Universal HTRF® phospho-protein platform: from 2D, 3D, primary cells to patient derived tumor cells

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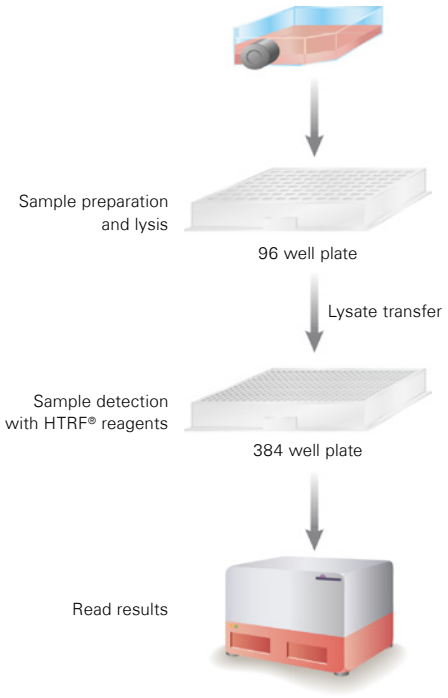
INTRODUCTION Why is monitoring key signaling nodes, such as the MAP-Kinase ERK or PI3 Kinase/AKT, of such fundamental importance in most therapeutic areas? The dysregulation of cellular signaling is the major cause or consequence of most diseases. Thus investigation of the signaling network is essential to understand the pathophysiology involved and to find new medicines. Phospho-proteins are key players in this complex network and can be exploited either as pathway readouts downstream from a cascade of signaling events or as primary targets for drugs. Therefore, the quantitative assessment of their phosphorylation status serves as a powerful marker for functional activation or inhibition of the corresponding pathway. For many years, Western Blot and ELISA were the most popular methods to detect protein phosphorylation. More recently, HTRF® assays based on homogeneous TR-FRET, an advanced fluorescence-based technology, have had great success because they facilitate the cell signaling research in many ways.

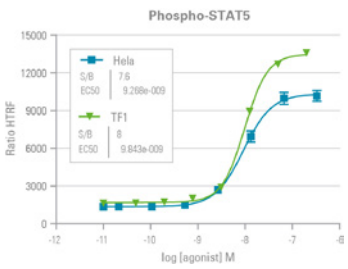
The simple add-and-read, no-wash protocol for time and labor saving processing is just one of the many benefits of HTRF® assays. They require only low sample volumes and offer a very high compatibility level with a variety of biological samples, multi-species and different cellular models. Here, this is demonstrated starting from well-established cell lines grown in 2D or 3D, over normal primary cells such as PBMC, up to primary patient-derived tumor cells, and prove the versatile application of Cisbio's cell signaling assays.

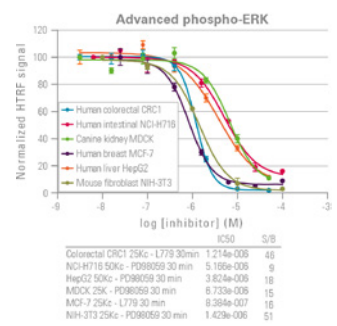
CELL MODELS	HTRF® PHOSPHO ASSAY	COMPOUND	INCUBATION TIME
Hela	P-STAT5	EGF	30 min
TF1	P-STAT5	IL3	10 min
BXPC3 3D	P-EGFR, T-EGFR	Erlotinib (+EGF 150 nM, 5 min)	1 h
Ins1E, Min6, Islets	Advanced P-ERK1/2, P-CREB	Glucose	10 min
CRC-1	Advanced P-ERK1/2	L-779,450	30 min
	P-AKT	Wortmanin (+2 nM IGF1)	10 min
	P-p38	IL-1b	10 min
	P-IKKb	IL-1b	10 min
CPP25, CPP44	Advanced-P-ERK1/2, P-S6RP,	EGF 100 ng/mL	30 min
	P-p38	Anisomycin 50 nM	30 min



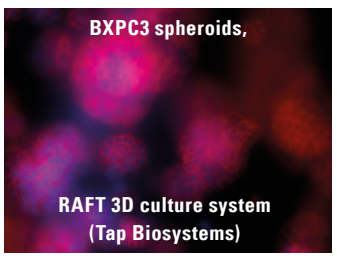
CELL LINES –DIFFERENT SPECIES

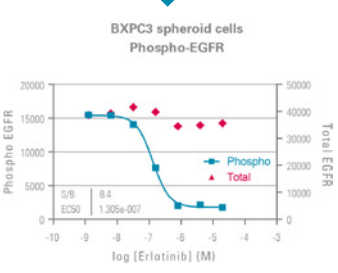
2D





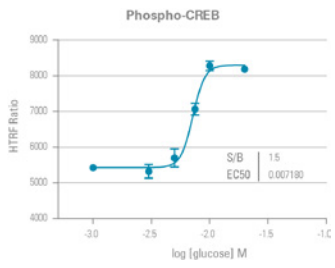
3D



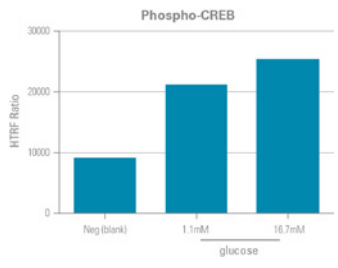


B-PANCREATIC CELLS AND ISLETS

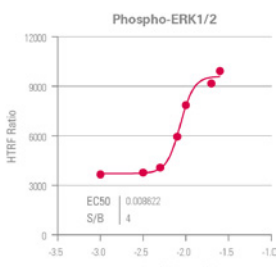
Ins1-E



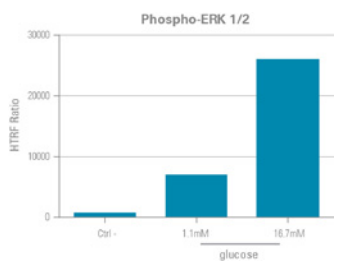
Islets from mice



Min-6

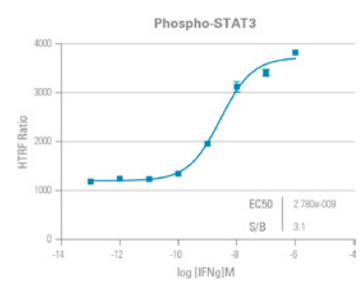


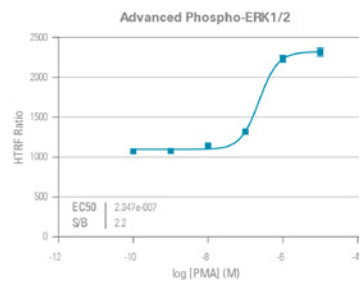
Islets from mice



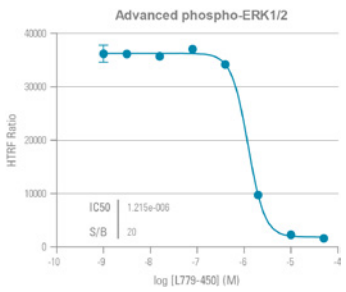
HEALTHY & TUMOR DERIVED PRIMARY CELLS

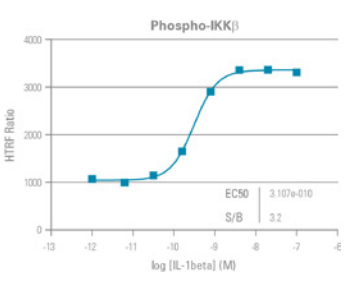
PBMC

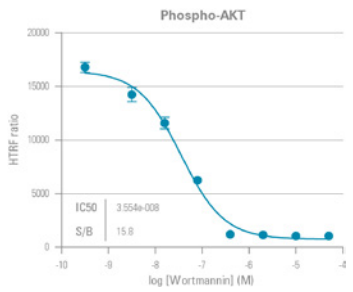


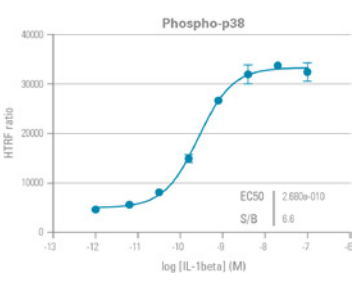


CRC1 (colorectal cancer cells)

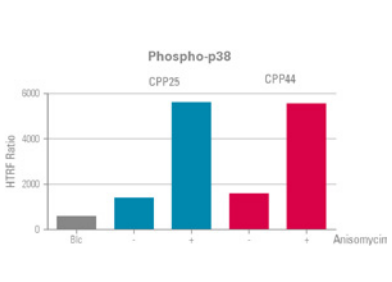


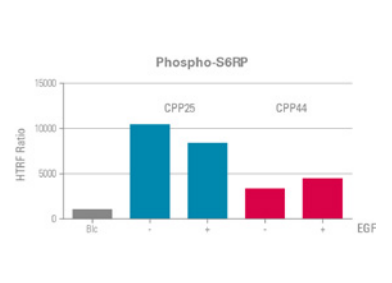






CPP (patient derived tumor cells)





CONCLUSION As shown here, HTRF® phospho-protein assays enable the exploration of cell signaling by making the analysis of a large panel of diverse biological samples and cellular models of different physiological complexity. Throughout research programs, HTRF® phospho-protein assays are versatilely applicable from well-established 2D cell lines, adherent or in suspension, and 3D grown cell culture tumor spheroids up to tissue-like models, such as pancreatic islets. Furthermore Cisbio's pathway readout assays have also proven to be valuable tools also for studies in primary cells such as PBMCs, for example, and patient derived primary tumor cells. With their high compatibility level, simple no-wash protocol, and low sample volumes, HTRF® assays are suitable for all research where time and labor saving processing is required.

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