Cellular Phosphorylation Assay for ROCK

ProQinase TARGETING CANCER

Cellular Assay Service

➢ The Target

The Rho-associated, coiled-coil containing serine/threonine kinases ROCK I and ROCK II (ROCK) are involved in the regulation of cytoskeletal activity. Activation of ROCK by binding to activated Rho-GTP results in the phosphorylation of numerous substrate proteins like regulatory myosin light chain (MLC) or the myosin binding subunit of MLC phosphatase (MYPT1). These phosphorylations lead to changes in cell morphology causing phenotypes like cellular contraction, formation of stress fibers or neurite retraction. Several of these processes play important roles in pathological conditions like cardiovascular diseases, neuronal degeneration or cancer.

➢ Cellular Phosphorylation Assay

The myosin light chain (MLC) in the rat aortic smooth muscle cell line A7r5 is constitutively bisphosphorylated at Thr^{18}/Ser^{19} by ROCK kinases, which directly phosphorylate MLC and simultaneously prevent its dephosphorylation through inhibition of MLC phosphatase. Inhibition of ROCK kinase by specific inhibitors like Y-27632 reduce the levels of phospho-MLC (see Fig.1). In the cellular assay phospho-levels of MLC-Thr^{18}/Ser^{19} are quantified by direct-ELISA technique.

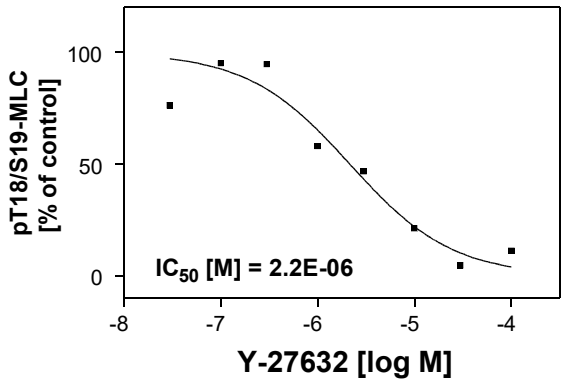
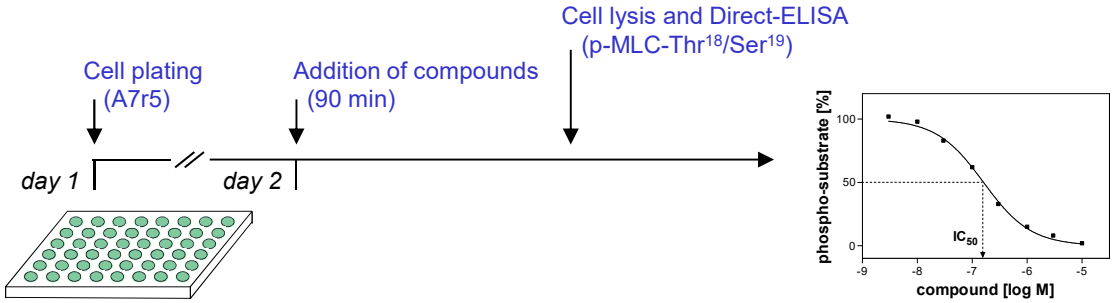


Figure 1: Assay validation.
The ROCK kinase inhibitor Y-27632 blocks ROCK and inhibits the cellular double-phosphorylation of the myosin light chain at Thr^{18} and Ser^{19} with highly reproducible IC_{50} values. The graph shows the result of a representative experiment.

➢ You ship your compounds – ProQinase 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.

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