

# Cellular Phosphorylation Assay for MNK1

## ➤ The Target

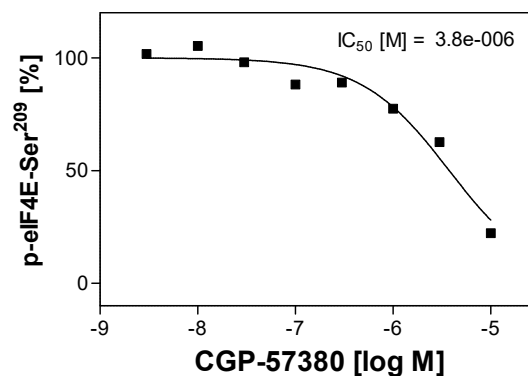
The MAP kinase interacting serine/threonine kinase 1 (MKNK1; MNK1) activates translation of proliferation-associated proteins by phosphorylation of elongation initiation factor 4E (eIF4E) at serine 209. MNK1 itself is phosphorylated and activated by the p38 kinases and kinases in the Erk pathway downstream of many receptor tyrosine kinases.

## ➤ Cellular Phosphorylation Assay

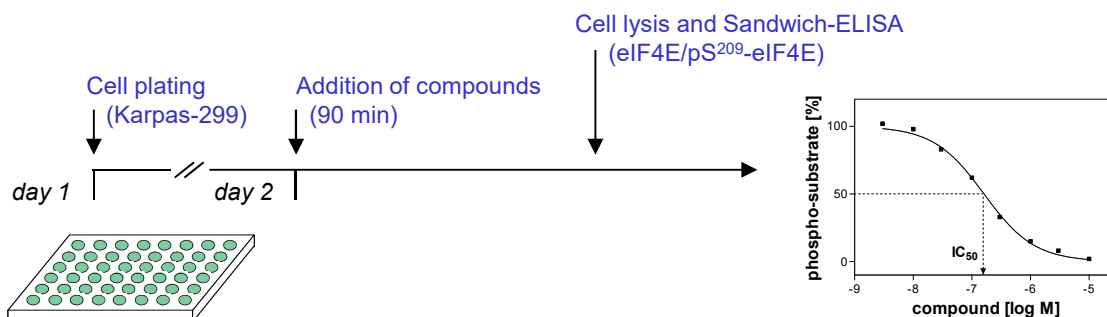
The human T cell lymphoma cell line Karpas-299 expresses a constitutively active fusion protein of the receptor tyrosine kinase ALK (NPM-ALK) which activates the ERK pathway and subsequently MNK1, resulting in phosphorylation of eIF4E at Ser<sup>209</sup>. Inhibition of MNK1 with the cognate MNK1 inhibitor CGP-57380 results in a decrease of phospho-levels of MNK1 substrate eIF4E (see Fig. 1). The cellular eIF4E phosphorylation assay is suited for the characterization of inhibitors of MNK1. In the assay, levels of phospho-Ser<sup>209</sup> of eIF4E are quantified by Sandwich-ELISA technique.

**Figure 1: Assay validation.**

The known MNK1 Inhibitor CGP-57380 (4-Amino-5-(4-fluoroanilino)-pyrazolo[3,4-d]pyrimidine) acted as inhibitor of phosphorylation of eIF4E at Ser<sup>209</sup> in Karpas-299 cells and generated highly reproducible IC<sub>50</sub> values in the cellular MNK1 activity assay. The graph shows a representative result.



## ➤ You ship your compounds – ProQinase performs the testing



- IC<sub>50</sub> 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<sub>50</sub> curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.