# Measure inhibitor binding to non-activated kinases

## LanthaScreen® Eu Kinase Binding Assay

Kinase activity assays are cost-effective and widely used to drive early drug discovery and lead identification efforts. However, activity-based assays are limited in that they are suitable only for identification and characterization of compounds that bind to active (e.g., phosphorylated) forms of a kinase. Thus, such methods are not well suited to study compounds such as imatinib (Gleevec®) that bind preferentially to the non-activated (dephosphorylated) state of Abl [1]. Targeting a non-activated state may be preferential for multiple reasons. For example, higher selectivity is observed for some compounds that preferentially bind to non-activated states due to interactions with less conserved residues that are only exposed in the non-active state. Targeting a non-activated state may also be beneficial because there is less competition from intracellular ATP and it may give the researcher the ability to discover novel chemotypes.

To this end, we have developed a TR-FRET binding assay platform (Figure 1) that enables the characterization of compound binding to non-activated as well as activated states of a kinase. We present examples of compound affinity comparisons for active and non-activated forms, including kinase inhibitors with various binding mechanisms.

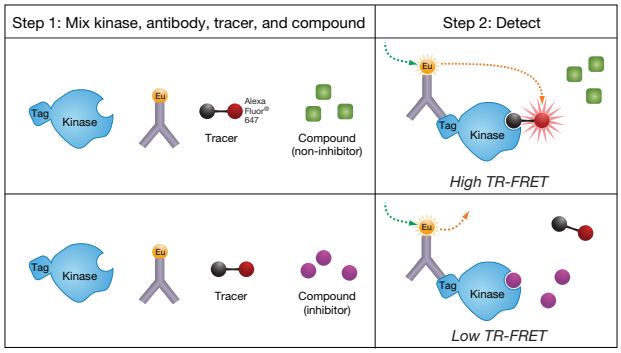


Figure 1. LanthaScreen® Eu Kinase binding assay schematic.

## LanthaScreen® Eu Kinase Binding Assay

The LanthaScreen® Eu Kinase Binding Assay (Figure 1) provides a simple means of assessing the ability of compounds to bind to specific kinases, which is particularly useful when investigating a target with no available activity assay, or when examining a compound binding to non-activated (e.g., non-phosphorylated) kinase states. Even in cases where an activity assay is available, the simplicity of the LanthaScreen® Eu Kinase Binding Assay and its ability to generate real-time binding data are advantages over many kinase assays. The basis of this technology is an ATP-competitive tracer, which binds to the kinase of interest and is detected by a europium-labeled anti-tag antibody also bound to the kinase of interest. When both tracer and antibody are bound, there is a high TR-FRET signal. When the tracer is displaced by a kinase inhibitor, there is a loss of the TR-FRET signal.

## Kinase inhibitor types

Most kinase inhibitors can be grouped into one of three types, based on the location of their binding sites (Figure 2). Type I inhibitors are the most common and bind exclusively to the ATP site. Type II inhibitors bind to both the ATP site and an adjacent hydrophobic site exposed in the non-activated kinase state. Type III inhibitors bind exclusively outside of the ATP-binding site.

## Targeting non-activated kinase states

While initial discoveries of inhibitors that bind preferentially to non-activated kinase states may have been serendipitous, such a binding mode may have advantages from a therapeutic research perspective. ATP pockets are highly conserved among kinases; thus, developing highly selective ATP-competitive inhibitors is challenging. However, some compounds that bind preferentially to non-activated states have been shown to interact with less conserved sites, leading to greater selectivity. Hence, a simple means of measuring binding to non-active states may have utility for primary screening and mechanistic studies. In addition, the ability to measure binding to both activated and non-activated kinase states can provide additional selectivity information not obtainable using activity assays alone. We provide three test cases comparing inhibitor affinities for active and non-activated kinase states of Type I, Type II, and Type III inhibitors.

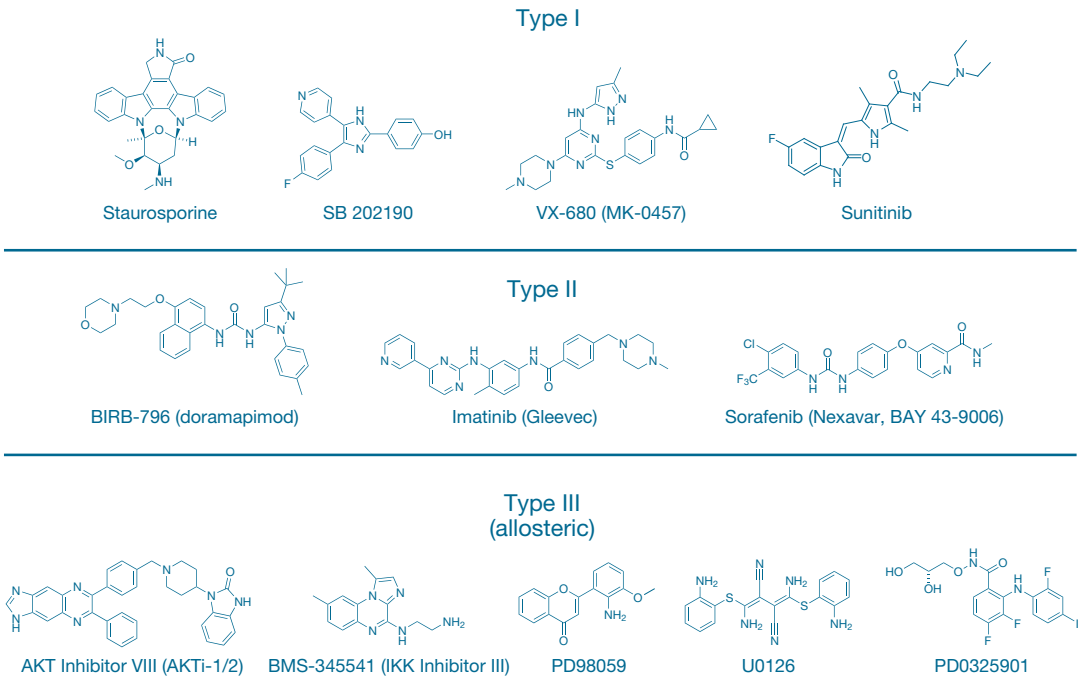


Figure 2. Examples of kinase inhibitor types.

## Abl: Preferential binding of Type II inhibitors

The classic case of preferential binding of imatinib to a non-phosphorylated form of Abl was studied with the LanthaScreen® Eu Kinase Binding Assay. To generate a dephosphorylated form of Abl, an active preparation was treated with a phosphatase. $IC_{50}$ curves show a strong preference for binding to the dephosphorylated Abl, not only for imatinib but also the Type II inhibitors sorafenib and BIRB-796 (Figure 3). In contrast, the Type I inhibtors staurosporine, sunitinib, and VX-680 display similar affinities for the two activation states.

In the case of Abl, dephosphorylation can be achieved with YOP or PTP1B phosphatases. Such treatments have also been successful for numerous other tyrosine kinases (data not shown). It is important to note that for such experiments the phosphatase can be left in the kinase preparation, as it will not impact assay results. This is in direct contrast to activity assays, which are generally hindered by the presence of phosphatase activity.

## RSK2: Preferential binding of Type I inhibitors

Although differential binding of Type I inhibitors to activated and non-activated kinase states is not widely recognized, RSK2 provides an example where binding of several Type I compounds is dependent on phosphorylation status. The inhibitors VX-680, sunitinib (Sutent®), and PP2 all show between 8- and 70-fold more potent $IC_{50}$ values for non-activated RSK2 as compared to the activated protein (Figure 4). The non-activated RSK2 used in this experiment was produced in *E. coli*. This production method provides another approach to producing dephosphorylated kinases for such studies.

## MEK: Preferential binding of Type III inhibitors

The third test case highlights an example of Type III compounds that bind preferentially to non-activated kinase states. The Type I inhibitors sunitinib and staurosporine display less than a 4-fold difference in affinity between constitutively active MEK1 (S218D, S222D) and non-activated MEK1, whereas the allosteric and non–ATP-competitive inhibitors PD98059 and PD0325901 display greater than 20-fold higher affinity for the non-activated state of MEK1 (Figure 5).

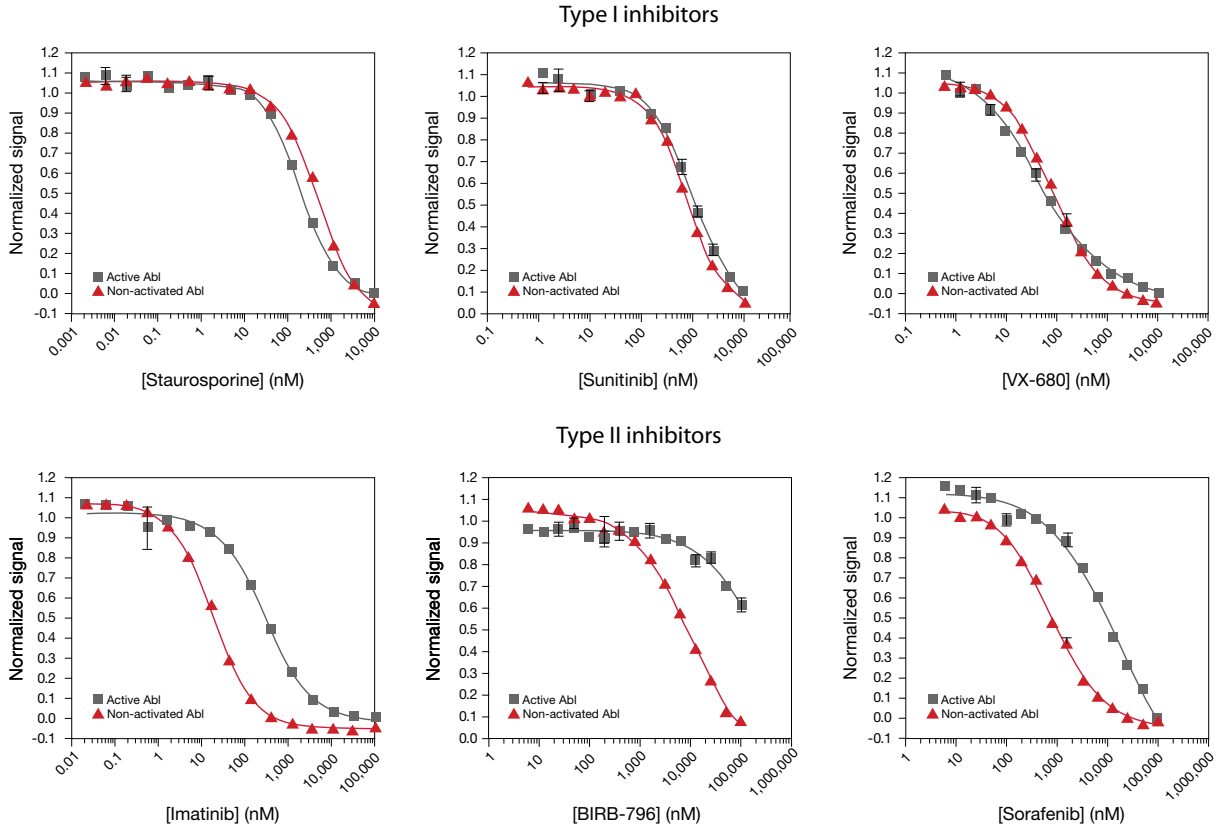


**Figure 3. Comparison of compound $IC_{50}$ values obtained for active and non-activated Abl.** Competitive binding experiments were performed under optimized conditions for active Abl and Abl that had been treated with phosphatase (non-activated Abl). The assay detects higher affinity interactions of non-activated Abl and the Type II inhibitors imatinib, BIRB-796, and sorafenib, whereas the $IC_{50}$ values detected for the Type I inhibitors staurosporine, sunitinib, and VX-680 are similar for the two activation states.

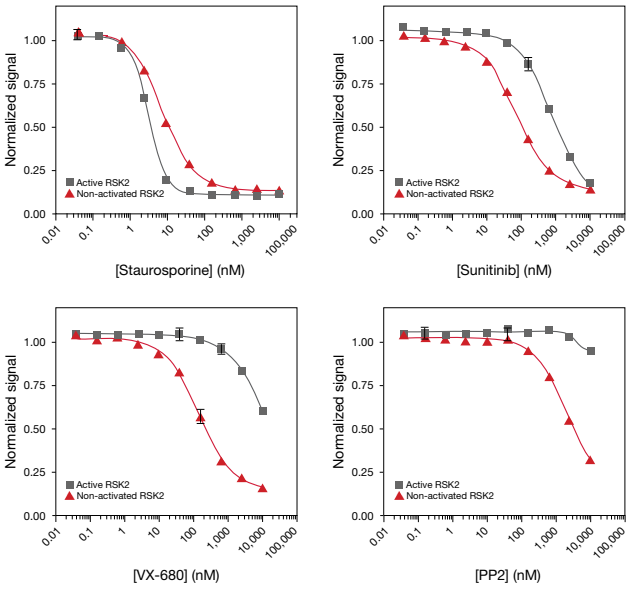


Figure 4. Preferential binding of Type I inhibitors to non-activated RSK2. Competitive binding experiments were performed under optimized conditions for active and non-activated preparations of RSK2 (RPS6KA3). The non-activated RSK2 used was wild-type protein that was not treated with any upstream activators. The active RSK2 used was wild-type protein that was activated by ERK2 and PDK1. Three Type I inhibitors tested bind with greater than 8-fold higher affinity to the non-activated state of RSK2.

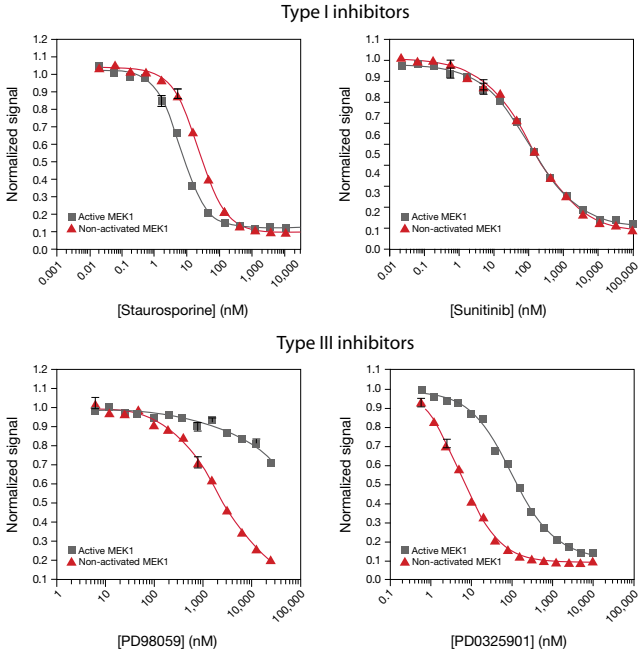


Figure 5. Preferential binding of MEK1 inhibitors PD98059 and PD0325901 to non-activated MEK1. Competitive binding experiments were performed under optimized conditions for active and non-activated preparations of MEK1. The non-active MEK1 used was wild-type protein that was not treated with any upstream activators. The active MEK1 used was a constitutively active mutant (S218D, S222D).

## Conclusions

The LanthaScreen® Eu Kinase Binding Assay provides a straightforward and flexible means of directly targeting non-activated forms of kinases not easily examined with other assay formats. In addition, these studies demonstrate that Type I, II, and III kinase inhibitors can all exhibit differential binding (based on kinase activation states), and the LanthaScreen® Eu Kinase Binding Assay makes it possible to measure these various compound affinities.

### Reference

1. Schindler T, Bornmann W, Pellicena P et al. (2000) Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 289(5486):1938–1942.

## Ordering information

| Product | Quantity | Cat. No. |
|---|---|---|
| 5X Kinase Buffer A | 4 mL | PV3189 |
| Kinase Tracer 178 | 25 µL | PV5593 |
| Kinase Tracer 199 | 25 µL | PV5830 |
| Kinase Tracer 236 | 25 µL | PV5592 |
| LanthaScreen® Eu-Anti-GST Antibody | 25 µg | PV5594 |
| LanthaScreen® Eu-Anti-His Antibody | 25 µg | PV5596 |
| LanthaScreen® Eu-Anti-DYKDDDDK Antibody | 25 µg | PV6026 |
| LanthaScreen® Eu-Streptavidin | 25 µg | PV5899 |

To learn more about the LanthaScreen® Eu Kinase Binding Assay, visit www.invitrogen.com/bindingassay.