Cellular Phosphorylation Assay for Haspin (GSG2)

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

➢ The Target

Haspin (GSG2) represents a serine/threonine-protein kinase that specifically phosphorylates histone H3 at Thr^3 during mitosis. Haspin associates with the chromosomes and thereby positions and activates Aurora B and other components of the chromosomal passenger complex (CPC). This activity ensures proper chromosome alignment and segregation and normal progression through the cell cycle. Haspin inhibitors such as CHR6494 have been shown to cause mitotic catastrophe in cancer cell lines such as HT-29 and to induce tumor regression in vivo, suggesting Haspin as promising target in tumor therapy.

➢ Cellular Phosphorylation Assay

Using HT-29 colon carcinoma cells, this cellular screening system monitors the phosphorylation level of Histone-H3 at Thr^3, a direct downstream target of endogenous Haspin / GSG2. To boost the phospho-Histone-H3 signal, phosphatase inhibitor Calyculin is added prior to cell lysis. Using this assay, we could successfully show the inhibitory effect of CHR6494 on the levels of phospho-Histone-H3-Thr^3 which was quantitated via direct ELISA (see. Fig.1).

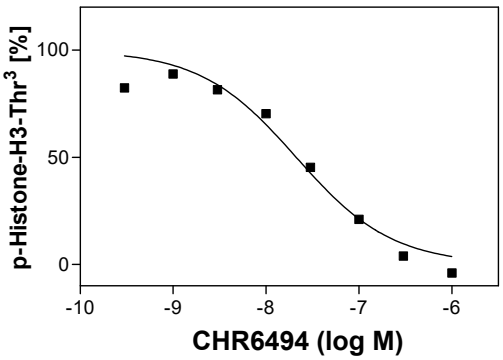
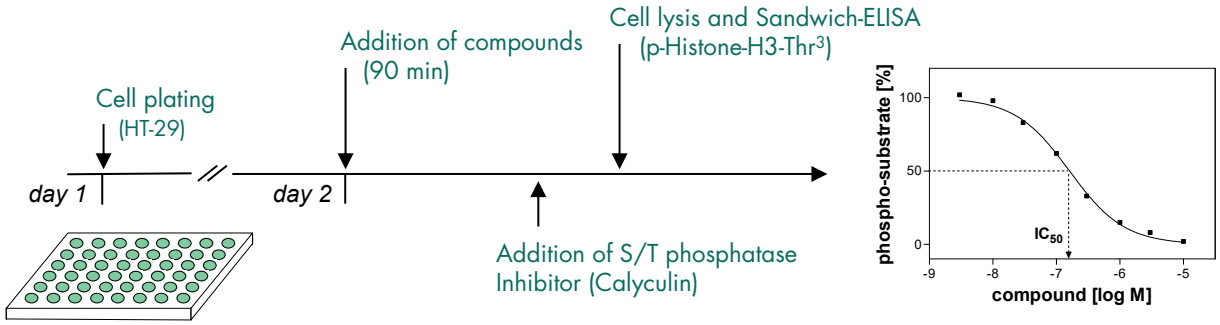


Figure 1: Assay validation.
The cognate Haspin inhibitor CHR6494 blocks Haspin and potently inhibits the cellular phospho-Histone-H3-Thr^3 signal. The graph shows the result of a representative experiment.

➢ You ship your compounds – Reaction Biology performs the testing



- IC_{50} values are determined by testing 8 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 IC_{50} curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.