A flexible kinase inhibitor assay platform for active, non-activated, and impure kinase preparations

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ABSTRACT

Kinase activity-based assays are cost-effective and widely used to drive early drug discovery and lead optimization. However, it is possible to satisfy these requirements for the well-studied kinases, there are many cases which lack one or more of these measures, such as CDK1 and STK38, making drug discovery efforts difficult. To address these assay development problems, we have developed a TR-FRET-based binding assay platform which allows for characterization of compounds with kinase that are not sufficiently purified or do not demonstrate measurable activity against potential or potential substrates. This assay addresses kinase assay requirements early in the drug discovery process, and should enable more targets to be screened sooner, resulting in compounds being made available for lead optimization faster. The ability to detect and characterize binding of compounds to non-activated kinase is also a benefit to the lead optimization process, as compounds that bind preferentially to kinases in their non-activated state, such as Imatinib (Gleevec®), are sometimes preferred due to their selectivity. In this paper, we present solutions for kinase that are difficult to address with activity assays and provide a comparison of compound activities for active and non-activated forms of a diverse set of kinase to enable faster lead optimization.

RESULTS

Competitive binding experiments were performed under optimized conditions for active Abl and Abl which had been treated with phosphatase (Non-activated Abl). The assay detects the higher affinity interactions of non-activated Abl and the Type II inhibitors inhibits MEK1 (Phospho-PKCa/PIK3CA/PIK3R1), CDK8, STK33, and TBK1 with IC50 values that comparable to literature values. One advantage of the binding assay over activity assay is the ability to rapidly develop assays for kinases with low intrinsic activity (e.g. CDK9) or without knowledge of the substrate (e.g. STK33).

Figure 1. Lanthascreen® EU Kinase Binding Assay

• Easy-to-optimize, mix-and-read assay format
• Broaden target coverage to include challenging kinases
• No substrate required
• Analyze low activity or non-active kinases
• Specificity-driven by epitope tag
• Complement existing work for downstream applications (easily detect Type I, II or III (allosteric) inhibitors)
• Track side binding inhibitors in real-time
• Compare on/off rates

Table 1. Assay Conditions for Inhibitor Screening

Lanthascreen® EU Kinase Binding Assay to evaluate assays are typically prepared by addition of 2 components at 2x the final concentration as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEP 1</td>
<td>Add 5 µL of test compound</td>
<td>100 nM</td>
</tr>
<tr>
<td>STEP 2</td>
<td>Add 5 µL of kinase/receptor mixture</td>
<td>100 nM</td>
</tr>
<tr>
<td>STEP 3</td>
<td>Add 5 µL of tracer</td>
<td>100 nM</td>
</tr>
<tr>
<td>STEP 4</td>
<td>Incubate for 1 hour at room temperature and read plate</td>
<td></td>
</tr>
</tbody>
</table>

Competitive binding experiments were performed under optimized conditions for active Abl and non-activated preparations of MEK1. The non-activated MEK1 used was wild-type protein that was not treated with any upstream activators. The active MEK1 used was a constitutively active mutant (S199E, D200E). The Type I inhibitors inhibited MEK1 (Phospho-PKCa/PIK3CA/PIK3R1), CDK8, STK33, and TBK1 with IC50 values for the Type I inhibitors similar to those observed for the Type II inhibitors. The Type I inhibitors had lower IC50 values for the non-activated MEK1.

Figure 2. Assays for challenging targets

Figure 3. Binding assays are resistant to contaminating kinases

Table 2. Assay Conditions for Inhibitor Screening

Lanthascreen® EU Kinase Binding Assay to evaluate assays are typically prepared by addition of 3 components at 3x the final concentration as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Incubate for 1 hour at room temperature and read plate</td>
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</tbody>
</table>

Typical final assay conditions are 3x MEK1, 2x [3H] ATP, and the tracer equal to 1x near the Kd value.

Protocols and assay conditions for specific kinases can be found at http://www.invitrogen.com/bindingassaytable.

REFERENCES


http://www.invitrogen.com/bindingassay

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