

➤ The Target

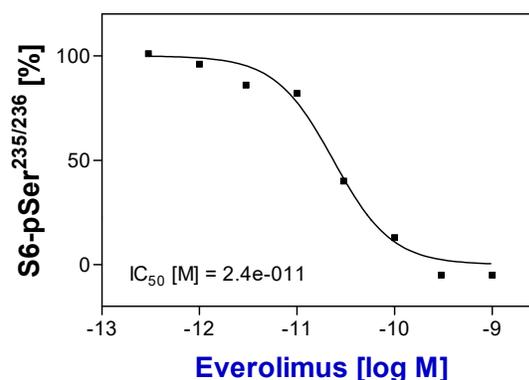
p70-S6K is a serine/threonine kinase downstream of the PI3K/AKT/mTOR pathway and belongs to the family of ribosomal S6 kinases (RSKs). It phosphorylates several residues of the 40S ribosomal protein S6 and of several other translational-regulatory factors, which results in increased protein synthesis and cell proliferation. The kinase is activated via direct phosphorylation by the protein complex mTORC1. In breast cancer cell lines and patient tumor samples overexpression of p70-S6K was found and correlated with poor prognosis.

➤ Cellular Phosphorylation Assay

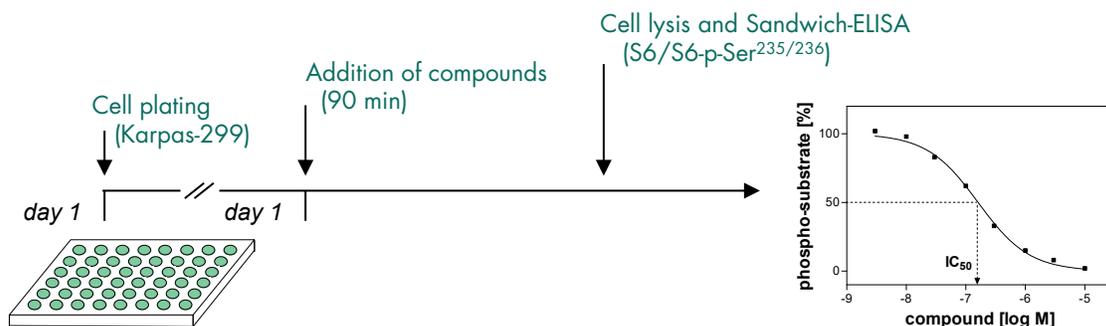
In the human T cell lymphoma cell line Karpas-299 the serine/threonine kinase p70-S6K is constitutively activated via its direct upstream kinase mTORC1. Inhibition of the mTORC1 protein complex with a specific inhibitor like Everolimus results in inactivation of S6K causing a decrease of phospho-levels of the S6K substrate 40S ribosomal protein S6 (see Fig. 1). Therefore, the cellular S6K phosphorylation assay can be used for the characterization of inhibitors of S6K as well as inhibitors of mTORC1. In the assay levels of phospho-Ser^{235/236} of S6 are quantified by Sandwich-ELISA technique.

Figure 1: Assay validation.

Everolimus specifically blocks the activation of p70-S6 kinase by inhibiting the mTORC1 protein, which is a direct upstream kinase complex of p70-S6K. Reduced activation of p70-S6 kinase subsequently results in reduction of phosphorylation of the S6 protein at Ser^{235/236}. Everolimus shows highly reproducible IC₅₀ values in the cellular p70-S6K assay. The graph shows the result of a representative experiment.



➤ You ship your compounds – Reaction Biology performs the testing



- IC₅₀ 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 IC₅₀ curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.