

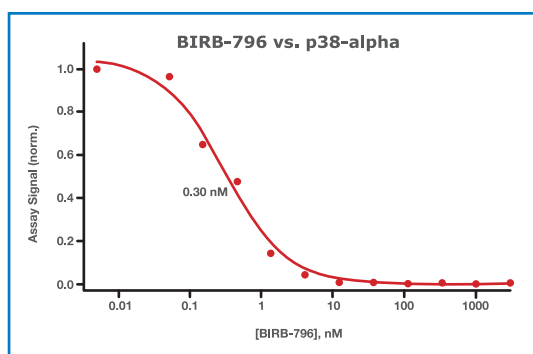
## Detection of Allosteric Inhibitors with KINOMEScan™

### Measure Binding to ATP-competitive & Non-ATP-competitive Kinase Inhibitors

A majority of kinase inhibitors developed to date are ATP-competitive and target the ATP binding site. Targeting kinases outside of the conserved ATP site is thought to be an effective strategy to achieve greater selectivity & cellular potency and to identify inhibitors with an attractive intellectual property position. Compounds of this class are often described as “allosteric inhibitors”. The definition of an allosteric kinase inhibitor is somewhat subjective and can vary among investigators. Five distinct classes of “allosteric kinase inhibitors” have been described that include both ATP-competitive and non-ATP-competitive compounds. KINOMEScan affords investigators a flexible screening solution for the detection of multiple inhibitor types and to extract the maximum value from their kinase-focused chemical assets.

#### ATP-competitive

**Inhibitors that penetrate into an allosteric pocket adjacent to the ATP site that is created when the activation loop adopts a catalytically inactive “DFG-out” conformation.** Most inhibitors in this class, including the type II p38-alpha inhibitor BIRB-796, also make direct contact within the ATP site. Members of this class that do not contact the ATP site nevertheless modify the shape of the ATP site by stabilizing the DFG-out inactive activation loop conformation and are indirectly ATP-competitive. The “allosteric” designation for some inhibitors of this class is controversial. KINOMEScan assays detect this inhibitor class.

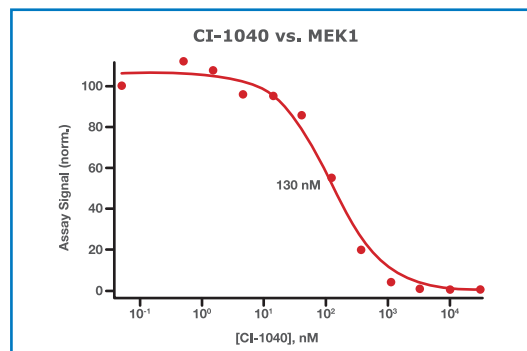


**Figure 1.** Binding constant (Kd) determinations for BIRB-796, a highly potent ATP-competitive type II inhibitor that contacts the ATP site and penetrates into an “allosteric” pocket, created when the activation loop adopts a “DFG-out” inactive conformation.

#### Non-ATP-competitive

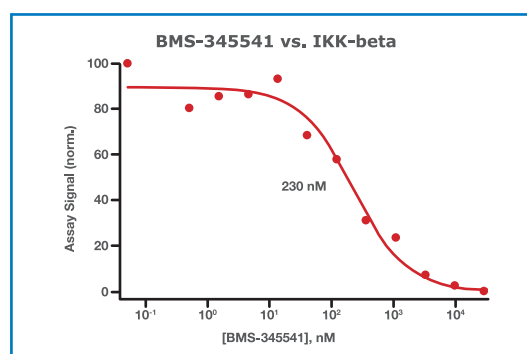
**Non-ATP-competitive inhibitors that bind in an “allosteric pocket” within the kinase domain that is distal to the ATP site and inhibit catalysis by repositioning key catalytic residues that are not required for ATP binding.** Inhibitors of this class, including CI-1040 (PD-184352), have been described for MEK. Apart from the MEK inhibitors, few examples of inhibitors targeting other kinases through this

mechanism have been reported. KINOMEScan can detect this inhibitor class.



**Figure 2.** Binding constant (Kd) determination for the non-ATP-competitive inhibitor CI-1040 against MEK1.

**Non-ATP-competitive inhibitors that are competitive with binding of protein/peptide substrate and have an allosteric effect on the active-site conformation.** The IKK-beta inhibitor BMS-345541 appears to exemplify this class, and, to our knowledge, few other examples have been reported. KINOMEScan can detect the IKK-beta-BMS-345541 interaction and should also detect other inhibitors that act through this mechanism.



**Figure 3.** Binding constant (Kd) determination for the non-ATP-competitive inhibitor BMS-345541 against IKK-beta.

**Inhibitors that are competitive with binding of protein/peptide substrate or bind to regulatory sites or domains that are distal to the active site cleft and do not affect the active-site conformation.** Examples of inhibitors in this class include the ABL inhibitor ON012380, the JNK1 inhibitor BI-78D3, GNF-2, an ABL inhibitor from GNF/Novartis which binds to the myristate site in the c-terminal lobe of the ABL kinase domain, and the PH-domain-binding AKT inhibitors. It is unlikely that current KINOMEScan assays will detect inhibitors that bind to domains other than the kinase domain (e.g. PH domains).