Implement HTRF® phospho-protein assays at every step of the drug discovery process: from in vitro to in vivo models

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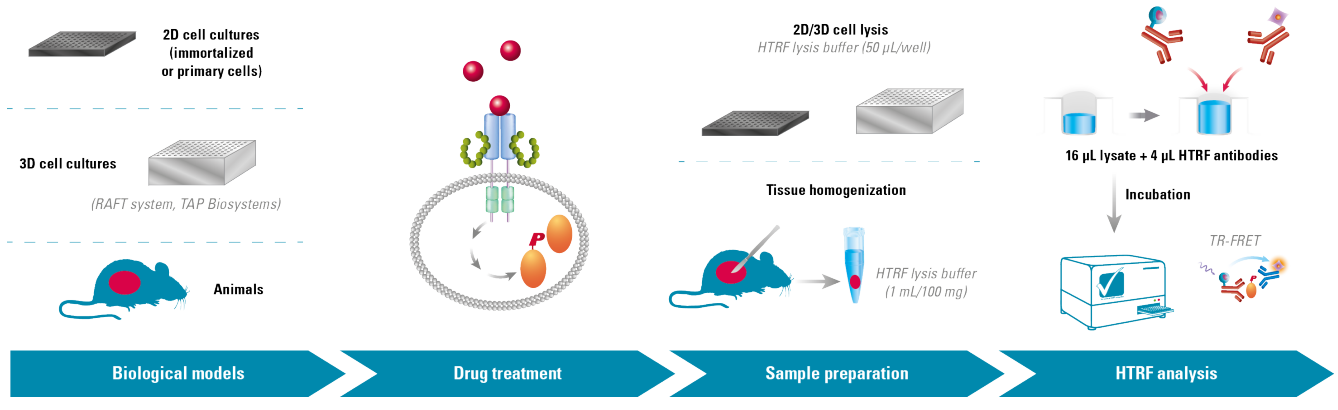
INTRODUCTION High-throughput screening (HTS) on two-dimensional (2D) immortalized cell cultures is frequently the starting point for identifying promising new drugs. To easily and rapidly translate drug discovery research from simplistic in vitro to physiologically relevant in vivo models, researchers need robust biochemical assays compatible with samples presenting different levels of complexity.

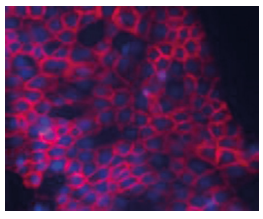
This study demonstrates that the HTRF phospho-/total protein platform is suitable for the rapid and robust analysis of cell signaling pathways in different kinds of biological models such as 2D, 3D cell cultures, patient-derived primary cells, and xenografts.



2D, 3D CELL CULTURES AND XENOGRAFT MODELS DERIVED FROM PANCREATIC TUMOR CELLS

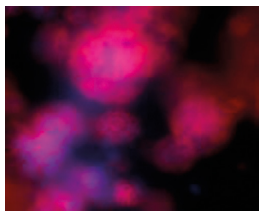
Human BxPC3 pancreatic tumor models / erlotinib treatment

2D



(1-hr treatment)

3D

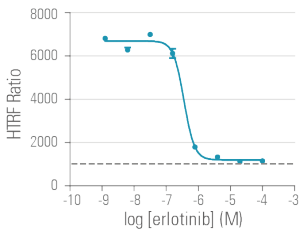


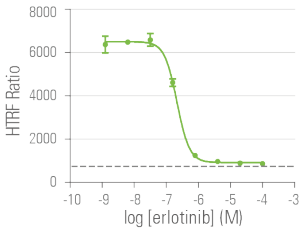
(1-hr treatment)

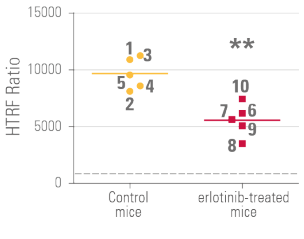
Xenograft

(21 days of treatment)

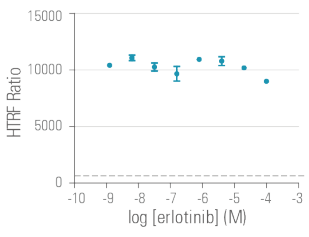
Phospho-AKT (S473)

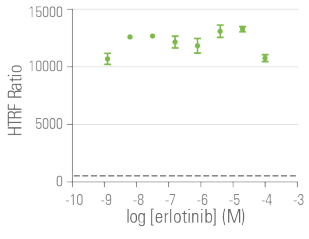


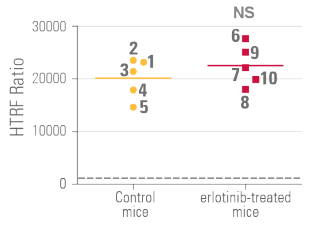




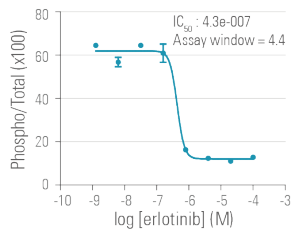
Total AKT

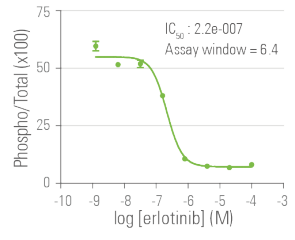


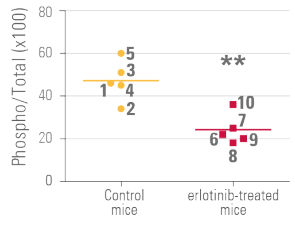




Phospho/Total



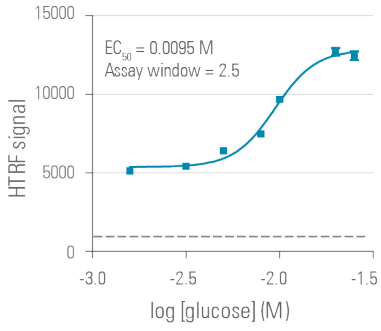




MOUSE PANCREATIC β-CELLS AND ISLETS

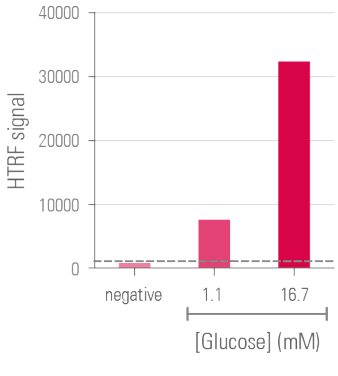
Min6 β-cell line / glucose treatment (5 min)

Phospho-CREB (S133)



Pancreatic islets / glucose treatment (10 min)

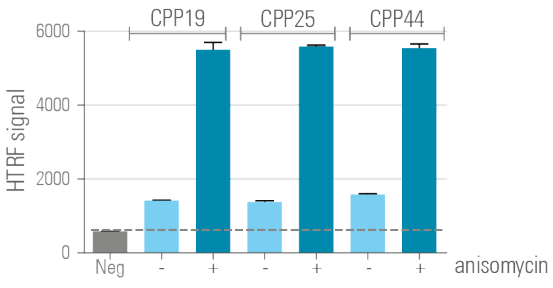
Advanced phospho-ERK1/2 (T202/Y204)



PATIENT-DERIVED COLORECTAL TUMOR PRIMARY CELLS AND XENOGRAFTS

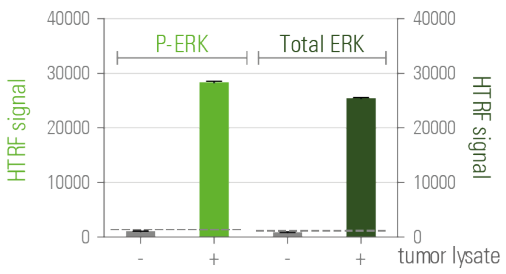
Patient-derived colorectal tumor primary cells / Anisomycin treatment

Phospho-p38 (T180/Y182)

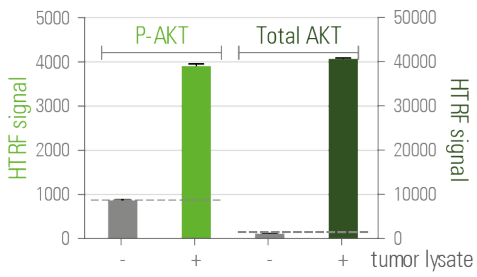


Patient-derived colorectal tumor xenograft

Advanced phospho-ERK1/2 (T202/Y204) and Total ERK



Phospho-AKT (S473) and Total AKT



CONCLUSION The data presented here shows that HTRF phospho-/total protein assays represent a smart solution for studying cell signaling pathways in biological models with different levels of complexity and from different species. Their homogeneous format enables a rapid and simple assay procedure, and their sensitivity is suitable for analyzing endogenous protein expression and phosphorylation.

Moreover, HTRF technology delivers accurate pharmacological parameters and reproducible statistical data.
In conclusion, the universal HTRF phospho-protein platform is compatible with every step of the drug discovery process, from HTS to preclinical studies, and thus facilitates research program transition from in vitro to in vivo models.

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