONDITIONS _____ **5**

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.

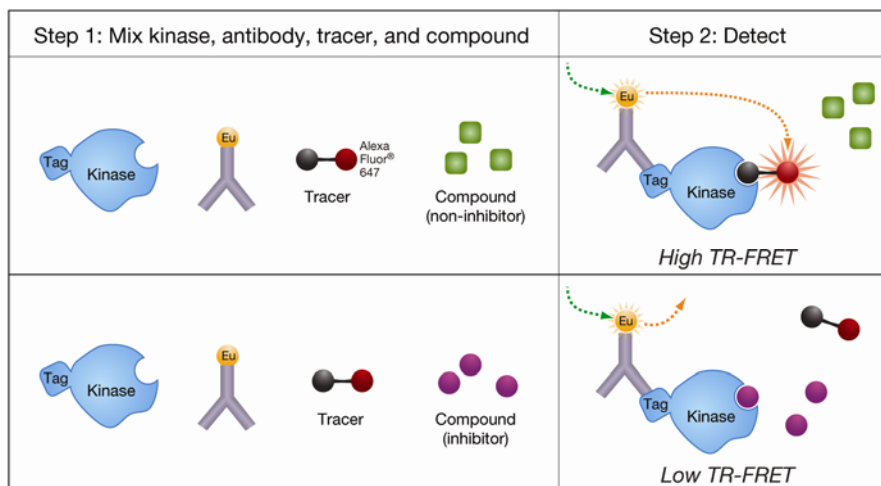


Figure 1. Schematic of the LanthaScreen® Eu Kinase Binding assay

Invitrogen’s 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. 4.0 µL – 4X Test Compound in Kinase Buffer or 160 nL 100X Test Compound in 100% DMSO plus 3.84 µL Kinase Buffer
2. 8.0 µL – 2X Kinase/Antibody Mixture
3. 4.0 µL – 4X Tracer
4. 30-second plate shake
5. 60-minute incubation at room temperature
6. 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 IC₅₀ 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	$\left\{ \frac{\text{ER}_{0\% \text{ Disp Ctrl}} - \text{ER}_{\text{Sample}}}{\text{ER}_{0\% \text{ Disp Ctrl}} - \text{ER}_{100\% \text{ Disp Ctrl}}} \right\} * 100$
Difference Between Data Points (single point only)	$ \% \text{ Displacement}_{\text{Point 1}} - \% \text{ Displacement}_{\text{Point 2}} $
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)	$1 - \frac{3 * \text{Stdev}_{0\% \text{ Disp Ctrl}} + 3 * \text{Stdev}_{100\% \text{ Disp Ctrl}}}{ \text{Mean}_{0\% \text{ Disp Ctrl}} - \text{Mean}_{100\% \text{ Disp Ctrl}} }$

Graphing Software

SelectScreen® Kinase Profiling Service uses *XLfit* 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)	Buffer	Known Inhibitor	IC50 (nM)
ACVR1 (ALK2)	5	Eu-anti-GST	2	Tracer 236	100	A	Staurosporine	73.2
BRAF	5	Eu-anti-GST	2	Tracer 178	20	A	Dasatinib	64.8
BRAF V599E	5	Eu-anti-GST	2	Tracer 178	20	A	Dasatinib	56.8
CAMKK1 (CAMKKA)	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	3.07
CAMKK2 (CaMKK beta)	5	Eu-anti-GST	2	Tracer 236	10	A	Staurosporine	1.98
CDK8/cyclin C	5	Eu-anti-His	2	Tracer 236	10	A	Staurosporine	10.6
CDK9/cyclin K	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	10.6
DDR2	5	Eu-anti-GST	2	Tracer 178	10	A	Dasatinib	11.2
DMPK	5	Eu-anti-GST	2	Tracer 236	30	A	Staurosporine	1.33
EPHA7	5	Eu-anti-GST	2	Tracer 236	100	A	Staurosporine	66.4
KIT V654A	5	Eu-anti-His	2	Tracer 178	10	A	Dasatinib	15.6
LIMK1	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	11.6
LIMK2	5	Eu-anti-GST	2	Tracer 178	50	A	Dasatinib	157
MAP2K1 (MEK1)	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	11.1
MAP2K1 (MEK1) S218D S222D	5	Eu-anti-His	2	Tracer 236	30	A	Staurosporine	5.52
MAP2K2 (MEK2)	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	13.8
MAP2K3 (MEK3)	5	Eu-anti-GST	2	Tracer 236	100	A	Staurosporine	5.18
MAP2K6 (MKK6)	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	5.21
MAP2K6 (MKK6) S207E T211E	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	2.19
MAP3K10 (MLK2)	5	Eu-anti-GST	2	Tracer 236	10	A	Staurosporine	4.41
MAP3K11 (MLK3)	5	Eu-anti-GST	2	Tracer 236	10	A	Staurosporine	0.918
MAP3K14 (NIK)	5	Eu-anti-GST	2	Tracer 236	100	A	Staurosporine	19.2
MAP3K2 (MEKK2)	5	Eu-anti-GST	2	Tracer 236	100	A	Staurosporine	4.08
MAP3K3 (MEKK3)	5	Eu-anti-GST	2	Tracer 236	100	A	Staurosporine	6.29
MAP3K5 (ASK1)	20	Eu-anti-GST	2	Tracer 236	100	A	Staurosporine	11.1
MAP3K7/MAP3K7IP1 (TAK1-TAB1)	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	21.1
MAPK10 (JNK3)	5	Eu-anti-GST	2	Tracer 236	30	A	Staurosporine	104
MAPK8 (JNK1)	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	134
MAPK9 (JNK2)	5	Eu-anti-His	2	Tracer 199	5	A	SB202190	265
MLCK (MLCK2)	5	Eu-anti-GST	2	Tracer 236	30	A	Staurosporine	16.0
MYLK (MLCK)	5	Eu-anti-GST	2	Tracer 236	30	A	Staurosporine	16.2
NLK	5	Eu-anti-GST	2	Tracer 178	50	A	Dasatinib	373
RAF1 (cRAF) Y340D Y341D	5	Eu-anti-GST	2	Tracer 178	10	A	Dasatinib	76.2
RIPK2	5	Eu-anti-His	2	Tracer 178	10	A	Dasatinib	3.59

**SelectScreen® Biochemical Kinase Profiling Service****LanthaScreen® Eu Kinase Binding Assay Screening Protocol and Assay Conditions**Revised 02-JUL-09
Page 6 of 6

Kinase	Kinase Conc (nM)	Antibody	Antibody Conc (nM)	Tracer	Tracer Conc (nM)	Buffer	Known Inhibitor	IC50 (nM)
SLK	5	Eu-anti-GST	2	Tracer 236	10	A	Staurosporine	2.40
STK16 (PKL12)	5	Eu-anti-His	2	Tracer 236	100	A	Staurosporine	95.7
STK17A (DRAK1)	5	Eu-anti-GST	2	Tracer 236	30	A	Staurosporine	3.49
STK33	5	Eu-anti-His	2	Tracer 236	30	A	Staurosporine	0.558
TAOK3 (JIK)	5	Eu-anti-GST	2	Tracer 236	100	A	Staurosporine	9.62
TEC	1	Eu-anti-His	2	Tracer 178	1	A	Dasatinib	38.5
TNK2 (ACK)	5	Eu-anti-GST	2	Tracer 236	30	A	Staurosporine	4.85
TTK	5	Eu-anti-GST	2	Tracer 236	30	A	Staurosporine	47.3
WEE1	5	Eu-anti-GST	2	Tracer 178	50	A	Dasatinib	214
WNK2	5	Eu-anti-GST	2	Tracer 178	50	A	Dasatinib	736
ZAK	5	Eu-anti-GST	2	Tracer 178	50	A	Dasatinib	22.2

Kinase Buffer A: 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA