

The target

Aurora kinases are cytosolic serine-/threonine kinases which are highly conserved in eukaryotes and involved in many processes during cell division. Three Aurora kinases have been identified in humans and designated as Aurora-A, -B, and -C. Aurora-B localizes at the kinetochore from G2 to metaphase, and relocates to the midbody after anaphase. Aurora-B plays roles in spindle dynamics, chromosome condensation, and cytokinesis. Overexpression of Aurora-B is frequently observed in various human cancer tissues and cells overexpressing Aurora-B are tumorigenic in nude mice. Inhibitors of Aurora-B kinase activity such as VX-680 are developed for therapeutic application.

Cellular phosphorylation assay

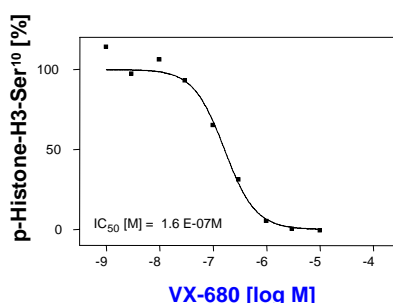
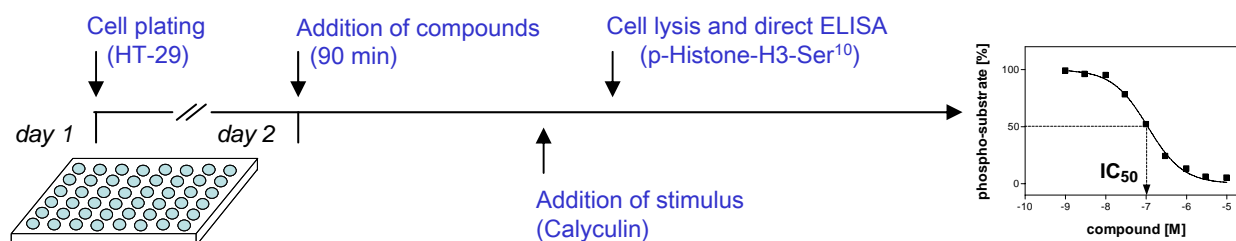


Figure 1: Assay validation. The known Aurora-B inhibitor VX-680 blocks Aurora-B and inhibits the cellular phospho-Histone-H3-Ser¹⁰ signal with highly reproducible IC₅₀ values. The graph shows the result of a representative experiment.

Using HT29 colon carcinoma cells, this cellular screening system monitors the phosphorylation level of Histone-H3 at Ser¹⁰, the direct downstream target of endogenous Aurora-B. To boost the phospho-Histone-H3 signal, phosphatase inhibitor Calyculin is added prior to cell lysis. Using this assay, we could successfully translate the effect of many Aurora-B inhibitors such as VX-680 on endoreduplication into decreased levels of phospho-Histone-H3-Ser¹⁰ which was quantitated via direct ELISA (see. Fig.1).

You ship your compounds – ProQinase performs the testing



- IC₅₀ values are determined by testing 9 compound concentrations in semi-logarithmic steps (each concentration in duplicates).
- Quality assurance is provided by calculation of Z' factors for Low/High controls on each assay plate and by including a full IC₅₀ curve for VX-680 to monitor adequate dose/response relation in your assay run.

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