HTRF® phospho-assays reveal subtle drug-induced effects in tumor xenografts. A method of choice beyond Western blot

INTRODUCTION Cell signaling pathways involving phosphoproteins are often over-activated in cancers, leading to aberrant cell proliferation and survival. Therefore, protein phosphorylation is frequently assessed while developing novel anti-tumor therapeutics, such as tyrosine kinase inhibitors (TKI) or monoclonal antibodies. In preclinical models, human tumor xenograft-bearing mice are routinely used to evaluate the in vivo efficacy of such anticancer drugs. Western blot is the standard technique for analyzing proteins in tumor lysates, but it is time- and labor-intensive.

This poster compares the use of HTRF phospho-assays with traditional Western blot for the analysis of AKT and ERK1/2 in human pancreatic tumor xenografts, after mouse treatment with the anti-EGFR TKI erlotinib (Fig 1). The data demonstrates that HTRF is a more convenient, accurate and sample-saving method than Western blot for assessing protein phosphorylation in tumor xenografts.

CONCLUSION The data presented here demonstrates that HTRF phospho-assays are completely suitable for revealing subtle drug-induced modulations of protein phosphorylation in tumor xenografts, and represent a more convenient and sample-saving method than Western blot. Most importantly, highly reproducible HTRF assay results rapidly lead to correct data interpretation, essential in evaluating the in vivo efficacy of candidate anti-tumor therapeutics.


ASSAY PROTOCOL

PERFORMANCE OF HTRF ASSAYS

SIDE-BY-SIDE COMPARISON OF WESTERN BLOT AND HTRF ASSAYS

For more information on sample preparation, assay protocol and data handling, please refer to the corresponding application note “HTRF® phospho-assays reveal subtle drug-induced effects in tumor xenografts. A method of choice beyond Western blot.”

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