



Why don't you evaluate your slow binding compounds in Carna's Preincubation Assay ?



Certain classes of kinase inhibitors exhibit slow binding kinetics as shown in Figure 1.

For such compounds, preincubation (Figure 2) is necessary to assess their real maximal effect (Figure 3 and 4).

Our preincubation kinase profiling service incorporates a thirty (30) minute preincubation time at room temperature with your test compound(s) prior to measuring activity in our standard Mobility Shift Assay. This preincubation condition is validated to best capture such slow binding compounds.

Please inquire us for more detailed information.

* As of Nov 01, 2018

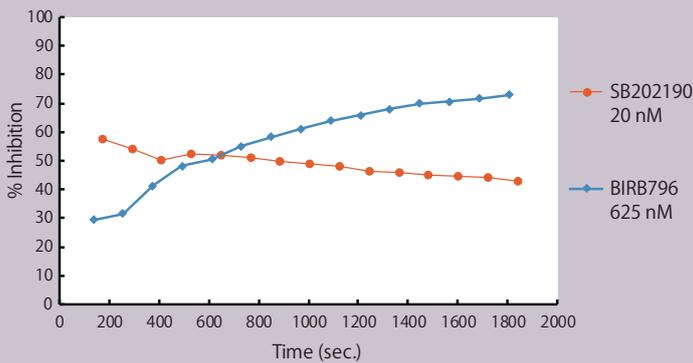
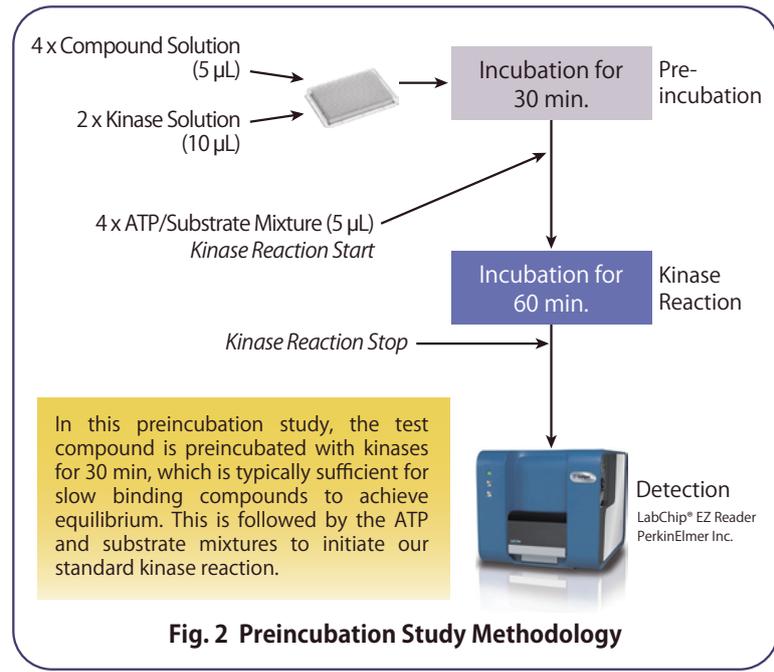


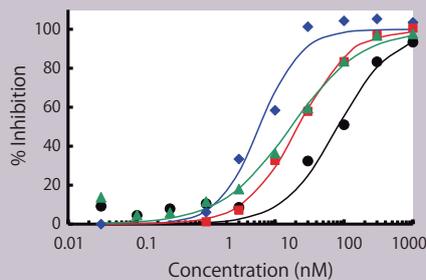
Fig. 1 Time course of p38 α inhibitors

Structurally unrelated SB202190 and BIRB796 are potent inhibitors of p38 α . BIRB796 interacts with p38 α in a manner different from SB202190, and its binding induces a slow conformational change that locks the protein into an inactive conformation. Therefore, the potency of BIRB796 increased with the period of incubation. The assay was performed at an ATP concentration of 1mM.



In this preincubation study, the test compound is preincubated with kinases for 30 min, which is typically sufficient for slow binding compounds to achieve equilibrium. This is followed by the ATP and substrate mixtures to initiate our standard kinase reaction.

Fig. 2 Preincubation Study Methodology



	Preincubation	
	+	-
BIRB796	9.5 (◆)	82 (●)
SB202190	19 (▲)	22 (■)

Fig. 3 IC₅₀ Comparison with or without Preincubation for p38 α Inhibitors

Preincubation did not affect the IC₅₀ of SB202190, however, a significant change was observed for the activity of BIRB796. The assay was performed at an ATP concentration of 1mM.

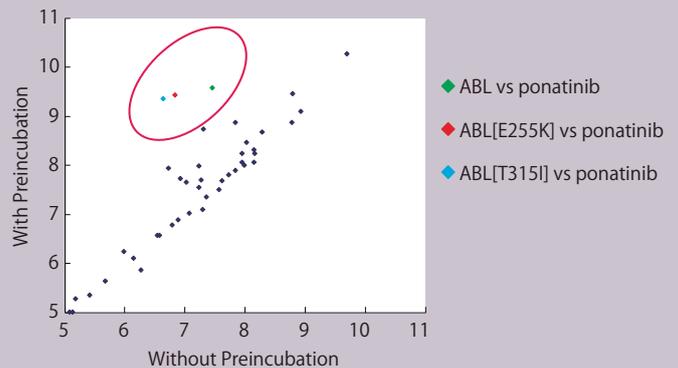


Fig. 4 IC₅₀ Comparison with or without Preincubation for Clinical Inhibitors

Several clinical kinase inhibitors and their corresponding target kinases were evaluated in a preincubation study; the resulting pIC₅₀ values are compared above. In most cases, good correlations were observed. However, Ponatinib showed enhanced inhibition of native and mutant isoforms of the ABL kinase following preincubation. All assays were performed at an ATP concentration of 1mM.

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