Kinases are key enzymes in signal transduction pathways. However, only a small fraction of more than 500 predicted human kinases is characterised so far. We developed a peptide micro-array based technology for extremely efficient profiling of kinase substrate specificity. This technology reduces set-up time for HTS assays and allows the identification of downstream targets and/or biological substrates. Moreover, peptide micro-arrays enable both identification of compatible phosphospecific antibodies and efficient mapping of the pharmacophoric space.